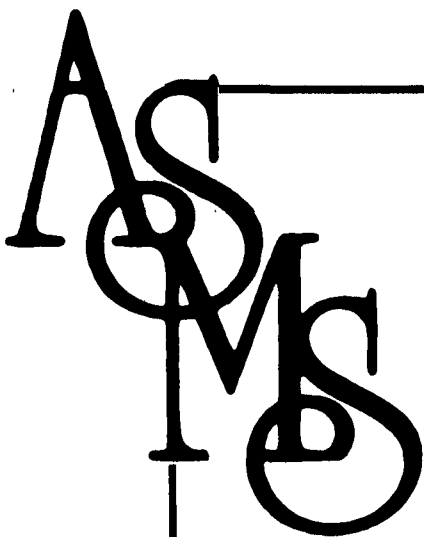




**Proceedings
of the
37th ASMS
Conference
on
Mass Spectrometry
and
Allied Topics**

**May 21 - 26, 1989
Miami Beach, Florida**

THE 37th ASMS CONFERENCE ON MASS SPECTROMETRY AND ALLIED TOPICS
May 21 - 26, 1989

TABLE OF CONTENTS

INTRODUCTION	iv
ASMS BOARD OF DIRECTORS	v
ASMS COMMITTEES	v
CORPORATE MEMBERS	vi
ASMS INTEREST GROUPS	viii
LISTING OF EXTENDED ABSTRACTS	ix
Plenary Lectures	1
Monday Morning	
Symposium: Advances in Instrumentation	22
Symposium: Introduction to Alternative Ionization Techniques	36
Symposium: Elemental Analysis	46
Monday Afternoon	
Symposium: Advances in Instrumentation	60
Symposium: Air Monitoring by Mass Spectrometry	72
Biochemistry I	82
Monday Posters	
LC/MS I	106
Clusters, Inorganic, Surface Analysis	155
Ion-Activation-Dissociation	214
Polymers	258
Fuels in Geosciences	294
Ion-Molecule Reactions	313
Computational	344
Tuesday Morning	
LC/MS	365
Ionization/Activation	387
Symposium: Isotope Measurements	411
Tuesday Afternoon	
Symposium: Formation & Analysis of Multicharged Ions	425
Symposium: MS/MS in Environmental Applications	436
FT/MS and Ion Traps	449
Symposium: Historical Developments in Instrumentation & Techniques	470
Tuesday Posters	
Ionization Methods	527
Isotope Ratios	588
Reaction Mechanisms	600
Pharmacology/Drugs	676
Oligosaccharides/Lipids	720

Wednesday Afternoon	
Symposium: Next Generation of Environmental Contaminants	798
Symposium: Lasers in Mass Spectrometry	812
Ion Molecule Reactions	824
Wednesday Posters	
Peptides and Proteins	844
LC/MS II	927
Instrumentation	1017
Thursday Morning	
Symposium: Tandem MS in the Solution of Peptide & Protein Structural Problems	1122
Inorganic Elemental Analysis and Instrumentation	1133
Symposium: Comparison of Ionic Reactions in Gas Phase & in Solution	1157
Thursday Afternoon	
Symposium: State of the Art Research in Mass Spectrometry	1165
Biochemistry II	1175
Symposium: Organometallic Mass Spectrometry	1198
Thursday Posters	
FT/MS and Ion Traps	1210
Metabolism and Toxicology	1280
Ion Molecule Reactions	1363
Environmental	1402
Friday Morning	
Symposium: Separations and Peptides	1472
Environmental Applications	1478
Fundamental Ion Chemistry Clusters and Surfaces	1498
INDEX OF AUTHORS	1517
INDEX OF SUBJECTS	1536
MINUTES OF THE ASMS BUSINESS MEETING	1545
SALARY SURVEY	1548
COMMITTEE, INTEREST GROUP AND WORKSHOP REPORTS	
Analytical Utility of High and Low Energy Collision Regimes	1550
Biomedical Interest Group	1551
Budget Blues or Where Do I Get Money to Buy a New Multiplier	1552
Desorption Processes Interest Group	1554
Environmental Interest Group	1556
Fundamentals of Peptide Sequencing	1557
History of Mass Spectrometry Interest Group	1559
Instrument-Dependent CAD Databases	1560
Instrumentation and Ion Physics Interest Group	1562
Laser Desorption of Biomolecules. How Does It Work?	1563
Young Mass Spectrometrists Interest Group	1564
1989 ASMS MEMBERS AND CONFERENCE ATTENDEES	1565

INTRODUCTION

By action of the ASMS Board of Directors, this official record of the annual meeting will henceforth be known as the Proceedings instead of the more informal term "bound volume". Thus, the 1989 designation is Proceedings of the 37th ASMS Conference on Mass Spectrometry and Allied Topics.

The extended abstracts that comprise the bulk of the Proceedings provide a valuable and timely view of the most current research in this very dynamic field. A large number (more than 90 %) of those presenting papers and posters at the conference contribute extended abstracts to the Proceedings. Extra space has been allotted to Plenary lecturers and symposia speakers to provide more details of their presentations. New this year is an index of subjects. Also included in the Proceedings are workshop and interest group reports and a directory of names, addresses, and telephone numbers of 1989 members and conference attendees.

The efforts of Judith A. Watson and her staff of Professional Association Management, Inc. in compiling the Proceedings and arranging for timely printing and distribution in a cost effective manner are gratefully acknowledged. The work presented here also reflects the hard work and creativity of the Vice-President for Programs, Robert C. Murphy, in arranging the technical program.

The Proceedings find a greater audience each year and form a valuable asset to the members of the Society. No doubt there are ways it could be improved, and suggestions or comments directed toward making this a more useful resource are invited.

Willard W. Harrison
Editor and Member-at-Large
ASMS Board of Directors

CITATION NOTICE

This volume contains the extended abstracts of papers presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics. The material is not copyrighted and appearance of material in this volume does not jeopardize the author's right to publish the information in scientific journals.

Reference to individual abstracts contained in this volume should be cited as follows:

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ASMS INTEREST GROUPS

The following is a brief description of the role of each interest group as provided by their coordinators.

Academic Laboratory Managers, *Thomas R. Sharp*

The problems and solutions encountered by those responsible for academic service mass spectrometry facilities.

Biomedical Applications, *Richard M. Caprioli*

Mass spectrometric relevance to biological and medical systems, including areas such as enzymology, macromolecular sequencing, metabolic screening, natural products, etc.

Computer Applications, *Jon D. Onstot*

Enhancement of the science of mass spectrometry by exploitation of computer technology either through electronic data acquisition, data processing, artificial intelligence or other technologies.

Desorption Ionization, *Peter J. Todd*

Investigation of, and applications for, processes by which gas phase ions are generated from condensed phases.

Elemental Analysis, *Gary M. Hieftje*

The application of mass spectrometry to elemental analysis, including applications to organic and inorganic samples, the use and development of isotope ratio technology and the development of instrumentation and methodology for elemental mass spectrometry.

Environmental Applications, *William L. Budde*

The development and application of mass spectrometry and related techniques to the qualitative and quantitative analysis of environmental systems.

Fundamentals, *Douglas P. Ridge*

The chemistry of gas phase ions including structural, spectroscopic, thermodynamic and kinetic properties and their influence on other areas of mass spectrometry.

History of Mass Spectrometry, *Michael A. Grayson*

The collection and archiving of information about the development of mass spectrometry, its practitioners, and its instrumentation.

Instrumentation and Ion Physics, *Scott A. McLuckey*

Techniques for forming, transporting, analyzing, and detecting charged particles with the goal of improving mass spectrometry as a research tool.

Isotopic Applications, *Lawrence W. Green*

The application and development of isotope ratio mass spectrometry in all areas of science, including (but not limited to) archeology, atmospheric science, geology, medicinal chemistry, and nuclear science.

Pharmacology, *James Vrbanc*

The structural and quantitative analysis of drugs and their metabolites, and of endogenous bioactive compounds and their metabolites.

Physical Organic, *Brian Chait*

The physical and chemical processes involved in the production of neutral and ionized organic molecules, their subsequent interaction with neutral and charged entities, and their fragmentation.

Surface Science, *Robert W. Odom*

The application of mass spectrometry to the study of the elemental and chemical composition and structure of solid surfaces and the characterization of atomic and molecular adsorbates.

Young Mass Spectrometrists, *Julie A. Leary*

The support and advancement of mass spectrometrists in the early stages of their careers.

PROCEEDINGS OF THE 37TH ASMS CONFERENCE TECHNICAL SESSIONS

PLENARY LECTURES

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY: PRECEPTS AND PROSPECTS *MARSHALL, A.G.	1
MOLECULAR ION SPECTROSCOPY: THE THIRD GENERATION *SAYKALLY, R.J.	6
MASS SPECTROMETRY: HOW DID WE GET HERE AND WHERE ARE WE GOING? *MCLAFFERTY, F.W.	8
ONE MAN'S JOURNEY IN MASS SPECTROMETRY *BEYNON, J.H.	16

MONDAY ORAL SESSIONS

Symposium: Advances in Instrumentation

IONIZATION BY POSITRONS: WHAT'S THE MATTER WITH ANTIMATTER? *MCLUCKEY, S.A., GLISH, G.L., DONOHUE, D.L., HULETT, L.D. AND MCKOWN, H.S.	22
ION/SURFACE COLLISIONS: INSTRUMENTATION AND APPLICATIONS *COOKS, R.G., HABIBI-GOUDARZI, S., MORAND, K.L., SCHEY, K.L. AND SCHWARTZ, J.C.	25
ARRAY DETECTORS FOR MAGNETIC MASS SPECTROMETERS *BIEMANN, K.	27
CAPILLARY ELECTROPHORESIS/FLOW FAB/MS *MINARD, R.D., CURRY, P. AND EWING, A.G.	30
ANALYSIS OF DATA RATE REQUIREMENTS FOR HIGH PERFORMANCE GC/MS: THE CASE FOR TOFMS WITH TIME-ARRAY DETECTION *ENKE, C.G., WATSON, J.T., ALLISON, J. AND HOLLAND, J.F.	32
ISOTOPE-RATIO-MONITORING GAS CHROMATOGRAPHY MASS SPECTROMETRY *HAYES, J.M., FREEMAN, K.H., RICCI, M.P., STUDLEY, S.A., MERRITT, D.A., BRAND, W.A. AND HABFAST, K.	33
COUPLING PLANAR CHROMATOGRAPHIES WITH MASS SPECTROMETRY, AND MASS SPECTROMETRY WITH OPTICAL SPECTROSCOPIC IMAGING *BUSCH, K.L.	35
MASS RANGE EXTENSION IN QUADRUPOLE ION TRAPS KAISER, R.E., COOKS, R.G. AND *HEMBERGER, P.H.	60
ION INJECTION INTO ION TRAPS *YOST, R.A. AND PEDDER, R.E.	62
TWO-DIMENSIONAL FT/MS *GAUMANN, T.	65
NEW METHODS OF ION ISOLATION AND EXCITATION IN FT/MS *CODY, R.B.	68

A TRIPLE STAGE QUADRUPOLE GLOW DISCHARGE MASS SPECTROMETER: THE DISSOCIATIVE REMOVAL OF POLYATOMIC INTERFERENCES *HARRISON, W.W. AND KING, F.L.	70
--	----

Symposium: Introduction to Alternative Ionization Techniques

A PRIMER ON ELEMENTAL MASS SPECTROMETRY: ICPMS(SOLUTIONS) VS GDMS(SOLIDS) *HARRISON, W.W.	36
RIMS AND TIMS AND APPLES AND ORANGES - A COMPARATIVE SUMMARY *MOORE, L.J.	38
PRODUCTION OF IONS FROM CHARGED LIQUID DROPLETS IN ELECTROSPRAY, THERMOSPRAY, AND RELATED TECHNIQUES *VESTAL, M.L.	41
DESORPTION TECHNIQUES: HOW THEY ARE DIFFERENT *COTTER, R.J.	43

Symposium: Elemental Analysis

INTERACTIONS OF SOLIDS, LIQUIDS, AND VAPORS IN ICP-MS: POTENTIAL FOR ADVANCES ZHU, G., PAN, C. AND *BROWNER, R.F.	46
PLASMA MASS SPECTROMETRY FOR CHROMATOGRAPHIC DETECTION *CARUSO, J.A., SHEPPARD, B., CREED, J., SUYANI, H. AND DAVIDSON, T.	48
STRUGGLING TO REDUCE MOLECULAR ION INTERFERENCES IN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) *DOUGLAS, D.J.	50
TIME RESOLVED STUDIES IN GLOW DISCHARGE MASS SPECTROMETRY *HARRISON, W.W. AND KLINGLER, J.A.	52
NEW DEVELOPMENTS IN SOURCES FOR PLASMA-SOURCE MASS SPECTROMETRY *HIEFTJE, G.M., CHAMBERS, D., JIN, Q. AND ROSS, B.	54
ION KINETIC ENERGIES IN THE LOW-PRESSURE ZONES OF AN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER *CHAMBERS, D.M. AND HIEFTJE, G.M.	56
SAMPLE AEROSOL, PLASMA GAS COMPOSITION AND SAMPLER-SKIMMER GEOMETRY EFFECTS IN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) *HORLICK, G.	58

Symposium: Air Monitoring by Mass Spectrometry - Present & Future

EXPECTED REGULATORY AND GUIDANCE REQUIREMENTS FOR CONTROL OF AIR TOXICS *WOOD, G.	72
ROLE OF MASS SPECTROMETRY IN EXPOSURE ASSESSMENT PROGRAMS *PELLIZZARI, E.D.	74
DIRECT AIR MONITORING USING AN ION TRAP MASS SPECTROMETER *GLISH, G.L. AND MCLUCKEY, S.A.	77
ATMOSPHERE ANALYSIS ON BOARD SUBMARINES *WYATT, J.R. AND KOSLIN, M.E.	80

Biochemistry I

A RAPID IMMUNOAFFINITY CLEANUP/GC-MS METHOD FOR DETERMINATION OF 3-METHYLADENINE IN URINE *FRIESEN, M., SHUKER, D.E.G., GARREN, L. AND PREVOST, V.	82
ISOLATION AND CHARACTERIZATION OF AN UNUSUAL NUCLEOSIDE FROM HUMAN URINE *CHHEDA, G.B., DUTTA, S.P., TWOREK, H.A., BHARGAVA, A.K., TAKITA, H., ANTKOWIAK, J.G. AND PATRZYC, H.B.	84
LASER IONIZATION FOURIER TRANSFORM MASS SPECTROMETRY FOR THE ISOMERIC DIFFERENTIATION OF ALKYLATED NUCLEOSIDES *HETTICH, R.L. AND BUCHANAN, M.V.	86
FAB/MIKES ANALYSIS OF CYCLIC NUCLEOTIDES AND RELATED COMPOUNDS NEWTON, R.P., WALTON, T.J., *BRENTON, A.G., HARRIS, F.M., GHOSH, D., KINGSTON, E.E., BEYNON, J.H., JENKINS, A. AND SALVAGE, B.J.	88
AN INVESTIGATION OF IONIZATION MECHANISMS DURING LIQUID SECONDARY ION MASS SPECTROMETRY (LSIMS): EVIDENCE FOR ONE-ELECTRON REDUCTION *LARAMEE, J.A., ARBOGAST, B. AND DEINZER, M.L.	90
QUANTIFICATION OF EPOXYEICOSATRIENOIC ACID BY CAPILLARY COLUMN CHROMATOGRAPHY/NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY *BLAIR, I.A., CAPDEVILA, J. AND YAMASHITA, K.	92
IDENTIFICATION OF CARROT PHOSPHOINOSITOLS BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY *VAN BREEMEN, R.B., WHEELER, J.J. AND BOSS, W.F.	94
ENHANCED DETECTION OF DIAGNOSTIC ACYLCARNITINES IN PLASMA AND TISSUE UTILIZING FAB COUPLED WITH TANDEM MASS SPECTROMETRY *MILLINGTON, D.S., NORWOOD, D.L. AND KODO, N.	96
SEQUENCE SPECIFIC PEPTIDE FRAGMENTATION OBSERVED USING MS/MS/MS SCAN MODES *SCHEY, K.L., SCHWARTZ, J.C. AND COOKS, R.G.	98
TANDEM MASS SPECTROMETRY OF MULTIPLY CHARGED PEPTIDES AND PROTEINS *BARINAGA, C.J., UDSETH, H.R. AND SMITH, R.D.	100
STRUCTURE DETERMINATION OF NOVEL CYCLIC PEPTIDES BY USING TANDEM MASS SPECTROMETRY *CERNY, R.L., GROSS, M.L., PETTIT, G.R., KAMONO, Y. AND HERALD, C.H.	102
HIGH VS LOW ENERGY CID OF PEPTIDE IONS ALEXANDER, A.J., THIBAUT, P., *BOYD, R.K., CURTIS, J.M. AND RINEHART, K.L.	104

MONDAY POSTER SESSIONS

LC/MS I

ANALYSIS OF SYNTHETIC PEPTIDE MIXTURES AND PROTEIN DIGESTS BY CZE/CF-FAB/MS *MOORE, W.T., MARTIN, M., DAGUE, B., CAPRIOLI, R.M., WILSON, K.J. AND MORING, S.E.	106
SENSITIVITY ENHANCEMENT OF PARTICLE BEAM LC/MS *WILLOUGHBY, R.C., BUCHNER, J.D. AND SHEEHAN, E.W.	108

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

PARTICLE BEAM LC/MS BY FAB *SANDERS, P.R.,WILLOUGHBY, R. AND MITROVICH, S.	110
DETERMINATION OF ALKYL BENZENESULFONATES BY CAPILLARY ZONE ELECTROPHORESIS - MASS SPECTROMETRY *VASSILIADOU, M.,MILLS, T.M. AND MISSLER, S.R.	112
CAPILLARY ZONE ELECTROPHORESIS AND CAPILLARY LIQUID CHROMATOGRAPHY COUPLED WITH SECTOR MASS SPECTROMETRY USING COAXIAL CONTINUOUS FLOW FAB INTERFACES *MOSELEY, M.A.,DETERDING, L.J.,TOMER, K.B.,BRAGG, N. AND JORGENSEN, J.W.	114
CAPILLARY ZONE ELECTROPHORESIS-MASS SPECTROMETRY OF PEPTIDES AND PROTEINS *LOO, J.A.,UDSETH, H.R. AND SMITH, R.D.	116
ANALYSIS OF TRYPTIC DIGESTS BY ON-LINE ZONE ELECTROPHORESIS TANDEM MASS SPECTROMETRY *HUANG, E.,COVEY, T. AND HENION, J.	118
THE ON-LINE COMBINATION OF CAPILLARY ISOTACHOPHORESIS WITH MASS SPECTROMETRY *UDSETH, H.R.,LOO, J.A. AND SMITH, R.D.	120
CHROMATOGRAPHY AND MASS SPECTROMETRY OF DOMOIC ACID, A MARINE NEUROTOXIN *PLEASANCE, S.,QUILLIAM, M.A.,LEBLANC, Y.,SIM, P.G.,THIBAUT, P. AND MEI, X.	122
QUALITATIVE AND QUANTITATIVE DATA OBTAINED FROM PACKED COLUMN SUPERCRITICAL FLUID CHROMATOGRAPHY/MASS SPECTROMETRY USING THERMOSPRAY JOSEPH, R.S.I.,*LANT, M.S. AND WOOLGAR, P.	124
HIGH TEMPERATURE VAPORIZATION EFFECTS IN PARTICLE-BEAM MASS SPECTROMETRY *HARRIS, W.E. AND BROWNER, R.F.	126
IN VITRO ANALYSIS OF ANTIMETABOLITES IN BLOOD PLASMA BY MAGIC LC/MS *HARRIS, W.E. AND BROWNER, R.F.	128
AEROSOL TRANSPORT STUDIES IN PARTICLE-BEAM MASS SPECTROMETRY *EDMAN, K.R.,KIRK, J.D. AND BROWNER, R.F.	130
FAST ATOM BOMBARDMENT: BROADENING THE CAPABILITIES OF PARTICLE-BEAM LC/MS *KIRK, J.D. AND BROWNER, R.F.	131
LC/API/MS BY PARTICLE BEAM LIQUID CHROMATOGRAPHY *SHEEHAN, E.W.,LO, H.H. AND WILLOUGHBY, R.C.	133
OPTIMIZATION AND EVALUATION OF PARTICLE BEAM HPLC/MS PERFORMANCE FOR THE ANALYSIS OF PESTICIDE COMPOUNDS AND METABOLITES *SMITH, C.S.,KNOX, P.C. AND VOYKSNER, R.D.	135
THE APPLICATION OF PARTICLE BEAM HPLC/MS TO THE ANALYSIS OF DERIVATIZED AND UNDERIVATIZED PROSTAGLANDINS *SCHILLING, A.B.	137
CHARACTERIZATION OF FLAVORS AND AROMAS BY GC/MS AND PARTICLE BEAM LC/MS *MARECIC, T.,SHEEHAN, E.W. AND DILTS, M.J.	139
DEVELOPMENT OF AN INTERNAL STANDARD AND A PERFORMANCE TEST MIXTURE FOR PARTICLE BEAM LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY SYSTEMS *BEHYMER, T.D.,BELLAR, T.A. AND BUDDE, W.L.	141
VARIOUS ASPECTS OF IONSpray MASS SPECTROMETRY/MASS SPECTROMETRY *SIU, K.W.M.,GARDNER, G.J. AND BERMAN, S.S.	143

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

IONSpray MASS SPECTROMETRY FOR THE ANALYSIS OF MARINE TOXINS *QUILLIAM, M.A. AND THOMSON, B.	145
A NON-AEROSOL VAPORIZATION INTERFACE FOR LC/MS AND LC/MS/MS *SLIVON, L.E., KENNY, D.V. AND SEVERANCE, R.A.	147
ATMOSPHERIC PRESSURE SPRAY IONIZATION FOR LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS) *SAKAIRI, M. AND KAMBARA, H.	149
SHEET DIRECT INSERTION MEMBRANE PROBE FOR SELECTIVE INTRODUCTION OF ORGANIC COMPOUNDS INTO A MASS SPECTROMETER BIER, M.E., *KOTIAHO, T. AND COOKS, R.G.	151
MATRIX-FREE FAB ANALYSIS UTILIZING THE MOVING BELT LC/MS INTERFACE *BAKER, T.R., KYRANOS, J.N. AND VOURES, P.	153

Clusters, Inorganic, Surface Analysis

COLLISION INDUCED DISSOCIATION STUDIES OF TRANSITION METAL OXIDE CLUSTERS *MALEKNIA, S.D. AND LAUDE, D.A.	155
CLUSTER ION FORMATION BY DIRECT LASER VAPORIZATION AND PARTICLE BOMBARDMENT *CALLAHAN, J.H., ROSS, M.M. AND BARONAVSKI, A.P.	157
FTMS STUDIES OF CARBON CLUSTER ANIONS MCELVANY, S.W.	159
DISSOCIATION AND REACTIVITY OF COBALT AND MOLYBDENUM OXIDE CLUSTER IONS *CASSADY, C.J. AND MCELVANY, S.W.	161
REACTIVITY OF BISMUTH CLUSTER IONS *ROSS, M.M., WYSOCKI, V.H. AND MCELVANY, S.W.	163
CLUSTER FORMATION IN THE FAST ATOM BOMBARDMENT (FAB) MASS SPECTRA OF DIOXOURANIUM (VI) DINITRATE AND DIACETATE *JENNINGS, K.R., KEMP, T.J. AND READ, P.A.	165
FORMATION OF CARBON CLUSTERS FROM LASER ABLATION OF POLYMERS *CREASY, W.R. AND BRENNAN, J.T.	167
TANTALUM CARBIDE CLUSTER IONS: PRODUCTION, DISSOCIATION, AND REACTIVITY *CASSADY, C.J. AND MCELVANY, S.W.	169
SAMPLE INTRODUCTION CHARACTERISTICS OF FLOW INJECTION WITH ICP-MS *ZHU, G., PAN, C. AND BROWNER, R.F.	171
COLLISION-INDUCED DISSOCIATION OF SOME RUTHENIUM COMPLEXES *LIANG, X. AND FREAS, R.B.	173
PURITY OF 1% SILANE AND 1% DICHLOROSILANE IN HYDROGEN *REENTS, W.D., GREEN, M.L. AND BRASEN, D.	175
APPLYING THE CONCERTED ISOTOPE PATTERN-EXACT MASS METHOD OF ESTABLISHING ELEMENTAL COMPOSITION *BLOM, K. AND CUMMISKEY, J.	176
INORGANIC FINGERPRINT OF RAT TISSUES *GELINAS, Y., FERRARIS, J. AND SCHMIT, J-P.	178
ELEMENTAL COMPOSITION OF MILK: FROM THE COW TO THE CONSUMER *MARTINEZ, C., AMER, M.A., GOSWAMI, T. AND SCHMIT, J-P.	180

THE DETERMINATION OF LOW-ABUNDANCE OXYGEN IN FLUORINE GAS MIXTURES *CISPER, M.E. AND HEMBERGER, P.H.	182
MATRIX EFFECTS ON THE FAST ATOM BOMBARDMENT MASS SPECTRA OF PALLADIUM COMPLEXES *MALLIS, L.M. AND SCOTT, W.J.	184
FAST ATOM BOMBARDMENT ANALYSIS OF SPECIES FORMED BY THE INSERTION OF CARBON DISULFIDE INTO Mn-H BONDS SCHAUER, S.J., EYMAN, D.P. AND *MALLIS, L.M.	186
EVALUATION OF A DOUBLE-QUADRUPOLE SPECTROMETER SYSTEM FOR GLOW DISCHARGE MASS SPECTROMETRY DUCKWORTH, D.C. AND *MARCUS, R.K.	188
THE RELATIVE ROLE OF PENNING IONIZATION IN GLOW DISCHARGE DEVICES SERXNER, D., SMITH, R.L. AND *HESS, K.R.	190
SEPARATION OF ATOMIZATION AND IONIZATION PROCESSES IN GDMS *BARSHICK, C.M. AND HARRISON, W.W.	192
STUDIES OF THIN FILMS IN GLOW DISCHARGE MASS SPECTROMETRY *RATLIFF, P.H. AND HARRISON, W.W.	194
ANALYSIS OF POWDERED METAL OXIDES USING RADIO FREQUENCY GLOW DISCHARGE MASS SPECTROMETRY *KLINGLER, J.A. AND HARRISON, W.W.	196
GETTERS AS PLASMA REAGENTS IN GLOW DISCHARGE MASS SPECTROMETRY *MEI, Y. AND HARRISON, W.W.	198
KINETICS OF ETHYLLIDYNE FORMATION USING LITD/FTMS *PETTIETTE-HALL, C.L., LAND, D.P., MCIVER, R.T. AND HEMMINGER, J.C.	200
CALCULATIONS OF THE TEMPERATURE JUMP AND DESORPTION PROBABILITY IN LASER-INDUCED THERMAL DESORPTION *WANG, D.T-S., HEMMINGER, J.C. AND MCIVER, R.T.	202
TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY (TOF-SIMS) OF POLYMER MATERIALS *VANLEYEN, D., NIEHUIS, E., HAGENHOFF, B. AND BENNINGHOVEN, A.	204
THE EFFECTS OF POLYATOMIC PRIMARY IONS ON THE YIELD AND EXTENT OF FRAGMENTATION OF SECONDARY IONS IN MOLECULAR SECONDARY ION MASS SPECTROMETRY *HAND, O.W., MAJUMDAR, T.K. AND COOKS, R.G.	206
ORGANIC SURFACE ANALYSIS WITH A TIME-OF-FLIGHT SECONDARY ION MICROSCOPE *SCHUELER, B., SANDER, P. AND ODOM, R.W.	208
EVIDENCE FOR ION NEUTRALIZATION BY ELECTRON TUNNELING IN THE DESORPTION OF SPECIES FROM POLYMER SURFACES *NORD, R.F. AND MACFARLANE, R.D.	210
ENHANCEMENT OF STATIC FAB SPECTRAL DATA OF AZO SULFONATED DYES NG, K., *SHIMANSKAS, C., KARLINER, J. AND JHO, C.	212

Ion Activation-Dissociation

ON THE DYNAMICS OF LOW AND MEDIUM ENERGY COLLISION INDUCED DISSOCIATION OF IONS *SHUKLA, A.K., QIAN, K., HOWARD, S.L., ANDERSON, S.G. AND FUTRELL, J.H.	214
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Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

DETECTION OF LONG-LIVED EXCITED STATES OF POLYATOMIC IONS IN MOLECULAR BEAM STUDIES OF COLLISION-INDUCED DISSOCIATION PROCESSES *QIAN, K., BIGGERSTAFF, J., ANDERSON, S., HOWARD, S., SHUKLA, A. AND FUTRELL, J.	216
ANGLE AND ENERGY RESOLVED COLLISION-INDUCED DISSOCIATION STUDIES OF THE PROPANE MOLECULAR ION *ANDERSON, S.G., SHUKLA, A.K., LEHMAN, T.H. AND FUTRELL, J.H.	218
ACCELERATION REGION PROCESSES MARCH, R.E. AND *HUGHES, R.J.	220
A COMPARISON OF INTERNAL ENERGIES OF IONS FORMED BY CHARGE INVERSION COLLISIONAL ACTIVATION AND DIRECT EXCITATION BY COLLISIONAL ACTIVATION *STROBEL, F., BRICKER, D.L. AND RUSSELL, D.H.	222
REINVESTIGATION OF KINETIC ENERGY LOSS ACCOMPANYING COLLISION ACTIVATION OF CHLOROPHYLL A USING A FLOATED CELL ALEXANDER, A.J., BOYD, R.K. AND *THIBAUT, P.	224
INTERNAL ENERGY UPTAKE OF SURVIVAL IONS IN KILOVOLT COLLISIONS ALEXANDER, A.J., BOYD, R.K., *THIBAUT, P. AND TOMER, K.B.	226
REFLECTION OF POLYATOMIC IONS UPON COLLISIONS WITH SURFACE *HABIBI-GOUDARZI, S. AND COOKS, R.G.	228
EVALUATION OF PHOTON INDUCED DISSOCIATION IN A FOUR-SECTOR MASS SPECTROMETER *MARTIN, S.A., HILL, J.A., KITTRELL, C. AND BIEMANN, K.	230
MEASUREMENTS OF DAUGHTER ION MASS SPECTRA IN A REFLECTING TIME-OF-FLIGHT MASS SPECTROMETER *TANG, X., ENS, W., MAYER, F., MAO, Y., POPPE, N. AND STANDING, K.G.	232
CHARGE SEPARATION AND ELECTRON CAPTURE INDUCED DISSOCIATION OF DOUBLY CHARGED PORPHYRIN IONS *VAN BERKEL, G.J., GLISH, G.L. AND MCLUCKEY, S.A.	234
DISSOCIATION OF LARGE IONS IN FTMS *WANG, B.H., WILLIAMS, E.R., HENRY, K.D. AND MCLAFFERTY, F.W.	236
ENERGETICS OF CHARGE-REMOTE FRAGMENTATION: A FURTHER INVESTIGATION *GIBLIN, D. AND GROSS, M.L.	238
CHARGE-REMOTE FRAGMENTATIONS OF A SERIES OF HOMOCONJUGATED OCTADECADIENOIC ACIDS: A CA MS/MS STUDY *CROCKETT, J.S. AND GROSS, M.L.	240
STRUCTURAL INFORMATION DERIVED FROM KINETICS OF DISSOCIATION REACTIONS *RUSSELL, D.H. AND SELLERS-HANN, L.	242
INFLUENCE OF CATIONIZING METAL IONS UPON FRAGMENTATION IN FOLIC ACID DERIVATIVES *JONES, A.D., CLIFFORD, A.J. AND BILLS, N.D.	244
CONTROLLED ENHANCEMENT OF STEROCHEMICAL EFFECTS BY MS/MS/MS: ACCESS TO ENDOTHERMICALLY FORMED DAUGHTER IONS *COLE, R.B., PREVOST, C. AND TABET, J.C.	246
COLLISIONALLY ACTIVATED DISSOCIATION OF LASER-DESORBED N-ALKYLPYRIDINIUM CATIONS *WATSON, C.H., KATRITZKY, A.R., DEGA-SZAFRAN, Z. AND EYLER, J.R.	248
TWO-LASER INFRARED MULTIPHOTON DISSOCIATION OF GASEOUS IONS STUDIED BY FTICR *WATSON, C.H. AND EYLER, J.R.	250

DETERMINATION OF INTERNAL-ENERGY AND ENERGY DISSIPATION RATES IN N-BUTYLBENZENE IONS BY COMPETITIVE PHOTODISSOCIATION *UECHI, G.T. AND DUNBAR, R.C.	252
TIME RESOLVED PHOTOFAGMENTATION OF THIOPHENOL IONS IN AN ICR MASS SPECTROMETER *FAULK, J.D. AND DUNBAR, R.C.	254
FRAGMENTATION MECHANISMS FOR HIGHLY FUNCTIONALIZED IONS: THE FAB MASS SPECTRA OF SOME CARDIAC GLYCOSIDES REVISITED SCHULTZ, G.,LIGHT, K. AND *ALLISON, J.	256

Polymers

LASER ABLATION MASS SPECTRA OF HIGH MOLECULAR WEIGHT POLYMERS DETERMINED BY FOURIER TRANSFORM MASS SPECTROMETRY *BRENNAN, J.T. AND CREASY, W.R.	258
FOURIER TRANSFORM SPECTRA OF POLYBUTADIENES AND POLYISOPRENES *CHOWDHURY, A.K. AND WILKINS, C.L.	260
LASER DESORPTION FT/ICR MASS SPECTRA OF POLYAMIC ACIDS *WEBER, W.D.	262
IDENTIFICATION OF POLYIMIDES BY MASS SPECTROMETRY *CHACE, M. AND PLAT, M.	264
EI AND CI SFC/MS OF ISOCYANATE PREPOLYMERS *CARY, A.S.	266
PHOTO- AND THERMAL DEGRADATION OF IODONIUM SALTS: COMPLICATIONS WITHIN SECONDARY ION MASS SPECTROMETRY AND FAST ATOM BOMBARDMENT MASS SPECTRA *SCHURZ, H.H. AND BUSCH, K.L.	268
IDENTIFICATION OF POLYMER INTERMEDIATES BY LINKED SCAN FAST ATOM BOMBARDMENT MASS SPECTROMETRY *KYRANOS, J.N.,VOUROS, P. AND HAGNAUER, G.L.	270
THE CHARACTERIZATION OF AROMATIC POLYMERS BY FIELD DESORPTION MASS SPECTROMETRY ROLLINS, K.,*SCRIVENS, J.H.,TAYLOR, M.,MAJOR, H.J. AND BATEMAN, R.H.	272
THE CHARACTERIZATION OF SYNTHETIC LUBRICANT FORMULATIONS BY FIELD DESORPTION MASS SPECTROMETRY ROLLINS, K.,TAYLOR, M.,*SCRIVENS, J.H. AND ROBERTSON, A.	274
MASS SPECTRAL CHARACTERIZATION OF COMPOUNDS FORMED DURING HIGH TEMPERATURE OXIDATION OF STYRENE BASED POLYMERS USING THERMAL CHROMATOGRAPHY *SUTTON, C. AND ZUMBERGE, J.E.	276
MASS SPECTRAL STUDY OF THE THERMAL BEHAVIOR OF POLYMERS OF OXALIC ACID WEININGER, S. AND *YEOMANS, W.	278
THERMAL DEGRADATION OF POLY[bis(PHENOXY)-PHOSPHAZINE] POLYMERS MAYNARD, S.J.,*SHARP, T.R. AND HAW, J.F.	280
THERMAL STABILITY OF ETFE COPOLYMER SYSTEMS *MORELLI, J.J.,FRY, C.,WOLF, C.J.,GRAYSON, M.A. AND LIND, A.C.	282
LIQUID CARBON DIOXIDE VS SOLVENT EXTRACTION OF ORGANIC POLYMER ADDITIVES FOR MASS SPECTRAL ANALYSIS *MILLER, M.L.	284

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

STEPWISE PYROLYSIS OF POLYMERIC MATERIAL BY ON-LINE PYROLYSIS/GC/MS CHUNG, H.L.,*MOORE D.S. AND ALDRIDGE, J.C.	286
BULK POLYMER ANALYSIS USING LASER IONIZATION MASS SPECTROMETRY (LIMS) AND PATTERN RECOGNITION TECHNIQUES *ODOM, R.W. AND DI BROZOLO, F.R.	287
STUDY OF REDOX PROPERTIES OF POLY-3-METHYLTHIOPHENE FILMS BY Cf-252 PLASMA DESORPTION MASS SPECTROMETRY *AI, J. AND MACFARLANE, R.D.	289
WATCHING REACTIONS WITH K+IDS *SIMONSICK, W.J. AND FULGINITI, M.	291
PYROLYSIS GAS CHROMATOGRAPHY MASS SPECTROMETRY OF POLYARYLACETYLENE RESINS *EASTON, M.P. AND GILMARTIN, D.	293

Fuels in Geosciences

PHOTOIONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY OF ORGANIC COMPOUNDS FROM MANTLE MINERALS AND METEORITES *BECKER, C.H. AND TINGLE, T.N.	294
HYDROCARBON-TYPE ANALYSIS BY FIELD IONIZATION MASS SPECTROMETRY *GALLEGOS, E.J.	296
THE ON-LINE DETERMINATION OF WATER BY MASS SPECTROMETRY *CRAWFORD, R.W., COBURN, T.T. AND ALCARAZ, A.	298
NAPHTHENIC ACIDS IN PETROLEUM BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY *FAN, T.P.	300
DEVELOPMENT OF ITD BASED GC/MS TECHNIQUES FOR RAPID PROFILING OF MICROBIAL CELL WALL LIPIDS *TAGHIZADEH, K., MEUZELAAR, H.L.C. AND HATCHER, H.H.	302
LASER PYROLYSIS MASS SPECTROMETRY OF SINGLE LEVITATED COAL PARTICLES MASWADEH, W., ROBERTS, K.A., MCCLENNEN, W.H., MEUZELAAR, H.L.C. AND *ARNOLD, N.S.	304
THE USE OF A HYBRID MASS SPECTROMETER IN DETERMINING BIOMARKER COMPOUNDS IN CRUDE OIL EXTRACTS WEIR, O. AND *WOOD, D.	306
THERMAL SLICING AS A TOOL TO STUDY THE THERMAL DECOMPOSITION OF COALS & KEROGEN *ZUMBERGE, J.E. AND SUTTON, C.	308
MASS-SPECTROMETRIC ANALYSIS OF HIGH-BOILING WAXES SEPARATED FROM SLUDGE FORMED DURING STORAGE OF CRUDE OIL IN UNDERGROUND RESERVOIRS *GRIGSBY, R.D., THOMSON, J.S., VOGH, J.W., WOODWARD, P.W. AND GILES, H.N.	310
FREE-RADICAL CHEMISTRY IN SUPERCRITICAL FLUIDS *OLESIK, S.V. AND PEKAY, L.	312

Ion Molecule Reactions

ADDUCTS OF d5 AND d10 TRANSITION METAL CATIONS WITH ALKANES: AB INITIO AND EXPERIMENTAL STUDIES *BEAUGRAND, C., BERTHIER, G., SUARD, M., MESTDAGH, H. AND ROLANDO, C.	313
GAS-PHASE ION-MOLECULE REACTIONS OF Cu+ AND C7H8 ISOMERS: A CA MS/MS STUDY *MACMILLAN, D.K., HAYES, R.N. AND GROSS, M.L.	315
LIGAND FLUXIONALITY AND CLUSTERING REACTIONS OF TRANSITION METAL CARBONYLS: Fe(13CO)+ CLUSTERING WITH Fe(CO)5 *KERELY, E.L. AND RUSSELL, D.H.	317
LIGAND EXCHANGE REACTIONS OF IONIC TRANSITION METAL CARBONYL CLUSTERS: REACTIONS FOR Cr2(CO)y+ AND Fe2(CO)y+ *SELLERS-HANN, L. AND RUSSELL, D.H.	319
DYNAMICS AND THERMODYNAMICS OF LASER ABLATED METAL IONS BY HIGH PRESSURE MASS SPECTROMETRY *BOUCHARD, F., MCMAHON, T.B. AND HEPBURN, J.W.	321
ION-MOLECULE CHEMISTRY OF METHYL, CYCLOPENTADIENYL CHROMIUM DINITROSYL *COMISAROW, M.B. AND CHEN, S-P.	323
THE PROPER CONSTRUCTION OF EQUILIBRIUM "LADDER" SCALES *SORENSEN, D.M. AND BARTMESS, J.E.	325
THE STRUCTURE OF THE (M-H) ANIONS OF NITROMETHANE AND METHYLNITRITE *THOMAS, D. AND BARTMESS, J.E.	326
THERMOCHEMISTRY FOR REACTIVE NEUTRAL SPECIES FROM APPEARANCE ENERGY MEASUREMENTS IN A FLOWING AFTERGLOW-TRIPLE QUADRUPOLE INSTRUMENT *PAULINO, J.A. AND SQUIRES, R.R.	327
DETERMINATION OF STABILITY AND STRUCTURE OF CLUSTER IONS C1-(RH) (C1- ATTACHED WITH ALKANES) *LI, C. AND MCMAHON, T.B.	329
FOURIER TRANSFORM ION CYCLOTRON RESONANCE INVESTIGATION OF HYDROGEN BONDING IN ALCOHOL-FLUORIDE ADDUCT IONS *WILKINSON, F.E. AND MCMAHON, T.B.	331
A HIGH PRESSURE MASS SPECTROMETRIC DETERMINATION OF EQUILIBRIUM ISOTOPE EFFECTS OF ASSOCIATION AND EXCHANGE REACTIONS OF F- AND RO- WITH SIMPLE OH/OD LABELLED ALCOHOLS *SZULEJKO, J.E., WILKINSON, F.E. AND MCMAHON, T.B.	333
STRUCTURES AND ENERGETICS OF NO3- FORMED IN A GLOW DISCHARGE IONIZATION SOURCE *FLURER, R.A., MCLUCKEY, S.A. AND GLISH, G.L.	335
RATE ENHANCEMENT OF -ELIMINATION REACTIONS BY DIPOLE-ANION INTERACTIONS *SUMPTER, T. AND BARTMESS, J.E.	337
AN FTMS STUDY OF ALKANE ELIMINATIONS FROM GAS PHASE IONS *AHMED, M.S., GIAM, C.S. AND MCADOO, D.J.	338
ASSIGNMENT OF HYDROXYHYDANTOIN ISOMERS BY LINKED SCAN, TANDEM, AND HIGH RESOLUTION MS	340

*ONISKO, B. AND LEWIS, S.

THE TRIMETHYLSILYLUM (Me₃Si⁺) AFFINITY OF ALKYLAMINES 342
STONE, J.A. AND *LI, X.

Computational

NON-FT/ICR/MS: AUTOREGRESSION AND BAYESIAN SPECTRAL ANALYSIS FOR ICR MASS 344
SPECTROMETRY
MEIER, J.E. AND *MARSHALL, A.G.

MSCOINT: A COMPUTER PROGRAM DEVELOPED FOR THE INTERPRETATION OF 346
COLLISIONALLY ACTIVATED MASS SPECTRA
*ZIDAROV, D. AND BERTRAND, M.J.

AN AUTOMATED CHEMICAL STRUCTURE ELUCIDATION SYSTEM (ACES) FOR MS/MS 348
SPECTRA
*HART, K.J. AND ENKE, C.G.

MASSPEC- A GRAPHICS BASED DATA SYSTEM FOR CORRELATING A MASS SPECTRUM 350
WITH A PROPOSED STRUCTURE
*SIEGEL, M.M. AND GILL, G.

MACPROMASS-A COMPUTER AID FOR THE ANALYSIS OF MASS SPECTRAL DATA OF 352
PEPTIDES AND PROTEINS
*LEE, T.D. AND VEMURI, S.

IMPROVED RETRIEVAL OF HIGH RESOLUTION UNKNOWN MASS SPECTRA WITH EXACT 354
MASS-PROBABILITY BASED MATCHING
*LOH, S. AND MCLAFFERTY, F.W.

QUALITATIVE EFFECTS OF ION ABUNDANCE TUNING ON MASS SPECTRAL MATCHING 356
ALGORITHMS, RESULTS FROM AN INTERLABORATORY STUDY
*SAUTER, A.D., DOWNS, J.J., ISAACSON, P., MCCARTY, H. AND FISK, J.

REPORTING MS RESULTS WITH PC SOFTWARE 358
*LOW, N.

DATA TRANSCRIPTION FROM HEWLETT PACKARD GCMS WORKSTATION TO IBM PERSONAL 359
COMPUTER (OR COMPATIBLE)
GARDNER, J.M., *QUICK A.N. AND FENNESSEY, P.V.

MASS SPECTROMETRY DATA CENTRE 361
*BELLARD, S., CORKILL, M., DOWN, S. AND REID, R.

QUANTITATION IN DATA EDITOR USING HP 3.2 CHEMSTATION SOFTWARE 363
*CODY, J.T.

TUESDAY ORAL SESSIONS

LC/MS

REAL-TIME REACTION MONITORING BY CONTINUOUS INTRODUCTION ION SPRAY TANDEM 365
MASS SPECTROMETRY
*LEE, E.D., MUCK, W. AND HENION, J.D.

DETERMINATION OF CHARGE STATE OF IONS IN THERMOSPRAY MASS SPECTROMETRY 367
*CHAN, K., WINTERGRASS, D., HO, T. AND STRAUB, K.

THERMOSPRAY LC/MS USING A QUADRUPOLE ION TRAP 369
KAISER, R.E., LAMMERT, S.A., WILLIAMS, J., COOKS, R.G. AND *ZAKETT, D.

MASS SPECTROMETRIC INSIGHTS INTO BIOLOGICAL REDOX REACTIONS *VOLK, K.J., BRAJTER-TOTH, A. AND YOST, R.A.	371
MASS-FLOW ENHANCEMENT APPROACHES IN LC/MS *VAN DER GREEF, J., NIESSEN, W.M.A. AND TJADEN, U.R.	373
MASS SPECTROMETRIC DETECTION FOR HIGH PERFORMANCE ANION EXCHANGE LIQUID CHROMATOGRAPHY *SIMPSON, R.C., FENSELAU, C.C., LEE, Y.C., TOWNSEND, R.R. AND COTTER, R.J.	375
DEVELOPMENTS IN SUPERCRITICAL FLUID CHROMATOGRAPHY MASS SPECTROMETRY *NIESSEN, W.M.A., VERHEIJ, E.R., VAN DER HOEVEN, R.A.M., TJADEN, U.R. AND VAN DER GREEF, J.	377
OPTIMIZATION AND APPLICATIONS OF PARTICLE BEAM HPLC/MS FOR COMPOUNDS OF PHARMACEUTICAL INTEREST *VOYKSNER, R.D., KNOX, P.C. AND SMITH, C.S.	379
INTERMEDIATE TERM STABILITY OF ION INTENSITIES AND ELECTRON IMPACT MASS SPECTRA WITH PARTICLE BEAM LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY SYSTEMS BELLAR, T.A., BEHYMER, T.D. AND *BUDDE, W.L.	381
INCORPORATION OF MS/MS DETECTION FOR THE CHARACTERIZATION OF TRYPTIC DIGESTS BY CONTINUOUS FLOW FAB LC-MS *ACKERMANN, B.L., CHEN, T-M. AND COUTANT, J.E.	383
TANDEM MASS SPECTROMETRY FOR THE ANALYSIS OF BIOMOLECULES VIA COAXIAL CONTINUOUS FLOW-FAST ATOM BOMBARDMENT AND CAPILLARY ZONE ELECTROPHORESIS *DETERDING, L.J., MOSELEY, M.A., TOMER, K.B. AND JORGENSEN, J.W.	385

Ionization/Activation

SURFACE-CATALYZED REACTIONS OF AROMATIC AMINES WITH OXYGEN AND NITROGEN IN NCI MASS SPECTROMETRY *STEMMLER, E.A. AND BUCHANAN, M.V.	387
IONIZATION EFFICIENCIES IN AN ELECTROSPRAY SOURCE WONG, S.F., MANN, M., MENG, C.K. AND *FENN, J.B.	389
SPECULATION ON THE MECHANISM(S) OF ELECTROSPRAY IONIZATION *SMITH, R.D., UDSETH, H.R., LOO, J.A., EDMONDS, C.G. AND BARINAGA, C.J.	391
VISCOSITY EFFECTS IN FAST ATOM BOMBARDMENT SPECTRA SHIEA, J-T. AND *SUNNER, J.	393
ON THE DISSOCIATION AND FORMATION DYNAMICS OF CLUSTER IONS IN FAST ATOM BOMBARDMENT MASS SPECTROMETRY *BERTRAND, M.J., PERREAULT, H. AND ZHA, Q.	395
SOLUTE INTRODUCTION AND LIQUID SECONDARY ION EMISSION *TODD, P.J.	397
NEGATIVE MOLECULAR IONS FROM SAMPLES ADSORBED ON NITROCELLULOSE FOR keV AND MeV PARTICLE BOMBARDMENT *ENS, W., STANDING, K.G., SUNDQVIST, B.U.R. AND HAKANSSON, P.	399
ELUCIDATION OF ELECTRONIC AND VIBRATIONAL ENERGY LEVELS OF MOLECULAR IONS BY HIGH RESOLUTION TRANSLATIONAL ENERGY SPECTROSCOPY HAMDAN, M. AND *BRENTON, A.G.	401

FRAGMENTATION OF DISTONIC RADICAL IONS BY LOW-ENERGY COLLISION-INDUCED DISSOCIATION *WYSOCKI, V.H. AND KENTTAMAA, H.I.	403
TANDEM MASS SPECTROMETRY OF LARGE BIOMOLECULES: WHAT IMPEDES ITS USE FOR STRUCTURAL CHARACTERIZATION? *RUSSELL, D.H.	405
ANALYSIS OF THE FRAGMENTATION INDUCED BY FAB VS PHOTODISSOCIATION IN A Q-FTMS AND LONG-LIVED METASTABLES FORMED VIA FAB *LEBRILLA, C.B., WANG, D.T-S. AND MCIVER, R.T.	407
EXCITED STATE PROTON TRANSFER IN LASER MASS SPECTROMETRY *CHIARELLI, M.P., SHARKEY, A.G. AND HERCULES, D.M.	409

Symposium: Isotope Measurements

FAB MASS SPECTROMETRIC TECHNIQUES INCREASE PRACTICALITY OF AND ACCESS TO TRACE METAL ANALYSIS IN MAN *FENNESSEY, P.V. AND MILLER, L.V.	411
NEW DEVELOPMENTS IN NEGATIVE THERMAL IONIZATION MASS SPECTROMETRY AND ITS APPLICATIONS *HEUMANN, K.G., KOPPE, M. AND WACHSMANN, M.	414
HIGH SENSITIVITY ISOTOPE DILUTION (IDMS) USING THERMAL IONIZATION, RESONANCE IONIZATION, AND SECONDARY IONIZATION MASS SPECTROMETRIES--TIMS, RIMS AND SIMS *FASSETT, J.D., MAYO, S., SIMONS, D.S., WALKER, R.J. AND KELLY, W.R.	417
RIMS APPLIED TO ENVIRONMENTAL AND NUCLEAR RESEARCH *SCHWEIKHARD, L.	419
SYNTHETIC SILICON ISOTOPE MIXTURES FOR ABSOLUTE ISOTOPE ABUNDANCE RATIO MEASUREMENTS *LENAERS, G., MURPHY, T.J., VALKIEERS, S. AND DE BIEVRE, P.	422

Symposium: Formation and Analysis of Multicharged Ions

FACILITATING DESORPTION OF MULTIPLY CHARGED IONS *COOK, K.D. AND REYNOLDS, J.D.	425
SOME MASS SPECTROMETRIC IMPLICATIONS OF MULTIPLY CHARGED IONS *FENN, J.B., MANN, M., MENG, C.K., WONG, S.F. AND WEBER, R.S.	428
ELECTROSPRAY PRODUCTION AND COLLISION INDUCED DISSOCIATION OF LARGE MULTIPLY CHARGED IONS *SMITH, R.D., UDSETH, H.R., BARINAGA, C.J. AND EDMONDS, C.G.	430
ROLE OF ELECTRON TUNNELING IN THE FORMATION OF MULTIPLY-CHARGED IONS AT SURFACES *MACFARLANE, R.D., NORD, R. AND BUNK, D.	432
MULTIPLY CHARGED NEGATIVE IONS: A CRITICAL REVIEW *COMPTON, R.N.	434

Symposium: MS/MS in Environmental Applications

LC/MS/MS AND CZE/MS/MS DETERMINATION OF ENVIRONMENTALLY IMPORTANT COMPOUNDS *HENION, J. AND CONBOY, J.	436
APPLICATIONS OF TRIPLE QUADRUPOLE GC/MS/MS TO THE DETERMINATION OF DIOXINS IN COMPLEX ENVIRONMENTAL SAMPLES *REINER, E.J., SCHELLENBERG, D.H., TAGUCHI, V.Y., MCCURVIN, D.M. AND CLEMENT, R.E.	438
CHOOSING BETWEEN HRMS AND MS/MS: ENVIRONMENTAL APPLICATIONS *TONDEUR, Y. AND CHARLES, M.J.	441
DEVELOPMENT OF A MOBILE ION TRAP MASS SPECTROMETER SYSTEM FOR ENVIRONMENTAL MONITORING *MEUZELAAR, H.L.C., MCCLENNEN, W.H., ARNOLD, N.S. AND MASWADEH, W.	444
ANALYSIS OF XENOBIOTIC CONJUGATES BY MS/MS *TOMER, K.B.	446

FT/MS and Ion Traps

HIGH MASS REMEASUREMENT ENHANCEMENT OF FOURIER TRANSFORM MASS SPECTRA *WILLIAMS, E.R., HENRY, K.D., WANG, B.H. AND MCLAFFERTY, F.W.	449
TWO DIMENSIONAL FT-ICR AND ITS REALIZATION ON A PC BASED INSTRUMENT *GUAN, S. AND JONES, P.R.	451
MINIMIZATION OF SPACE CHARGE EFFECTS IN AN FTICR TRAPPED ION CELL *LAUDE, D.A., MALEKNIA, S. AND HOGAN, J.D.	453
EXACT AND NEARLY EXACT INTERPOLATION OF APODIZED FT-ICR SPECTRA *COMISAROW, M.B. AND KEEFE, C.D.	455
AN EXTERNAL ION SOURCE PROVIDING ION ACCUMULATION AND TRAPPING OF IONS WITH BROAD ENERGY RANGE FOR ION CYCLOTRON RESONANCE SPECTROMETRY BAMBERG, M. AND *WANCZEK, K.P.	456
THE EFFECTS OF PRESSURE ON ION EXCITATION IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE (FT-ICR) SPECTROMETRY *RUSSELL, D.H., KERLEY, E.L., HANSON, C.D. AND CASTRO, M.E.	458
SIMULATED RESONANCE EXCITATION OF TRAPPED IONS *MARCH, R.E., MCMAHON, A.W., LONDY, F.A., ALFRED, R.L., VEDEL, F. AND TODD, J.F.J.	460
NEGATIVE ION CHEMICAL IONIZATION IN AN ION TRAP MASS SPECTROMETER USING REAGENT ANIONS INJECTED FROM AN EXTERNAL ION SOURCE *ECKENRODE, B.A., GLISH, G.L. AND MCLUCKEY, S.A.	462
DAUGHTER ION FORMULA DETERMINATION BY MS _n EXPERIMENTS ON NATURALLY OCCURRING ISOTOPES GLISH, G.L., *ASANO, K.G. AND MCLUCKEY, S.A.	464
HOW TO USE A STANDARD QUADRUPOLE FILTER AS A TWO DIMENSIONAL ION-TRAP BEAUGRAND, C., *DEVANT, G., ELABBASSI, B., JAOUEN, D., MESTDAGH, H. AND ROLANDO, C.	466

INJECTION OF IONS INTO A QUADRUPOLE ION TRAP FROM A CHEMICAL IONIZATION SOURCE USING A DC QUADRUPOLE	468
*PEDDER, R.E., YOST, R.A. AND WEBER-GRABAU, M.	

Symposium: Historical Developments of Instrumentation and Techniques

A HISTORICAL REVIEW OF THE ON-LINE COMBINATION OF CHROMATOGRAPHY WITH MASS SPECTROMETRY	470
*MCFADDEN, W.H.	
THE DEVELOPMENT OF SELECTIVE METHODS OF IONIZATION	474
*MUNSON, B.	
THE HISTORY OF QUADRUPOLE MASS SPECTROMETRY DEVELOPMENT	479
*DAWSON, P.H.	
A HISTORY OF GC/MS INTERFACES	485
*GRAYSON, M.A.	
THE DEVELOPMENT OF METHANE CHEMICAL IONIZATION MASS SPECTROMETRY	491
*MUNSON, B.	
CHEMICAL IONIZATION WITH TETRAMETHYLSILANE, PAST AND PRESENT	493
*ORLANDO, R. AND MUNSON, B.	
TANDEM MASS SPECTROMETRY FOR FUN, NOT PROFIT	496
*FUTRELL, J.H.	
TRACING THE HISTORY OF ANALYTICAL MS/MS	502
*BUSCH, K.L.	
THE DETECTION AND RECORDING OF ION BEAMS: FROM THE GOLD-LEAF ELECTROSCOPE TO THE INTERACTIVE GRAPHICS WORKSTATION	506
*HOGG, A.	
ARTIFICIAL INTELLIGENCE IN MASS SPECTROMETRY	512
*KISER, R.W.	
A RESOURCE FOR BIOMEDICAL MASS SPECTROMETRY	517
*SHERMAN, W.R. AND BIER, D.M.	
MASS SPECTROMETERS IN SPACE. ROCKETS TO SATELLITES TO MARS 1960-1976	523
BIEMANN, K., *FENNESSEY, P.V., FLORY, D.A., NIER, A.D.C. AND RUSHNECK, D.A.	

TUESDAY POSTER SESSIONS

Ionization Methods

POTASSIUM ION IONIZATION OF DESORBED SPECIES (K+IDS) BY FAB: A USEFUL METHOD FOR PROBING THE SELVEDGE REGION, AND FOR OBTAINING MOLECULAR WEIGHT INFORMATION	527
*SCHULTZ, G. AND ALLISON, J.	
THE CHARGE STRIPPING OF ALKALINE EARTH SPECIES GENERATED BY SOLID FAB	529
*CALDWELL, K.A. AND GROSS, M.L.	
ON PERFORMED IONS AND ION-MOLECULE REACTIONS IN FAB	531
SHIEA, J., NESTOR, J. AND *SUNNER, J.	

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

MITIGATION OF BEAM-INDUCED REDUCTION IN DESORPTION IONIZATION MASS SPECTROMETRY *REYNOLDS, J.D. AND COOK, K.D.	533
EFFECT OF SORBITOL CRITICAL BEHAVIOR ON FAB TOTAL ION CURRENT *BENNETT, B.D. AND DAY, R.	535
EFFECTS OF SAMPLE PROBE SURFACE TEMPERATURE ON THE DETECTION OF PEPTIDES BY FAB/MS *PAPAYANNOPOULOS, I.A. AND BIEMANN, K.	537
NON-IONIC SURFACTANTS USED AS INTERNAL STANDARDS FOR EXACT MASS MEASUREMENTS IN FAB MASS SPECTROMETRY *SIEGEL, M.M., TSAO, R., OPPENHEIMER, S., CHANG, T.T. AND DEVASTRO, J.	539
LIQUID CHROMATOGRAPHY/HIGH PRESSURE FAST-ATOM BOMBARDMENT MASS SPECTROMETRY *THOMPSON, T., FINK, S.W. AND FREAS, R.B.	541
PEPTIDE DIMERIZATION REACTIONS IN THERMOSPRAY AND FAST-ATOM BOMBARDMENT IONIZATION *FINK, S.W., DYCKES, D.F. AND FREAS, R.B.	543
EFFECT OF PEPTIDE HYDROPHOBICITY ON MOLECULAR ION YIELDS IN NITROCELLULOSE ADSORPTION, PLASMA DESORPTION MASS SPECTROMETRY *WANG, R., CHEN, L. AND COTTER, R.J.	545
ION VS NEUTRAL DESORPTION IN PLASMA DESORPTION MASS SPECTROMETRY *COTTER, R.J. AND WANG, R.	547
FACTORS AFFECTING EH ION SOURCE FLOW AND EMISSION STABILITY: RELEVANCE TO MASS SPECTROMETRY *MAHONEY, J.F., PEREL, J., LEE, T.D. AND LEGESSE, K.	549
DISTINGUISHING ISOMERIC AND ISOBARIC IONS BY THERMOSPRAY MS PRECEDED BY ELECTROCHEMICAL ELECTROLYSIS *GETEK, T.A., KORFMACHER, W.A. AND FREEMAN, J.P.	551
CO ₂ AS A MODERATING GAS IN ELECTRON CAPTURE CHEMICAL IONIZATION *MOLLEKEN, M.E. AND HARRISON, A.G.	553
USE OF CO ₂ AS A BUFFER GAS IN HIGH PRESSURE ELECTRON CAPTURE MASS SPECTROMETRY. THE ELIMINATION OF UNUSUAL IONS *SEARS, L.J. AND GRIMSRUD, E.P.	554
SENSITIVITY STUDY OF PERFLUOROCARBONS AND PERFLUOROCARBONS CONTAINING NON-FLOUROCARBON GROUPS *HUANG, S., DESPOT, K., KLEIN, D.H. AND BIERL, T.W.	556
COMPARISON OF GAS PHASE AND LIQUID PHASE IONIZATION: CHEMICAL IONIZATION AT ATMOSPHERIC PRESSURE VS FIELD ASSISTED ION EVAPORATION *COVEY, T.R., SUSHAN, B.I., THOMSON, B.A. AND HENION, J.D.	558
FIELD IONIZATION AT LOW VOLTAGE *COGGIOLA, M.J. AND SPINDT, C.A.	560
LD/FTMS. A STUDY OF VARIABLES AFFECTING PERFORMANCE WEIL, D.A., CAMPANA, J.E., WELLER, R.R. AND *BJARNASON, A.	562
MULTIPHOTON IONIZATION OF LARGE MOLECULES: STUDY OF FRAGMENTATION AS FUNCTION OF PHOTON ENERGY AND INTENSITY *HOLLE, A., FREY, R., KAMINSKI, H., WEISS, G., FRANZEN, J. AND KOCH, D.	564
DESORPTION OF SMALL BIOMOLECULAR IONS BY UV LASER IRRADIATION *MAYER, F., ENS, W., MAO, Y., TANG, X. AND STANDING, K.G.	566

**Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL**

PULSED LASER INDUCED ELECTRON GENERATION FOR ELECTRON IONIZATION MASS SPECTROMETRY BEAVIS, R.C., ROHWER, E.R., LINDNER, J., KOSTER, C., GROTEMEYER, J. AND SCHLAG, E.W.	568
WAVELENGTH DEPENDENCE IN THE DESORPTION OF NEUTRAL MOLECULES LINDNER, J., GROTEMEYER, J. AND SCHLAG, E.W.	570
RESONANCE ENHANCED MULTIPHOTON IONIZATION DETECTION OF THE THERMAL DECOMPOSITION PRODUCTS INDUCED BY LASER DESORPTION OF OLIGOPEPTIDES LI, L. AND LUBMAN, D.M.	572
FRAGMENTATION OF CATIONIZED BIOMOLECULES IN LASER DESORPTION USING A CO ₂ LASER EMARY, W.B. AND COTTR, R.J.	574
ISOTOPE ANALYSIS IN INVOLATILE SAMPLES BY LASER DESORPTION ELECTRON IMPACT YUAN, Z.-M., FENSELAU, C., MARTIN, W.B. AND COTTER, R.J.	576
AN EFFECTIVE METHOD FOR ASSISTING LASER DESORPTION OF NON-VOLATILE COMPOUNDS ZHAO, S., SHARKEY, A.G. AND HERCULES, D.M.	578
CHARACTERIZATION OF A GLOW DISCHARGE PLASMA AS A FUNCTION OF SAMPLING ORIFICE POTENTIAL TAYLOR, W.S., DULAK, J.G. AND KETKAR, S.N.	580
ELECTROHYDRODYNAMIC EMISSION MASS SPECTRA OF PEPTIDES LEE, T.D., LEGESSE, K., MAHONEY, J.F. AND PEREL, J.	582
POSITRON IONIZATION MASS SPECTROMETRY DONOHUE, D.L., HULETT, L.D., GLISH, G.L., MCLUCKEY, S.A. AND MCKOWN, H.S.	584
ELECTROSPRAY IONIZATION-MASS SPECTROMETRY OF LARGE BIOMOLECULES LOO, J.A., BARINAGA, C.J., EDMONDS, C.G., UDSETH, H.R. AND SMITH, R.D.	586

Isotope Ratios

ISOTOPE RATIO MEASUREMENTS BY RESONANCE IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY WITH A COPPER VAPOUR LASER PUMPED DYE LASER SYSTEM GREEN, L.W., SMYTH, M.H. AND SOPCHYSHYN, F.C.	588
DIODE LASER APPLICATION TO RESONANCE IONIZATION MASS SPECTROMETRY SMITH, D.H., YOUNG, J.P. AND SHAW, R.W.	590
MEASUREMENT OF MAGNESIUM ISOTOPIC RATIOS IN THE STUDY OF CELLULAR MAGNESIUM FLUXES VIEIRA, N.E., MONTES, J.G., SJODIN, R.A. AND YERGEY, A.L.	592
THE IMPORTANCE OF ABUNDANCE SENSITIVITY IN THE MEASUREMENT OF LARGE ISOTOPE RATIOS CANTLE, J.E. AND PALACZ, Z.A.	594
THE EFFECT OF ULTRA-HIGH VACUUM ON THERMAL IONIZATION PROCESSES TACKETT, J.R., ELLEFSON, R.E. AND WIEDENHEFT, C.J.	595
EVALUATION OF MEMORY EFFECT IN THE DETERMINATION OF ALTERED ISOTOPE RATIOS OF METALS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AGGARWAL, S.K., KINTER, M., WILLS, M.R., SAVORY, J. AND HEROLD, D.A.	597
MEASUREMENT OF TRACES OF CHD ₃ IN CD ₄ MAMER, O.A. AND KOCH, H.	599

Reaction Mechanisms

EI INDUCED FRAGMENTATION OF 2-ARYLOXY-3-CYCLOHEXYL-6-(1,1-DIMETHYLETHYL)-3, 4-DIHYDRO-2H-1,3,2-BENZOXAZA PHOSPHORINE-2-OXIDES *RAJU, C.N., DASS, C. AND REDDY, G.S.	600
MASS SPECTRAL FRAGMENTATION OF 2,10-DICHLORO-6-ARYLOXY-DIBENZO [d,g] [1,3,6,2] DIOXATHIAPHOSPHOCIN-6-OXIDES REDDY, C.D., ANURADHA, D., *REDDY, G.S. AND DASS, C.	602
MASS SPECTRAL FRAGMENTATION OF SOME 1,4-DITHIANE-1,1-DIOXIDE AND 1,1,4,4-TETROXIDE AND RELATED HETEROCYCLIC COMPOUNDS REDDY, D.B., SEENAIHAH, B., REDDY, M.V.R. AND *REDDY, G.S.	604
PHENYL AND PYRIDYL GROUP MIGRATION ON ELECTRON IONIZATION OF ENAMIDES DERIVED FROM 1,3-BENZOTHAZINES AND ISOQUINOLINES MACLEAN, D.B., WANG, J., FODOR, L. AND *SMITH, R.W.	606
INVESTIGATION OF THE HINDRED AMINE LIGHT STABILIZER MECHANISM USING ISOTOPIC LABELING *NG, K., PIATEK, B., GANDE, M. AND KLEMCHUK, P.	608
1,2-SHIFTS, IN C ₃ H ₈ O ⁺ IONS *HUDSON, C.E. AND MCADOO, D.J.	610
THE OXIRANE ISOMERS CH ₂ OCH ₂ , OCH ₂ CH ₂ , AND CH ₃ OCH AND THEIR IONIZED COUNTERPARTS *WESEMIOTIS, C., LEYH, B., FURA, A. AND MCLAFFERTY, F.W.	612
TRIMETHYLSILYL GROUP MIGRATIONS AND EPOXIDE RING FRAGMENTATIONS IN THE MASS SPECTRA OF DERIVATIZED CHOLESTEROL OXIDATION PRODUCTS *ANNAN, M., WASILCHUK, B., LEQUESNE, P.W. AND VOURES, P.	614
SUBSTITUENT EFFECTS IN THE MASS SPECTRA OF SUBSTITUTED DIPHENYL-1-PHENYLAMINO-1-PHENYLMETHANEPHOSPHONATES AL-RAHI, M., *JAYASIMHULU, K., SMITH, S.J. AND ZIMMER, H.	616
REARRANGEMENTS OF FLUORINE-CONTAINING CATIONS *SHALER, T.A., STAMS, D.A. AND MORTON, T.H.	618
BENZOPHENONE IMINE FORMATION FROM BENZOPHENONE OXIME *MA, Y.C. AND MUNSON, B.	620
DEUTERIUM ISOTOPE EFFECT IN SOLID PROBE MS ANALYSIS *WANG, J.-S. AND QUA, A.	622
THE ELECTRON IONIZATION MASS SPECTRA OF NOVEL 2,3:4,5-BIS-O-(1-METHYLETHYLIDENE)- D-FRUCTOPYRANOSE DERIVATIVES AND RELATED SUGAR SULFAMATES *CALDWELL, G.W. AND MASUCCI, J.A.	624
THE INVESTIGATION OF [M-35] ⁺ IONS IN 2-ALKYL-4,6-DINITROPHENOLS *RILEY, J. AND MARBURY, D.	626
THE GAS-PHASE REACTION OF THE CYCLOPROPANE RADICAL CATION AND CYCLOPROPANE: FORMATION OF THE HEXAMETHYLENE DISTONIC ION *WARNER, C.D., HAYES, R.N. AND GROSS, M.L.	628
MASS SPECTROSCOPIC STUDY OF SOME FLUOROCARBONS *HUANG, S. AND TUINMAN, A.	630

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

A STUDY OF THE FRAGMENTATION PATHWAY OF A SERIES OF FROG ALKALOIDS OF THE PUMILIOTOXIN CLASS *PU, Q-L., PANNELL, L.K., FALES, H.M. AND MASON, R.T.	632
CLEAVAGE MECHANISMS OF CHROMIUM- DIKETONATES BY ELECTRON IONIZATION *WENCLAWIAK, B.	634
FUNDAMENTAL STUDIES OF CHEMICAL IONIZATION MECHANISMS OF PORPHYRINS AND METALLOPORPHYRINS *BEATO, B.D. AND YOST, R.A.	636
STUDIES OF THE ION/MOLECULE REACTION OF ESTERIFICATION BY ION TRAP MASS SPECTROMETRY *CHANG, J.S-C. AND YOST, R.A.	638
IONIC DISSOCIATION OF 7-R-O-METHYLNORGAROL AND ITS DEGRADATION PRODUCTS *NISHIMURA, T., ROBINS, R.H., CROW, F.W. AND SONODA, A.	640
PHOTOCHEMICALLY-INDUCED REACTION OF CHLORPROTHIXENE WITH OXYGEN UNDER METHANE NEGATIVE CHEMICAL IONIZATION CONDITIONS *FUKUDA, E.K., CALLERY, P.S. AND DAVIS, P.P.	642
THE METHANE CHEMICAL IONIZATION MASS SPECTRA OF A SERIES OF URETHANES WRIGHT, A.D., BOWEN, R.D. AND *JENNINGS, K.R.	644
REACTIONS OF BIDENTATE LIGAND ANIONS WITH LEWIS ACIDS AS PROBES OF POTENTIAL ENERGY SURFACES *BABCOCK, L.M. AND TAYLOR, W.S.	646
PROTONATION ON PHENOXY OXYGEN IN METHANE CHEMICAL IONIZATION OF ALKYL PHENYL ETHERS *KONDRAT, R.W., STAMS, D.A. AND MORTON, T.H.	648
OBSERVATION OF DIAZONIUM ION-CROWN ETHER MOLECULAR COMPLEXES IN THE GAS PHASE BY FIELD DESORPTION AND FAST ATOM BOMBARDMENT MASS SPECTROMETRY *LAALI, K. AND LATTIMER, R.P.	650
DIFFERENCES IN THE MECHANISM OF FORMATION OF PARENT-MOLECULAR IONS IN CHEMICAL IONIZATION AND FAST ATOM BOMBARDMENT MASS SPECTROMETRY *ZHA, Q. AND BERTRAND, M.J.	652
MECHANISMS OF THERMALLY ANALOGOUS COLLISION-INDUCED CHARGE-REMOTE FRAGMENTATIONS *CONTADO, M.J., ADAMS, J., JENSEN, N.J. AND GROSS, M.L.	654
COLLISION-INDUCED DISSOCIATION OF MULTIPLY-CHARGED DIATOMIC CATIONS *HARBOL, K.L. AND KISER, R.W.	656
THE ROLE OF GAS-PHASE BASICITY AND SURFACE ACTIVITY IN THE FORMATION OF FAB AND PD MASS SPECTRA *KEOUGH, T. AND LACEY, M.P.	658
UNIMOLECULAR DISSOCIATION OF METASTABLE $[Mn_2(CO)_{10}]^+$ *LIANG, X. AND FREAS, R.B.	660
MASS SPECTROMETRY OF RHODIUM CHELATES: HYDROGEN TRANSFER DURING ION DECOMPOSITION *WESTMORE, J.B. AND STERN, G.A.	662
MASS SPECTRAL STUDIES OF IRIIDIUM COMPLEXES OF 2,4-PENTANEDIONE, 1,1,1-TRIFLUORO-2,4-PENTANEDIONE AND 1,1,1,5,5,5-HEXAFLUORO-2,4-PENTANEDIONE *STERN, G.A. AND WESTMORE, J.B.	664

STRUCTURAL IMPLICATIONS REGARDING PHOTOFRAGMENTATION OF 12-CROWN-4 FROM PHOTOIONIZATION MASS SPECTROMETRY *MOINI, M., ALLISON, J. AND LEORI, G.E.	666
A COMPETITION BETWEEN TWO ISOMERIZATION PROCESSES IN UNIMOLECULAR DISSOCIATION *ZHA, Q., BERTRAND, M.J., NISHIMURA, T. AND MEISELS, G.C.	668
HIGH MASS CARBON CLUSTER FORMED FROM POLYCYCLIC AROMATIC HYDROCARBONS OBSERVED BY LASER MASS SPECTROMETRY *LINEMAN, D.N., VISWANADHAM, S.K., SHARKEY, A.G. AND HERCULES, D.M.	670
THE KINETIC ENERGIES OF IONS FORMED BY RESONANT AND NON-RESONANT MULTIPHOTON IONIZATION *ZIMMERMAN, J.A. AND O'MALLEY, R.M.	672
FRAGMENTATION OF THE PARENT ION OF METHYL ESTERS AND KETONES BY MS/MS IN AN ION-TRAP MASS SPECTROMETER: CHANGE IN MECHANISM WITH CHAIN LENGTH *MASON, R.T., FALES, H.M., PANNELL, L.K. AND PU, Q.-L.	674

Pharmacology/Drugs

MASS SPECTROMETRIC TECHNIQUE APPLIED TO ASSIGNMENT OF STRUCTURES IN THREE OR FOUR STEP - LACTAM SYNTHESSES *DAY, R.A. AND BENNETT, B.	676
RAPID IDENTIFICATION OF URINARY METABOLITES OF ADINAZOLAM IN HUMANS USING TANDEM MASS SPECTROMETRY *VRBANAC, J.J., PENG, G.W. AND O'LEARY, I.A.	678
EFFECT OF PHTHALATE IMPURITIES ON PEAK AREAS DURING NEGATIVE ION METHANE GC/MS ANALYSIS OF NABILONE AND DEUTERATED NABILONE IN HUMAN PLASMA SAMPLES *DHAHIR, P.H., BARTON, R., WOLEN, R.L. AND BREAU, A.P.	680
HYBRID MS/MS OF AROMATIC DIAMIDINO ANALOGS *MARBURY, G.D., CHARLES, J.C., BELL, C., HALL, J.E. AND TIDWELL, R.	682
A SELECTED CAD-DAUGHTER ION RECORDING ASSAY FOR FLUPHENAZINE *EDOM, R.W., MCKAY, G., HAWES, E.M. AND MIDHA, K.K.	684
PURIFICATION OF INHIBITORS OF OUABAIN BINDING FROM BOVINE POSTERIOR PITUITARIES USING HIGH PRESSURE LIQUID CHROMATOGRAPHY & MASS SPECTROMETRY *MCQUADE, P.S., LABELLA, F.S. AND STANDING, K.G.	686
THE STRUCTURE ELUCIDATION AND MASS SPECTROMETRY OF PENICILLINIC ACID THIOLACTONES *CHESS, E.K., PEREIRA, D. AND SMITH, W.J.	688
MASS SPECTROMETRY OF MITOMYCIN C AND SELECTED ANALOGS *HAMMARGREN, W.M., IYENGAR, B.S., REMERS, W.A. AND SCHRAM, K.H.	690
MASS SPECTROMETRY IN PREHISTORIC RESEARCH *ORLEY, C.O. AND MESTER, M.	692
ANALYSIS OF BLOODROOT EXTRACT USING TLC/SIMS, HIGH RESOLUTION MS, AND MS/MS *DOHERTY, S.J. AND BUSCH, K.L.	694
DERIVATIZATION OF PUTATIVE BRADYKININ METABOLITES FOR GC-MS ANALYSIS *VRBANAC, J.J., BALLARD, K.D. AND KNAPP, D.R.	696

APPLICATION OF AUTOMATED GC/MS/MS FOR THE QUANTITATIVE DETERMINATION OF NE-11740 IN PLASMA *DOBSON, R.L.M., NEAL, D.M., DEMARK, B.R. AND WARD, S.R.	698
CHARACTERIZATION OF DERIVATIVES OF CHIRAL ANTIARRHYTHMIC DRUGS AND THEIR METABOLITES USING FAB-MS *SWEETMAN, B.J., PRAKASH, C. AND BLAIR, I.A.	700
NEGATIVE ION FAST ATOM BOMBARDMENT MASS SPECTROMETRIC ANALYSIS OF PLATINATED OLIGONUCLEOTIDES *MARTIN, L.B., SCHREINER, A.F. AND VAN BREEMEN, R.B.	702
GAS CHROMATOGRAPHY-ION TRAP MASS SPECTROMETRY DETERMINATION OF ARECOLINE IN HUMAN PLASMA *SHETTY, H.U., GREIG, N.H., SONCRANT, T.T. AND RAPOPORT, S.I.	704
SELECTED REACTION MONITORING FOR RAPID QUANTITATION OF DEXAMETHASONE IN PLASMA *KAYGANICH, K.A., HEATH, T.G. AND WATSON, J.T.	706
NEGATIVE CHEMICAL IONIZATION VERSUS ELECTRON IMPACT IONIZATION DETECTION OF SCOPOLAMINE BY GC/MS IN HUMAN AND RAT PLASMA *DEUTSCH, J., SONCRANT, T.T., GREIG, N.H. AND RAPOPORT, S.E.	708
STRUCTURE ELUCIDATION OF ISOMERIC HYDROXY-ALOINS AND OXANTRHONE GLUCOSYLES BY THE USE OF HIGH RESOLUTION AND TANDEM MASS SPECTROMETRY *SCHRODER, E. AND RAUWALD, U.	710
SELECTIVE DETECTION OF SULFUR-CONTAINING COMPOUND BY REACTION INTERFACE/MASS SPECTROMETRY AND ITS APPLICATIONS TO DRUG METABOLISM *MOINI, M., CHACE, D.H. AND ABRAMSON, F.P.	712
DETECTION OF ¹⁴ C-LABELED DRUGS AND THEIR METABOLITES BY CAPILLARY GAS CHROMATOGRAPHY - REACTION INTERFACE / MASS SPECTROMETRY (GC-RIMS) *CHACE, D.H. AND ABRAMSON, F.P.	714
MASS SPECTROMETRIC STUDIES ON AZIDOTHYIMIDINE (AZT) USING FAB, THERMOSPRAY, PLASMASPRAY AND MS-MS *ROBOZ, J., LAPPIN, G. AND NIEVES, E.	716
USE OF PYROLYSIS CI/MS OVER THREE DIFFERENT TEMPERATURE RANGES TO IDENTIFY BACTERIA *CONWAY, D.C., LOPEZ, H. AND QUARLES, J.M.	718

Oligosaccharides/Lipids

DYNAMIC LIQUID SIMS OF UNDERIVATIZED OLIGOSACCHARIDES: IMPROVEMENTS IN MASS RANGE AND SENSITIVITY *GENO, P.W.	720
CF-FAB/MS MONITORING OF COMBINED PEPTIDASE HYDROLYSIS FOR THE CHARACTERIZATION OF SMALL AND MEDIUM-SIZED PEPTIDES *WHALEY, B.S. AND CAPRIOLI, R.M.	722
TANDEM MASS SPECTROMETRY OF LIPOSIDOMYCIN ANTIBIOTICS *NELSON, C.C., MCCLOSKEY, J.A., UBUKATA, M. AND ISONO, K.	724
STRUCTURE DETERMINATION A NOVEL ANTIFUNGAL NUCLEOTIDE ANTIBIOTIC *PHILLIPS, D.R., MCCLOSKEY, J.A., URAMOTO, M. AND ISONO, K.	726
STUDIES OF OLIGONUCLEOTIDE IONIZATION BY CONTINUOUS-FLOW FAST ATOM BOMBARDMENT *SHEIL, M.M., KOWALAK, J.A. AND MCCLOSKEY, J.A.	728

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

FAB-MS VS TIME-OF-FLIGHT SIMS: NEGATIVE ION STUDIES OF O-ALKYL DI-AND TRINUCLEOTIDES	730
*LAFORTUNE, F., BUCHKO, G., HRUSKA, F.E., SADANA, K.L., STANDING, K.G. AND WESTMORE, J.B.	
THE IDENTIFICATION OF 5,6-DIHYDROURIDINE IN NORMAL HUMAN URINE BY COMBINED GC/MS	732
*REIMER, M.L.J., SCHRAM, K.H., NAKANO, K. AND YASAKA, T.	
GC/MS ANALYSIS OF SPE ISOLATED URINARY NUCLEOSIDES	734
*MYKYTYN, V.V. AND SCHRAM, K.H.	
STRUCTURAL CHARACTERIZATION OF PENTAFLUOROBENZYL DERIVATIVES OF ALKYL AND HYDROXYALKYL MODIFIED DNA BASES	736
*ANNAN, R.S., GIESE, R. AND VOURES, P.	
FAB MASS SPECTRA OF SOME NATURALLY OCCURRING MODIFIED NUCLEOTIDES AND THEIR ANALOGS	738
DUTTA, S.P., WESOLOWSKI, J. AND *CHHEDA, G.B.	
COMPARISON OF THE MAJOR LIPIDS OF SCENT GLAND SECRETIONS FROM THE WESTERN DIAMONDBACK RATTLESNAKE (CROTALUS ATROX) AND DUMERIL'S GROUND BOA (ACRANTOPHIS DUMERILI) BY GC-MS: EVIDENCE FOR TAXONOMIC AND EVOLUTIONARY VARIATION	740
*SIMPSON, J.T., TANNER, M.J., SHARP, T.R., DAHM, K.H. AND WELDON, P.J.	
ELECTRON IMPACT MASS SPECTRA OF STEROIDS: ANDROSTANES AND DERIVATIVES	742
*SHARP, T.R., SUTTON, M., FERGUSON, A. AND MARSH, K.N.	
FATTY ACID AND HYDROCARBON CONTENT OF SALMONELLA TYPHIMURIUM BY GC/MS	744
*BEIER, R.C., OYOFI, B.A., SPATES, G.E. AND DELOACH, J.R.	
DETERMINATION OF ANOXIA-INDUCED PHOSPHOLIPASE A2 (PLA2) ACTIVATION IN FRESHLY ISOLATED RABBIT PROXIMAL TUBULES USING NICI	746
*PORTILLA, D., MILLINGTON, D.S. AND MANDEL, L.	
DIRECT ANALYSIS OF MODIFIED FATTY ACIDS THROUGH THEIR OXAZOLINE DERIVATIVES BY COMBINED IN-BEAM ELECTRON IMPACT (IBEI)-B/E LINKED SCANNING TECHNIQUE	748
*YANG, Y.M., ZHANG, J.Y. AND HUANG, Z.H.	
CHARACTERIZATION OF FATTY ACYL HYDROXYLATION OF TUMOR CELL GANGLIOSIDES BY FAB CAD MS/MS	750
*GAGE, D.A., SWEELEY, C.C., LADISCH, S. AND BECKER, H.	
EC-NI CAD MS/MS ANALYSIS OF THE FRAGMENTATION OF ABSCISIC ACID	752
*HEATH, T.G., ZEEVAART, J.A.D. AND GAGE, D.A.	
TANDEM MASS SPECTROMETRY OF UNDERIVATIZED PROSTAGLANDINS	754
*DAVOLI, E., BETTAZZOLI, L., GROSS, M.L. AND MURPHY, R.C.	
NEGATIVE ION FABMS/MS OF PLATELET ACTIVATING FACTOR	756
*CLAY, K.L. AND ZIRROLI, J.A.	
DETERMINATION OF ENDOTHELIAL CELL DERIVED PLATELET ACTIVATING FACTOR BY CONTINUOUS FLOW FAB/TANDEM MS WITH SELECTED REACTION MONITORING	758
*HAROLDSEN, P.E., TAYLOR, A.A. AND GASKELL, S.J.	
ANALYSES FOR LEUKOTRIENES USING CONTINUOUS FLOW FAST ATOM BOMBARDMENT/TANDEM MASS SPECTROMETRY	760
*RAFTERY, M.J., ORKISZEWSKI, R.S. AND GASKELL, S.J.	
LIPID OXYGENATION OF POLYUNSATURATES BY HUMAN AND RAT PLATELETS	762
*KIM, H-Y., KARANIAN, J.W. AND SALEM, N.	

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL .

NOVEL METABOLITES OF LTB ₄ FROM GUINEA PIG EOSINOPHILS *MATHEWS, W.R., GUIDO, D.M., SUN, F.F., CZUK, C.I. AND TAYLOR, B.M.	764
CHARACTERIZATION OF A-RING HYDROXY METABOLITES OF VITAMIN D BY CID ANALYSIS OF "STANDARD" IONS YOUNG, D., *VOUROS, P. AND HIGUCHI, T.	766
STRUCTURAL DETERMINATION OF MODIFIED CHLOROPHYLLS BY USING TANDEM MASS SPECTROMETRY *GRESE, R.P. AND GROSS, M.L.	768
DERIVATIZATION OF GANGLIOSIDES AND THEIR ANALYSIS BY HIGH PERFORMANCE TANDEM MASS SPECTROMETRY VATH, J.E., DOMON, B. AND *COSTELLO, C.E.	770
ANALYSIS OF CAROTENOID COMPOUNDS BY ELECTRON CAPTURE NEGATIVE ION MASS SPECTROMETRY *LUSBY, W.R., KHACHIK, F. AND BEECHER, G.R.	772
ETHANOL INDUCED CHANGES IN CENTRAL NERVOUS SYSTEM PROSTAGLANDIN PRODUCTION *YERGEY, J.A. AND HEYES, M.P.	774
BACTERIAL PHOSPHOLIPID ANALYSIS BY FAB/MS/MS: CHARACTERIZATION OF THE EFFECTS OF GROWTH CONDITIONS *COLE, M.J. AND ENKE, C.G.	776
ANALYSIS OF BACTERIAL CELL WALL GLYCOLIPIDS BY LASER DESORPTION AND PLASMA DESORPTION MASS SPECTROMETRY *EMARY, W.B., WANG, R., CHEN, L. AND COTTER, R.J.	778
TANDEM MASS SPECTROMETRY OF ERGOSTEROL AND RELATED COMPOUNDS *KINOSHITA, T., NAGAKI, H. AND NAKAMURA, T.	780
PEPTIDE MAPPING AND CARBOHYDRATE STRUCTURAL ANALYSIS OF RECOMBINANT SOLUBLE CD4 BY MASS SPECTROMETRY *HEMLING, M.E., CARR, S.A., ROBERTS, G.D. AND DODIA, V.	782
CONTROLLED FRAGMENTATION OF PERACETYLATED OLIGOSACCHARIDES IN 252Cf PLASMA DESORPTION MASS SPECTROMETRY *ADURU, S. AND CHAIT, B.T.	784
STRATEGIES TO DISTINGUISH CARBOHYDRATE SEQUENCE IONS *ORLANDO, R., MURPHY, C. AND FENSELAU, C.	786
LSIMS ANALYSIS OF OLIGOSACCHARIDES AS NEOGLYCOLIPID DERIVATIVES *LAWSON, A.M., CHAI, W., CASHMORE, G., STOLL, M., HOUNSELL, E.F. AND FEIZI, T.	788
CHARACTERIZATION OF OLIGOSACCHARIDES FROM MUCIN GLYCOPROTEINS BY LSIMS USING AN AUTOMATED TLC TARGET STAGE *LAWSON, A.M., CHAI, W., STOLL, M., HOUNSELL, E.F., FEIZI, T., BATEMAN, R.H. AND MAJOR, H.J.	790
ORIGIN OF THE (M-H) ⁺ IONS IN THE FAST ATOM BOMBARDMENT MASS SPECTRA OF SOME CYCLIC SUGARS *ZHA, Q. AND BERTRAND, M.J.	792
SEQUENCE ANALYSIS OF HIGHLY SULFATED, HEPARIN-DERIVED OLIGOSACCHARIDES USING FAST ATOM BOMBARDMENT MASS SPECTROMETRY *MALLIS, L.M., WANG, H.M., LOGANATHAN, D. AND LINHARDT, R.J.	794
PORPHYRIN PYRROLE SEQUENCING BY LOW ENERGY CID OF (M+7H) ⁺ GENERATED IN SITU DURING AMMONIA CHEMICAL IONIZATION VAN BERKEL, G.J., *TUINMAN, A.A., GLISH, G.L. AND MCLUCKEY, S.A.	796

WEDNESDAY ORAL SESSIONS

Symposium: Next Generation of Environmental Contaminants

AZODYES: ENVIRONMENTAL CONCERNS, ANALYTICAL CHEMISTRY, & MASS SPECTROMETRY *BUDE, W.L.	798
POSSIBLE WIDESPREAD ENVIRONMENTAL CONTAMINATION BY BROMO AND BROMOCHLORO DIOXINS AND DIBENZOFURANS *SOVOCOL, G.W. AND DONNELLY, J.R.	800
ALKYLINS; GOOD FOR ANTIFOULING PAINT, NOT SO GOOD FOR OYSTERS *GREAVES, J.	803
THE ROLE OF MASS SPECTROMETRY IN THE ANALYSIS OF EXPLOSIVES *MCLUCKEY, S.A. AND GLISH, G.L.	806
THE IDENTIFICATION OF BIZARRE ALKYLPHENOLS IN THE DETROIT RIVER CARTER, D., SHIRAISHI, H. AND *HITES, R.A.	809

Symposium: Lasers in Mass Spectrometry

LASER IONIZATION MASS SPECTROMETRY (LIMS): TECHNIQUE AND APPLICATIONS *ODOM, R.W. AND EVANS, C.A.	812
CO ₂ AND EXCIMER LASER DESORPTION AND PHOTODISSOCIATION IN A FTMS NUWAYSIR, L.M. AND *WILKINS, C.L.	816
MECHANISTIC STUDIES IN LASER IONIZATION MASS SPECTROMETRY WILKERSON, C.W., YANG, M., COLBY, S.M. AND *REILLY, J.P.	819
LASERS AND QUADRUPOLE FOURIER TRANSFORM MASS SPECTROMETRY APPLIED TO PROTEIN SEQUENCE ANALYSIS *SHABANOWITZ, J., HUNT, D.F., GRIFFIN, P.R. AND ZHU, N.Z.	821

Ion Molecule Reactions

PHOTOINDUCED ION MOLECULE REACTIONS IN THE GAS PHASE USING FTMS GORD, J.R. AND *FREISER, B.S.	824
DEPENDENCE OF CAR SPECTRA OF DIASTEREOMERIC MH ⁺ IONS UPON INITIAL INTERNAL ENERGY *TABET, J.-C.	826
EFFECTIVE ION TEMPERATURES IN A QUADRUPOLE ION TRAP NOURSE, B.D., *KENTAMAA, H.I. AND COOKS, R.G.	828
MECHANISTIC AND ENERGETIC IMPLICATION OF KINETIC ENERGY RELEASE DISTRIBUTIONS FOR REACTIONS OF TRANSITION METAL IONS WITH SMALL MOLECULES *BRODBELT-LUSTIG, J., VAN KOPPEN, P.A.M., BEAUCHAMP, J.L. AND BOWERS, M.T.	830
HYDROGEN BONDED, BONDED AND BONDED COMPLEXES (X.A)- FROM X- AND A, WHERE X- = CH ₃ O-, F-, Cl-, Br-, I-, AND A = SUBSTITUTED BENZENES AND QUINONES *PAUL, G.J.C. AND KEBARLE, P.	832

UNIMOLECULAR REARRANGEMENTS IN THE GAS PHASE: RING OPENING OF CYCLOPROPYL ANIONS CHOU, P. AND *KASS, S.	834
A THEORETICAL STUDY OF NUCLEOPHILIC SUBSTITUTION REACTIONS IN THE GAS PHASE *HIERL, P.M., MEYER, S. AND SIGLER, J.	836
SELECTIVITY AS A FUNCTION OF ANIONIC BASE PROPERTIES IN THE REACTIONS OF DIMETHYL SULFITE *GRABOWSKI, J.J. AND LUM, R.C.-J.	838
GAS-PHASE NUCLEOPHILIC ADDITION REACTIONS OF NITRILES: A CA MS/MS STUDY OF THE THORPE REACTION *HAYES, R.N. AND GROSS, M.L.	840
GAS-PHASE REACTIONS OF FIRST-ROW TRANSITION METAL IONS (Sc ⁺ -Zn ⁺) WITH PHENYL HALIDES *BJARNASON, A.	842

WEDNESDAY POSTER SESSIONS

Peptides and Proteins

ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF SMALL OLIGONUCLEOTIDES *EDMONDS, C.G., BARINAGA, C.J., LOO, J.A., UDSETH, H.R. AND SMITH, R.D.	844
FAB TANDEM MS OF PROTEASE AUTOLYSIS PRODUCTS *VESTLING, M.M. AND FENSELAU, C.	846
SEQUENTIAL MASS SPECTROMETRY ON A HYBRID TANDEM INSTRUMENT OF BEqQ CONFIGURATION *THORNE, G.C. AND GASKELL, S.J.	848
APPLICATIONS OF STABLE ISOTOPES AND TANDEM MASS SPECTROMETRY TO THE CHARACTERIZATION OF REACTIVE METABOLITE PEPTIDE INTERACTIONS *PEARSON, P.G., SLATTER, J.G., HAN, D.-H. AND BAILLIE, T.A.	850
ALTERNATE MASS SPECTROMETRIC METHODS FOR PEPTIDE SEQUENCE DETERMINATION *BOLGAR, M.S., DIDONATO, G.C., JOHNSON, J.H., TYMIK, A.A., UNGER, S.E. AND WARRACK, B.M.	852
SELECTIVE IDENTIFICATION OF DISULFIDE-CONTAINING PEPTIDES BY ELECTROCHEMICAL HPLC AND FAB/MS *SUN, Y., ANDREWS, P.C. AND SMITH, D.L.	854
AMINO-TERMINAL DERIVATIZATION OF PEPTIDES YIELDS IMPROVED CAD SPECTRA *STULTS, J.T., HALUALANI, R. AND WETZEL, R.	856
INVESTIGATION OF THE FRAGMENTATION OF PEPTIDES AS A FUNCTION OF COLLISION ENERGY IN A FOUR-SECTOR TANDEM MASS SPECTROMETER *MARTIN, S.A.	858
EFFECT OF TRIFLUOROACETIC ACID ON THE REDUCTION OF DISULFIDE BRIDGES IN PEPTIDES ANALYZED BY FAB/MS *VISENTINI, J., GAUTHIER, J. AND BERTRAND, M.J.	860
A MASS SPECTROMETRIC TECHNIQUE FOR DETECTING AND LOCATING ERRORS IN SYNTHETIC PEPTIDES *CHOWDHURY, S. AND CHAIT, B.T.	862
PULSED ION BOMBARDMENT TIME-OF-FLIGHT MASS SPECTROMETER WITH OPTIMUM SENSITIVITY FOR PEPTIDES IN 1-2 PICOMOLE RANGE *KATTA, V. AND CHAIT, B.T.	864

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

THE EFFECT OF PRIMARY IONIZATION ON THE MS/MS SPECTRA OF SMALL PEPTIDES USING A TRIPLE QUADRUPOLE MS *ROBINS, R.H. AND CROW, F.W.	866
THREE DIMENSIONAL CELLULAR MAPPING OF ANTIBODY-ANTIGEN INTERACTIONS BY SECONDARY ION MASS SPECTROMETRY LEE, J.J., LINTON, R.W., *VOYKSNER, R.D., HUNTER, J.L. AND WILSON, D.C.	868
TOPOGRAPHY OF MEMBRANE-BOUND PROTEINS: PHOTOAFFINITY STUDIES OF COLICIN Ia *FALICK, A.M., MEL, S.F., STROUD, R.M. AND BURLINGAME, A.L.	870
DERIVATIZATION AS AN AID TO PEPTIDE MAPPING BY LSIMS FALICK, A.M. AND *MALTBY, D.A.	872
LIQUID SECONDARY ION MASS SPECTROMETRIC (LSIMS) MASS MAPPING OF HUMAN HEMOGLOBIN FROM RED BLOOD CELLS MODIFIED IN VITRO BY STYRENE-7,8-OXIDE *KAUR, S., BURLINGAME, A.L., HOLLANDER, D. AND HAAS, R.	873
IDENTIFICATION OF POST-TRANSLATIONAL ARTIFACTS IN THE PRODUCTION OF BIOSYNTHETIC HUMAN GROWTH HORMONE BECKER, G.W., GILLIAM, J.M. AND *OCCELOWITZ, J.L.	875
OLIGOPEPTIDE FRAGMENTATION VIEWED AS CONSTANT NEUTRAL LOSS *MURPHY, C.M., ORLANDO, R.C., FENSELAU, C.C. AND COTTER, R.J.	877
INVESTIGATION OF ARTIFACTS FORMED DURING CYANOGEN BROMIDE DIGESTION OF PROTEINS *GOODLETT, D.R., CREECH, J. AND VAN BREEMEN, R.B.	879
NITRO-PHENYLISOTHIOCYANATE AS A REAGENT FOR PEPTIDE SEQUENCING BY NEGATIVE ION MASS SPECTROMETRY *WAIDYANATHA, S. AND ANDEREGG, R.J.	881
QUANTIFICATION OF SUBSTANCE P BY FAB-MS *KUSMIERZ, J. AND DESIDERIO, D.M.	883
QUANTIFICATION OF BETA-ENDORPHIN IN HUMAN PITUITARY BY FAB *DASS, C., KUSMIERZ, J. AND DESIDERIO, D.M.	885
MATRIX REACTIONS AND THEIR EFFECTS ON THE ISOTOPIC PEAK RATIOS OF PEPTIDES MEASURED BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY *VISENTINI, J., THIBAUT, P. AND BERTRAND, M.J.	887
ANALYSIS OF CANCER CELL LINES FOR THE PRESENCE OF PEPTIDE GROWTH HORMONES AND OTHER PEPTIDES BY ION EVAPORATION IONIZATION/TANDEM MASS SPECTROMETRY *TRESTON, A., KASPRZYK, P., CUTTITTA, F., COVEY, T. AND MULSHINE, J.	889
ANALYSIS OF PHOSPHORYLATED AND SULFATED PEPTIDES BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY *GUIDO, D.M. AND MATHEWS, W.R.	891
CESIUM ION LIQUID (SIMS) AND CALIFORNIUM-252 PLASMA DESORPTION MASS SPECTROMETRY STUDIES OF SOME NATURAL AND RECOMBINANT PROTEINS PRAMANIK, B.N., *TSARBOPoulos, A., SIEGEL, M., REICHERT, P., DAS, P., BARTNER, P., HER, G., TSAO, R., DOELLING, V. AND TROTTA, P.P.	893
SOLVING DIFFICULT PEPTIDE STRUCTURAL PROBLEMS BY TANDEM MASS SPECTROMETRY *BEAN, M.F., CARR, S.A., ESCHER, E., JOHNSON, W. AND MOORE, M.	895
NEW TECHNIQUES IN THE HYBRID TANDEM MASS SPECTROMETRY OF PEPTIDES *ORKISZEWSKI, R.S., THORNE, G.C., DOURDEVILLE, T.A. AND GASKELL, S.J.	897

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

BRAIN CALBINDINS AND A MONOCYTE CHEMOTACTIC AGENT; STRUCTURAL ANALYSIS BY TANDEM MASS SPECTROMETRY *MCCORMACK, A.L., HUNT, D.F., SHABANOWITZ, J., ZHU, N.Z. AND GRIFFIN, P.R.	899
TOF-SIMS AND FAB OF MODIFIED PEPTIDES: A COMPARISON OF SENSITIVITIES HAGENHOFF, B., *NIEHUIS, E., BENNINGHOVEN, A., WUNSCH, C. AND MUSCHE, H.	901
STRUCTURAL CHARACTERIZATION OF LENS PROTEINS BY FAB/MS *LEEDS, J., QIN, W. AND SMITH, D.L.	903
COLLISION-INDUCED DISSOCIATION OF MULTIPLY CHARGED PEPTIDES *COVEY, T.R., CONBOY, J.J. AND HENION, J.D.	905
COLLISION-INDUCED DECOMPOSITIONS OF PEPTIDE IONS CATIONIZED WITH ALKALINE EARTH AND ALKALI METALS *TEESCH, L.M. AND ADAMS, J.	907
PD/FAB-MAPPING OF BOVINE NEUROPHYSINS *FEISTNER, G.J., HOJRUP, P., EVANS, C.J., BAROFSKY, D.F., ROEPSTORFF, P. AND FAULL, K.F.	909
RESOLUTION AND IDENTIFICATION OF DERIVATIZED ENANTIOMERS OF α -AMINO ACIDS ON A CHIRAL DIAMIDE PHASE BY GC/MS *CHANG, J.S.-C. AND LIN, S.-R.	911
IDENTIFICATION OF AN AMINO ACID REPLACEMENT IN A MUTANT PHYCOCYANIN BY MS/MS WITH A HYBRID INSTRUMENT *WILLIAMS, T., SWANSON, R., WILBANKS, S., ZHOU, J., BRYANT, D., GLAZER, A.N. AND LEARY, J.A.	913
FAST ATOM BOMBARDMENT MASS SPECTROMETRIC CHARACTERIZATION OF RECOMBINANT HUMAN CYCLOPHILIN *LARSEN, B.S., LISCHWE, M., DAVIS, G. AND GALBRAITH, W.	915
MODIFIED PHENYLISOTHIOCYANATES FOR PEPTIDE SEQUENCING BY NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY *GHOSH, A. AND ANDEREGG, R.J.	917
LSIMS ON MULTIPLE CYSTINE-LINKED PEPTIDES FROM RECOMBINANT HIRUDIN VARIANT 2-Lys (rHV2-Lys 47) DIGESTION LEPAGE, P., ROITSCH, C., ACKER, M., BITSCH, F. AND *VAN DORSSELAER, A.	919
CONFIRMATION OF INDOLE-3-ACETIC ACID BOUND TO ZEIN LEVERONE, L.A., CARUSO, J.L. AND *JAYASIMHULU, K.	921
CHARACTERIZATION OF PHENYLGLYCIDYL ETHER NUCLEOSIDE ADDUCTS USING FAB/MS/MS CLAEREBOUDT, J., *ESMANS, E.L., VAN DEN EECKHOUT, E.G., BAETEN, W. AND CLAEYS, M.	923
INTERCHAIN DISULFIDE BOND FRAGMENTATIONS OF DIMERIC PEPTIDES BITSCH, F., HIETTER, H. AND *VAN DORSSELAER, A.	925

LC/MS II

QUANTIFICATION OF 1,25-DIHYDROXY-VITAMIN D ₃ BY STABLE-ISOTOPE DILUTION USING COMBINED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY THERMOSPRAY MASS SPECTROMETRY *WATSON, D., SETCHELL, K., ROSS, R. AND BJORKHEM, I.	927
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Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

THE ANALYSIS OF NONIONIC, CATIONIC, ANIONIC AND AMPHOTERIC SURFACTANTS USING THERMOSPRAY MS/MS AND FAB MS/MS *MABUD, Md.A., DREIFUSS, P.A., KILLINGER, W.E. AND SMITH, M.W.	929
THERMOSPRAY LC/MS INTERFACE FOR TIME-OF-FLIGHT MASS SPECTROMETRY *YERGEY, A.L. AND LAMPE, F.W.	931
USE OF DEUTERATED SOLVENTS IN THERMOSPRAY LC/MS TO DISTINGUISH CLUSTER COMPOSITION, ADDUCT FORMATION, DIMERIZATION AND EXCHANGEABLE PROTONS GOODWIN, R.M. AND *BREAU, A.P.	933
OPTIMIZATION OF SENSITIVITY AND INFORMATION OF THERMOSPRAY LC/MS *NIESSEN, W.M.A., HEEREMANS, C.E.M., VAN DER HOEVEN, R.A.M., TJADEN, U.R. AND VAN DER GREEF, J.	935
THE EFFECT OF ION SOURCE CONFIGURATION ON THE GAS-PHASE IONIZATION AND ION-EVAPORATION COMPONENTS OF THERMOSPRAY *MCLEAN, M.A. AND VESTAL, M.L.	937
DESIGN AND PERFORMANCE OF A NEW COMBINED THERMOSPRAY-EI LC-MS SYSTEM *VESTAL, M.L., WINN, D. AND WILKES, J.G.	939
APPLICATION OF THERMOSPRAY HPLC/MS TO ORGANIC SYNTHESIS *YANG, L.	941
OPTIMIZATION OF ON-LINE ENZYMATIC HYDROLYSIS HPLC/MS FOR PEPTIDE MAPPING AND DISULFIDE BOND LOCATION *VOYKSNER, R.D., CHEN, D.C. AND SWAISGOOD, H.E.	943
ANALYSIS OF ANTIBIOTICS USING SUPERCRITICAL FLUID EXTRACTION WITH SUPERCRITICAL FLUID CHROMATOGRAPHY/MS AND HPLC/MS TECHNIQUES *VOYKSNER, R.D., RAYMER, J., VELEZ, G. AND TYCZKOWSKA, K.	945
ANALYSIS OF CATIONIC HEAVY METAL ALKYLATES BY THERMOSPRAY LC/MS *LINSCHIED, M.	947
HPLC-MS ANALYSIS OF COCONUT AMINE SALTS *YELLE, L.M. AND FARROW, P.E.	949
ANALYSIS OF ENVIRONMENTALLY SIGNIFICANT COMPOUNDS BY THERMOSPRAY EI LC-MS *WINN, D. AND VESTAL, M.L.	951
PEPTIDE MAPPING USING THERMOSPRAY LC/MS/MS *KANAI, M. AND RUDEWICZ, P.	953
A FACILE METHOD FOR THE PREPARATION AND LC/MS CHARACTERIZATION OF BENZYLOXYCARBONYL METHYL ESTERS DERIVATIVES OF REDUCED GLUTATHIONE, CYSTEINE AND CORRESPONDING XENOBIOTIC-THIOETHER CONJUGATES *RASHED, M.S., TALAAT, R.E. AND BAILLIE, T.A.	955
STRUCTURE DETERMINATION OF IMPURITIES IN A SYNTHESIS OF A PROTECTED DIAZABICYCLOHEPTANE BY LC/MS *ROSNACK, K.J., STROH, J.G. AND COHEN, M.J.	957
INVESTIGATION OF PRAVADOLINE METABOLITES BY THERMOSPRAY MASS SPECTROMETRY *JOHNSON, J.A., GONYEA, G.J. AND BAKER, J.F.	959
METABOLISM AND CARCINOGENICITY OF HAIR DYES: A TSP LC/MS STUDY *PARKER, C.E., TOMER, K.B., KARI, F.W., DRISCOLL, S.M. AND RUDD, K.M.	961
A COMPARISON OF MASS SPECTROMETRY OF DYES GENERATED BY PARTICLE BEAM AND THERMOSPRAY LC/MS YINON, J., JONES, T.L. AND *BETOWSKI, L.D.	963

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

A STUDY OF CHLORINATED HERBICIDES BY HPLC/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TSP/MS) *JONES, T.L., BETOWSKI, L.D. AND YINON, J.	965
THERMOSPRAY LC-MS ANALYSIS OF AN INSECTICIDE CONTAINING A SERIES OF NEREISTOXIN ANALOGS HANSEN, E.B., GETEK, T.A., *KORFMACHER, W.A., XIA, S.J., KUO, W.S., WANG, X.T. AND CHOU, M.W.	967
GC/MS AND LC/MS IN THE ANALYSIS OF NPPD ("SPY DUST") *BURSEY, J., MOSEMAN, R., PARKER, C., TOMER, K. AND JAMESON, C.	969
A DERIVATIVE WITH ENHANCED NCI-MS SENSITIVITY FOR OLIGOSACCHARIDE SAMPLES: SYNTHESIS, SAMPLE DERIVATIZATION, AND SFC-MS SENSITIVITY STUDIES *CAESAR, J.P., SHEELEY, D.M., JOHNSON, R.S. AND REINHOLD, V.N.	971
INTERFACING CHROMATOGRAPHIC TECHNIQUES TO MULTIPHOTON IONIZATION MASS SPECTROMETRY KOSTER, C., ROHWER, E.R., *GROTEMEYER, J. AND SCHLAG, E.W.	973
SUPERCritical FLUID CHROMATOGRAPHY/CHEMICAL IONIZATION MASS SPECTROMETRY (SFC/CIMS) IN A THERMOSPRAY SOURCE *MUSSER, S.M. AND CALLERY, P.S.	975
ELECTRON IMPACT MASS SPECTRA WITH SFC/MS *CHUNG, H.L., MOORE, D.S., ALDRIDGE, J.C., GORDON, B.M. AND COLEMAN, W.M.	977
HIGH PRESSURE LIQUID NH ₃ INJECTION OF POLAR MOLECULES INTO A MASS SPECTROMETER WITH RESONANT TWO-PHOTON IONIZATION DETECTION PANG, H.M. AND *LUBMAN, D.M.	978
APPLICATION OF SUPERCRITICAL FLUID CHROMATOGRAPHY-MASS SPECTROMETRY TO N-LINKED GLYCANS *SHEELEY, D.M. AND REINHOLD, V.N.	980
SUPERCritical FLUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR CHARACTERIZATION OF COMMERCIAL SURFACTANT MIXTURES *KALINOSKI, H.T. AND HARGISS, L.O.	982
ION SPRAY LC/API-MS OF MORFINANS LANTING, A., DRENTH, B.F.H., BOSMAN, J. AND *BRUINS, A.P.	984
IDENTIFICATION OF TRACE LEVELS OF UNKNOWN CONJUGATED QUINONES USING NICI ENHANCEMENT BY DIRECT LC/MS ANALYSIS *NG, K., PIATEK, B., PATRIANAKOS, C. AND KARLINER, J.	986
COMPARISON OF THERMOSPRAY AND CONTINUOUS FLOW FAB LC/MS METHODS FOR THE ANALYSIS OF ACYL-CoA COMPOUNDS *NORWOOD, D.L., BUS, C.A. AND MILLINGTON, D.S.	988
EFFICIENT INTRODUCTION OF HPLC FRACTIONS INTO A HIGH PERFORMANCE TANDEM MASS SPECTROMETER *CAPPIELLO, A., PALMA, P., PAPAYANNOPOULOS, I.A., BIEMANN, K., CRESCENTINI, G. AND BRUNER, F.	990
CONDITIONS FOR MICROBORE LIQUID CHROMATOGRAPHY OF PEPTIDES FOR CONTINUOUS-FLOW FAB PRAMANIK, B.C., DOURDEVILLE, T.A. AND *SLAUGHTER, C.A.	992
ABSORBENT EVAPORATOR FOR CONTINUOUS FLOW LIQUID SIMS *SHIH, M., WANG, T.-C.L. AND MARKEY, S.P.	994
LINKED SCAN AND CONTINUOUS FLOW FAB ANALYSIS OF NUCLEOSIDE-PAH DNA ADDUCTS *ANNAN, R.S., NELSON, R.J., GIESE, R.W. AND VOURES, P.	996

CONTINUOUS-FLOW FAST ATOM BOMBARDMENT LC/MS IN BIOANALYSIS KOKKONEN, P., NIESSEN, W.M.A., TJADEN, U.R. AND *VAN DER GREEF, J.	998
CONTINUOUS FLOW FAB-MS FOR RAPID STRUCTURAL COMPARISON OF ANALOGOUS THERAPEUTIC PROTEINS MOCK, K., FIRTH, J., EVANS, S. AND *COTTRELL, J.	1000
CF-FAB AND MICRODIALYSIS FOR ANALYSIS BLOOD LEVELS OF DRUGS IN LIVE ANIMALS: MULTIPLE DOSAGE PHARMACOKINETICS *LIN, S-N., CHANG, S. AND CAPRIOLI, R.M.	1002
ANALYSIS OF URINARY ACYLCARNITINES BY DYNAMIC FIB MS/MS *CLENCH, M., WILSON, S., WEIR, O., MILLS, G. AND WALKER, V.	1004
ANALYSIS OF PROTEOLYTIC DIGESTS AND PEPTIDE MIXTURES USING COAXIAL CONTINUOUS FLOW FAB MASS SPECTROMETRY DETERDING, L.J., JORGENSEN, J.W., MOSELEY, M.A., *PLEASANCE, S., THIBAUT, P. AND TOMER, K.B.	1006
MICROBORE AND MICROCAPILLARY HPLC ANALYSIS OF GLYCOPEPTIDE ANTIBIOTICS USING CONTINUOUS FLOW FAB LC-MS COUTANT, J.E., CHEN, T-M. AND *ACKERMANN, B.L.	1008
MICROCOLUMN LC/CONTINUOUS FLOW FAB MS - APPLICATIONS TO AGRICULTURAL CHEMICALS RESEARCH *REISER, R.W.	1010
EXAMPLES OF THE USE OF A PARTICLE BEAM INTERFACE FOR THE HPLC/MS ANALYSIS OF NON-GAS CHROMATOGRAPHABLE ANALYTES *HUGHES, B.M., MCKENZIE, D.E., DOI, J.D. AND ZINKL, M.A.	1012
LC/MS DETERMINATION OF PROTEINS AND LARGE PEPTIDES *HENION, J., COVEY, T., MUCK, W. AND HUANG, E.	1014
ANALYSIS OF ENZYMATICALLY DIGESTED PROTEINS BY ON LINE CAPILLARY HPLC/FAB/MS *BOURELL, J.H., HENZEL, W.J. AND STULTS, J.T.	1015

Instrumentation

A NEW INSTRUMENT FOR THE ANALYSIS OF HIGH MOLECULAR WEIGHT BIOMOLECULES *JARVIS, S., BATEMAN, R.H. AND GREEN, B.N.	1017
DESIGN AND PERFORMANCE OF A NEW MAGNETIC SECTOR MASS SPECTROMETER WITH DOUBLE FOCUSING DIMIDATE GEOMETRY *BERTRAND, M.J., BOTT, P.A., PORTER, C.J. AND BATEMAN, R.H.	1019
A HIGH SENSITIVITY/HIGH RESOLUTION TANDEM MASS SPECTROMETER FOR HIGH MASS BIOMOLECULES *RUSSELL, D.H.	1021
A TANDEM QUADRUPOLE MASS SPECTROMETER OF NOVEL GEOMETRY OPTIMISED FOR INTELLIGENT DATA ACQUISITION *MCDOWALL, M.A., MERREN, T.O. AND BUTLER, I.	1023
ION TRANSMISSION CONSIDERATIONS IN TANDEM QUADRUPOLE MS-MS *MCDOWALL, M.A., MERREN, T.O. AND HAMMOND, M.J.	1025
A PENTAQUADRUPOLE MASS SPECTROMETER: INSTRUMENTATION, SYSTEM CONTROL, AND APPLICATIONS TO MS/MS/MS SCANS *SCHWARTZ, J.C., SCHEY, K.L. AND COOKS, R.G.	1027

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

HIGH RESOLUTION DAUGHTER ION ANALYSIS APPLICATIONS IN HYBRID MASS SPECTROMETRY *GRANT, B.C., MCBAY, E.H., MCLUCKEY, S.A. AND GLISH, G.L.	1029
RESIDUAL GAS ANALYZERS AS INEXPENSIVE SOURCES OF MASS-SELECTED ION BEAMS *GILBERT, J. AND ALLISON, J.	1031
NEW B-Q TANDEM MASS SPECTROMETER *LAUE, H.J. AND HABFAST, K.	1033
PERFORMANCE OPTIMIZATION OF A KRATOS MS80RFAQ MASS SPECTROMETER FOR HIGH MASS OPERATION TO MASS 6,000 *HANSEN, G. AND MCKELLOP, K.	1035
INTERFACING MULTIPLE ION SOURCES WITH A QUADRUPOLE ELECTROSTATIC DEFLECTOR *MAHAFFY, P. AND LAI, K.	1037
DESIGN OF A FOCUSED CESIUM ION SOURCE FOR CONTINUOUS FLOW LIQUID SIMS WANG, T.-C.L., *MARKEY, S.P. AND BONI, R.	1039
MASS SPECTROMETRIC DETECTION OF THIN LAYER CHROMATOGRAPHY USING PULSED LASER EVAPORATION WITH UV LASER IONIZATION LI, L. AND *LUBMAN, D.M.	1041
DIRECT FAB-MS FROM EMPORE TLC SHEETS *MINARD, R.D. AND LONG, E.J.	1043
COMPARISON OF LC/MS PARTICLE BEAM SPECTRA WITH DCI AND DEI PROBE SPECTRA *DILTS, M.J. AND MARECIC, T.	1045
COMPARISON OF GC/MS AND GC/MS/MS FOR THE ANALYSIS OF CGS-18416A *HAYES, M.J., KHEMANI, L. AND GAUDRY, D.	1047
DIRECTLY COUPLED SUPERCRITICAL FLUID EXTRACTION-GAS CHROMATOGRAPHY/MASS SPECTROMETRIC ANALYSIS OF ORGANIC COMPOUNDS FROM SOLID SAMPLES *MILLER, D.J., HAWTHORNE, S.B. AND KRIEGER, M.S.	1049
DIRECT COUPLING OF SUPERCRITICAL FLUID CHROMATOGRAPHY WITH SECONDARY ION MASS SPECTROMETRY *WENCLAWIAK, B., SICHTERMANN, W. AND BENNINGHOVEN, A.	1051
AN OPTICAL CCD CAMERA INTEGRATED WITH A SECONDARY ION MASS SPECTROMETER FOR ANALYSIS OF PLANAR CHROMATOGRAMS *BROWN, S.M., DOHERTY, S.J. AND BUSCH, K.L.	1053
EVALUATION AND OPTIMIZATION OF A CCD DETECTOR FOR SIMS IMAGING *MANTUS, D.S., TURNER, L.K., LING, Y.-C. AND MORRISON, G.H.	1055
DIRECT INTERFACE OF A RUSKA THERMA-CHROM WITH AN HP 5985B GC/MS *GALE, P.J., BENTZ, B.L., WORDEN, R.D., SMITH, D., KING, D.B., SIZEMORE, E. AND IPPOLITO, M.L.	1057
A DATA SYSTEM FOR DAUGHTER ION MEASUREMENTS WITH A REFLECTING TIME-OF-FLIGHT MASS SPECTROMETER *ENS, W. AND STANDING, K.G.	1059
A NEW TIME-OF-FLIGHT SECONDARY ION MICROSCOPE *SCHUELER, B.W., REED, D.A. AND SANDER, P.	1061
GC-MS BY TIME-OF-FLIGHT MASS SPECTROMETRY WITH TIME ARRAY DETECTION *SCHULTZ, G.A., TECKLENBURG, R., HOLLAND, J.F., WATSON, J.T. AND ALLISON, J.	1063

ION KINETIC ENERGY RELEASE MEASUREMENT BY POST-SECTOR BEAM DEFLECTION IN TIME-RESOLVED ION MOMENTUM SPECTROMETRY WITH TIME ARRAY DETECTION *LOPSHIRE, R.F., ECKENRODE, B.E., STULTS, J.T., ENKE, C.G. AND HOLLAND, J.F.	1065
MASS DEPENDENCE OF FOCUSING PARAMETERS IN TIME-OF-FLIGHT MASS SPECTROMETRY *ERICKSON, E.D., YEFCHAK, G.E. AND ENKE, C.G.	1067
POST SOURCE PULSE FOCUSING-A SIMPLE METHOD TO ACHIEVE IMPROVED RESOLUTION IN A TIME-OF-FLIGHT MASS SPECTROMETER *KINSEL, G.R. AND JOHNSTON, M.V.	1069
ORGANIC SECONDARY ION TIME-OF-FLIGHT MASS SPECTROMETRY WITH A FOCUSED LIQUID METAL ION PRIMARY BEAM JIANG, L.F., YEN, T.Y. AND *BAROFSKY, D.F.	1071
EXTENDED LIFETIME CONTINUOUS DYNODE ELECTRON MULTIPLIERS KURZ, E.A., LORETZ, T.J. AND *ROY, R.L.	1073
HIGH SENSITIVITY ION DETECTOR PARAMETRIC STUDY *PEREL, J., MAHONEY, J.F., LEE, T.D. AND LEGESSE, K.	1075
A FLEXIBLE ARRAY DETECTOR OF EXTENDED MASS RANGE FOR A HIGH PERFORMANCE TANDEM MASS SPECTROMETER *HILL, J.A., BILLER, J.E., MARTIN, S.A., BIEMANN, K. AND ISHIHARA, M.	1077
A VERSATILE ARRAY DETECTION SYSTEM *PESCH, R., JUNG, G., ROST, K. AND TIETJE, K.-H.	1079
DETECTION OF HIGH MOLECULAR WEIGHT IONS BY CHANNELPLATE DETECTORS *NIEHUIS, E., DEIMEL, M. AND BENNINGHOVEN, A.	1081
DEVELOPMENT OF AN ARRAY DETECTOR FOR WIDE MASS RANGE DETECTION *MUSSELMAN, B.D., DIXON, M., ELLIS, G., SATO, K. AND ISHIHARA, M.	1083
PERFORMANCE RELATED CHARACTERISTICS OF LINEAR ARRAY DETECTORS FOR MASS SPECTROMETRY *GRAY, J.W. AND HOOPER, J.B.	1085
DESIGN OF AN ION OPTICS USING A QUADRUPOLE DOUBLET(QPD) FOR ZOOMING MASS RANGE ON AN ARRAY DETECTOR *ISHIHARA, M., KUNITHIRO, F., OTSUKA, K., KAMMEI, Y., KUBOTA, E., ITAGAKI, Y., HIGUCHI, T. AND MUSSELMAN, B.C.	1087
DESIGN CONSTRUCTION AND CHARACTERIZATION OF ION DECELERATION LENSES. WHAT ARE THE SIMPLICITY/PERFORMANCE TRADEOFFS? *O'CONNOR, P. AND ALLISON, J.	1089
IONIZATION TECHNIQUES BASED ON PROPERTIES OF SUPERCRITICAL FLUIDS: II INSTRUMENTATION *LARKINS, W. AND OLESIK, S.V.	1091
HIGH ENERGY DYNODE (HED) ELECTRON MULTIPLIER DELIVERS INCREASED HIGH MASS SENSITIVITY FOR QUADRUPOLE MASS SPECTROMETERS FJELDSTEAD, J., *GOODLEY, P.C. AND HANSEN, S.	1092
ION TRAJECTORY MODELLING IN AN ION MOBILITY SPECTROMETER EMPLOYING NONLINEAR ELECTRIC FIELDS *ECKENRODE, B.A., MCLUCKEY, S.A. AND GLISH, G.L.	1094
NUMERICAL CALCULATION OF ION TRAJECTORIES IN A QUADRUPOLE MASS FILTER WITH CIRCULAR CROSS SECTION ELECTRODES *TAYLOR, L.C.E. AND SEDGWICK, R.D.	1096

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

A PC-BASED LINKED SCAN CONTROL AND DATA ACQUISITION SYSTEM CAPABLE OF REAL-TIME SIGNAL AVERAGING *MARTIN, W.B., COTTER, R.J., HENDERSON, L.E. AND FRASURE, W.	1098
AN IMPROVED INTERFACE DESIGN FOR DETERMINING RADIOISOTOPE PURITY BY RADIOGAS CHROMATOGRAPHY MASS SPECTROMETRY *KASTL, P.E., LANGVARDT, P.W., BARTELS, M.J. AND BRZAK, K.A.	1100
CHARACTERISTICS OF A HIGH RESOLUTION ICP-MS INSTRUMENT *BRADSHAW, N., SANDERSON, N.E. AND CANTLE, J.E.	1102
ICP-MS MEASURABILITY LIMITS IN CONCENTRATED SOLUTIONS *BEAUGRAND, C., DELAPORTE, T. AND DEVANT, G.	1104
PRECISE MASS MEASUREMENT OF FAB IONS USING AN ALTERNATING SAMPLE PROBE AND PEAK MATCHING ON THE KRATOS MS50 *HOGG, A.	1106
FIELD DESORPTION OF ORGANIC POLYMERS *DAVIS, S.C. AND BEATY, T.C.	1108
AN AUTOMATED SOLIDS INSERTION PROBE SYSTEM FOR AN ORGANIC MASS SPECTROMETER *SMITH, D.C., GOULD, V., MISTRY, H. AND TODD, D.E.	1110
PREDICTION OF RELATIVE SENSITIVITIES IN ELECTRON IONIZATION MASS SPECTROMETRY *ALLGOOD, C. AND MUNSON, B.	1112
INTERNAL STANDARD SYSTEM FOR USE WITH APCI MASS SPECTROMETERS *NELSON, P.R.	1114
NON-CONTAMINATING CI MASS CALIBRATION STANDARDS FOR QUADRUPOLE AND MAGNETIC INSTRUMENTS *HSU, F-F., TYLER, A.N. AND SHERMAN, W.R.	1115
THE DYNAMICS OF IONS IN THE DRIFT REGION OF A CORONA-TYPE MS SOURCE: SOME MATHEMATICAL MODELS SUNNER, J. AND *BUSMAN, M.	1118
INSTRUMENT-INDEPENDENT CAD DATABASE FOR XQQ INSTRUMENTS: CH ₃ CO+ --Ar CAD-CH ₃ + +CO *MARTINEZ, R.I. AND GANGULI, B.	1120

THURSDAY ORAL SESSIONS

Symposium: Tandem MS in the Solution of Peptide & Protein Structural Problems

STRUCTURE DETERMINATION OF PEPTIDES AND PROTEINS BY HIGH PERFORMANCE TANDEM MASS SPECTROMETRY *BIEMANN, K.	1122
PROTEIN STRUCTURAL PROBLEMS, THE ROLE OF TANDEM MASS SPECTROMETRY *HUNT, D.F., SHABANOWITZ, J., GRIFFIN, P.R., ZHU, N.Z., YATES III, J.R. AND MARTINO, P.	1125
FOUR-SECTOR MS/MS OF PEPTIDE TOXINS, INVERTEBRATE NEUROPEPTIDES AND TRYPTIC DIGESTS *RINEHART, K.L., THOMPSON, A.G., RONG, L., MILBERG, R.M. AND CURTIS, J.M.	1128

- AN INTEGRATED APPROACH TO PEPTIDE AND PROTEIN STRUCTURE ANALYSIS 1131
UTILIZING TANDEM HIGH PERFORMANCE MASS SPECTROMETRY
*CARR, S.A., BEAN, M.F., HEMLING, M.E. AND ROBERTS, G.D.

Inorganic Elemental Analysis and Instrumentation

- 252 CF-PLASMA DESORPTION MASS SPECTROMETRY OF Au67(PPH3)14C18 1133
*MCNEAL, C.J., WINPENNY, R.E.P., FACKLER, J.P. AND MACFARLANE, R.D.
- FABMS AND FABMS/MS STUDIES ON INORGANIC AND ORGANOMETALLIC COMPOUNDS 1135
*THOMPSON, A.G., RONG, L.-S., CURTIS, J.M., SUN, F., MILBERG, R.M.,
SUSLICK, K.S., BIBLE, K.C., SHAPLEY, J.R. AND RINEHART, K.L.
- FTMS STUDY OF THE ELECTRON AFFINITY OF TRANSITION METAL TRIS 1137
(ACETYLACETONATO) COORDINATION COMPOUNDS
RICHARDSON, D.E., *SHARPE, P., RYAN, M.F. AND EYLER, J.R.
- SOLIDS ANALYSIS VIA GLOW DISCHARGE MASS SPECTROMETRY 1139
*CHRISTIE, W.H.
- ANALYTICAL COMPARISONS OF DIRECT CURRENT AND RADIO FREQUENCY GENERATED 1141
GLOW DISCHARGE ION SOURCES FOR DIRECT SOLIDS ANALYSES
*DUCKWORTH, D. AND MARCUS, R.K.
- PRECISE ATOMIC MASS MEASUREMENTS AND NEUTRINO PHYSICS: RECENT RESULTS AT 1143
THE UNIVERSITY OF MANITOBA
*SHARMA, K.S., BARBER, R.C., DYCK, G.R., SIDKY, M.S., HYKAWY, J.G.,
HALL, B.J., LANDER, C.A. AND DUCKWORTH, H.E.
- A MASS-SELECTED REAGENT ION CHEMICAL IONIZATION SOURCE 1145
DOLNIKOWSKI, G.G., FALCINI, M.R.A. AND *JENNINGS, K.R.
- GC SAMPLE INTRODUCTION INTO THE COLLISION CELL OF A TRIPLE QUADRUPOLE 1147
MASS SPECTROMETER FOR MASS-SELECTED REACTANT ION CI
*HAIL, M.E., BERBERICH, D.W. AND YOST, R.A.
- ONE QUADRUPOLE ANALYSER, MS_n+1, ZERO-ANGLE (SID)_n 1149
*BEAUGRAND, C., DEVANT, G., JAOUEN, D., MORIN, N., MESTDAGH, H.
AND ROLANDO, C.
- USE OF A FOCUSED LIQUID METAL ION COLUMN WITH A HIGH PERFORMANCE MAGNETIC 1151
SECTOR MASS SPECTROMETER
*ARBOGAST, B., BAROFSKY, E., JIANG, L.F. AND BAROFSKY, D.F.
- EXACT MASS DETERMINATION BY HIGH RESOLUTION TIME-OF-FLIGHT SIMS 1153
*NIEHUIS, E., HELLER, T., JURGENS, U. AND BENNINGHOVEN, A.
- HIGH PRECISION CARBON 13/12 RATIOS BY GC/COMBUSTION/MASS SPECTROMETRY 1155
*BRAND, W.A., RAUTENSCHLEIN, M., HABFAST, K., RICCI, M., FREEMAN, K.L.
AND HAYES, J.

Symposium: Comparison of Ionic Reactions in Gas Phase and in Solution

- AROMATIC SUBSTITUTION REACTIONS BY RADICAL CATIONS 1157
*GRUTZMACHER, H.-F. AND THOELMANN, D.
- SOLVOLYSIS AND PROTONATION: POSITIVE ION FORMATION IN THE GAS PHASE AND 1159
IN SOLUTION
*MORTON, T.H.

SOLVENT AND STRUCTURAL EFFECTS IN GASEOUS ION ENERGETICS AND REACTIONS *MCMAHON, T.B.	1160
BIMOLECULAR REACTIONS AND STRUCTURES OF IONS IN THE GAS PHASE *NIBBERING, N.M.M.	1163

Symposium: State of the Art Research in Mass Spectrometry

DRUG METABOLISM MADE EASY (SOMETIMES): THE USE OF TANDEM MS TECHNIQUES FOR RAPID STRUCTURE ELUCIDATION *STRAUB, K.	1165
ULTRAVIOLET LASER DESORPTION OF BIOMOLECULES IN THE HIGH MASS RANGE *HILLENKAMP, F. AND KARAS, M.	1168
RECENT DEVELOPMENTS IN ISOTOPE ABUNDANCE MEASUREMENTS *DELAETER, J.R.	1170
RECENT COMPUTER APPLICATIONS IN MASS SPECTROMETRY *WILKINS, C.L.	1173

Biochemistry II

FAB/MS AND FAB/MS OF PROTEOLYTIC DIGESTS OF BOVINE ALDOSE REDUCTASE *THOMPSON, A.G., RONG, L-S., MILBERG, R.M., SUN, F. AND RINEHART, K.L.	1175
MOLECULAR WEIGHT DETERMINATION OF RECOMBINANT HUMAN GROWTH HORMONE WITH AN ATMOSPHERIC PRESSURE ION SOURCE QUADRUPOLE MASS SPECTROMETER *CHAKEL, J. AND COVEY, T.	1177
QUANTIFICATION BY GC/MS OF SEPARATED ENANTIOMERS OF ARABINITOL TO AID THE DIFFERENTIAL DIAGNOSIS OF DISSEMINATED CANDIDIASIS *ROBOZ, J., NIEVES, E. AND HOLLAND, J.F.	1179
RELATIONSHIP OF THE STRUCTURE OF SUBUNIT CONTAINING PROTEINS TO THEIR ION EVAPORATION SPECTRA *COVEY, T.R., SUSHAN, B.I., BONNER, R.F. AND HENION, J.	1181
INFLUENCE OF THE RATIO OF GLYCEROL TO ANALYTE ON FAB-MS ANALYSES *HEINE, C.E., HOLLAND, J.F. AND WATSON, J.T.	1182
DIFFERENTIATION OF LEAFY SPURGE (EUPHORBIA ESULA L.) ACCESSIONS BASED ON CHEMICAL CONSTITUTION USING CURIE POINT PYROLYSIS-MASS SPECTROSCOPY-PATTERN RECOGNITION VALCARCE, R.V., *SMITH, G.G., TORELL, J.M. AND EVANS, J.O.	1184
MATRIX ASSISTED UV LASER DESORPTION OF BIOLOGICALLY INTERESTING MOLECULES *CHAIT, B.T.	1186
STRUCTURAL ANALYSIS OF COMPLEX BACTERIAL OLIGOSACCHARIDES BY TWO-AND FOUR-SECTOR LSIMS *JOHN, C.M. AND GIBSON, B.W.	1188
HIGH ENERGY CID OF OLIGOSACCHARIDES (MS-MS) USING MULTICHANNEL ARRAY DETECTION *GILLECE-CASTRO, B. AND BURLINGAME, A.L.	1190
SEPARATION OF HIGH MANNOSE OLIGOSACCHARIDE ISOMERS AND THEIR CHARACTERIZATION BY MASS SPECTROMETRY *JOHNSON, R.S., COSTELLO, C.E. AND REINHOLD, V.N.	1192

LDI-FT-ICR MASS SPECTROMETRY OF OLIGOSACCHARIDES *COMISAROW, M.B., DUTTON, G.G.S., LAM, Z., WEIL, D.A. AND BJARNASON, A.	1194
FAST ATOM BOMBARDMENT MASS SPECTRAL BEHAVIOR OF SACCHARIDES AND NUCLEOSIDES: MATRIX EFFECT *OSEI-TWUM, E.Y. AND MAMER, O.A.	1196

Symposium: Organometallic Mass Spectrometry

CHEMISTRY OF IONS DERIVED FROM METAL CARBONYLS PAN, Y.H. AND *RIDGE, D.P.	1198
"DAMAGE CONTROL" IN CHARACTERIZING COMPLEXES *COOK, K.D. AND REYNOLDS, J.D.	1201
STRUCTURE AND CHEMISTRY REVEALED BY THE MS AND MS/MS SPECTRA OF INORGANICS AND ORGANOMETALLICS *COSTELLO, C.E.	1204
MASS SPECTROMETRY OF METAL-LINKED DIMERS AND COVALENT HEME PRODUCTS *COTTER, R.J. AND CHEN, L.	1207

THURSDAY POSTER SESSIONS

FT/MS and Ion Traps

ADVANCED FEATURES OF A FAST BUFFER BOARD FT-ICR INTERFACE TO A PERSONAL COMPUTER *GUAN, S. AND JONES, P.R.	1210
ENHANCED MASS RESOLUTION OF BROADBAND FT-ICR MASS SPECTRA BY THE MAXIMUM ENTROPY METHOD (MEM) *GUAN, S. AND JONES, P.R.	1212
AN ADVANCED DATA SYSTEM FOR FT/ICR *MEEK, J.T., MILLEN, W.G., STOCKTON, G.W. AND WAYNE, R.S.	1214
A COMPARISON OF SEVERAL APODIZATION FUNCTIONS FOR SWIFT WAVEFORMS *HEARN, B.A. AND EYLER J.R.	1216
A NEW METHOD FOR GENERATING SWIFT WAVEFORMS FOR FTMS *GOODMAN, S.D.	1218
THEORY OF IMPULSE EXCITATION FOR FOURIER TRANSFORM MASS SPECTROMETRY *BAYKUT, G., MCIVER, R.T. AND HUNTER, R.L.	1220
INCORPORATION OF AVERAGED ION-ION INTERACTION INTO A MODEL FOR PEAK SHAPES IN FTMS *REMPER, D.L. AND GROSS, M.L.	1222
A MODEL FOR THE REDUCTION OF MASS MEASUREMENT ERRORS DUE TO ION-ION INTERACTION IN FTMS *YANG, S.S., REMPEL, D.L. AND GROSS, M.L.	1224
ELIMINATION OF Z-EJECTION IN FT/ICR BY RF ELECTRIC FIELD SHIMMING *WANG, M. AND MARSHALL, A.G.	1226
A SCREENED STATIC ELECTROMAGNETIC ION TRAP FOR FT/ICR: ENHANCED MASS RESOLUTION, MASS ACCURACY, MS/MS, REPRODUCIBILITY, AND UPPER MASS LIMIT *WANG, M. AND MARSHALL, A.G.	1228

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

SOLUTION OF THE EQUATIONS OF ION MOTION DURING THE ION CYCLOTRON RESONANCE EXCITATION EVENT GROSSHANS, P.B. AND *MARSHALL, A.G.	1230
A NEW METHOD FOR CALCULATING THE SIGNAL INDUCED ON ICR DETECTOR PLATES GROSSHANS, P.B., *MARSHALL, A.G. AND SHIELDS, P.	1232
PRECISION IN DETERMINATION OF RELATIVE ION ABUNDANCES FROM FT/ICR MASS SPECTRA: ABSORPTION PEAK AREA IS SUPERIOR TO PEAK HEIGHT OR COMPLEX AREA LIANG, Z. AND *MARSHALL, A.G.	1234
EFFECT OF SAMPLING RATE ON FT/ICR MASS SPECTRA: IS OVERSAMPLING OVERRATED? ALBER, G.M. AND *MARSHALL, A.G.	1236
ISOTOPE-RATIO MEASUREMENTS BY FT/ICR: ESTIMATING INITIAL AMPLITUDES AND DAMPING CONSTANTS FROM SEGMENTED TIME SERIES *MITCHELL, D.W. AND DELONG, S.E.	1238
ICR EXPERIMENTS ON SHORT-LIVED ISOTOPES AND CLUSTERS: NEW THEORETICAL AND EXPERIMENTAL RESULTS *SCHWEIKHARD, L., AUDI, G., BECKER, S.T., BLUNDSCHLING, M., BOLLEN, G., EGELHOF, P., JERTZ, R., KLUGE, H.-J., KERN, F. AND KUNZ, K.	1240
IN-SITU PYROLYSIS AND THERMAL DESORPTION IN AN ION TRAP MASS SPECTROMETER *YOUNG, S. AND COGGIOLA, M.J.	1242
ION INJECTION INTO AN FTMS *CAMPANA, J.E., SHABANOWITZ, J. AND SHOHET, J.L.	1244
EXTERNAL ION SOURCES ON ION CYCLOTRON RESONANCE (ICR) MASS SPECTROMETERS ION-OPTICAL CONSIDERATIONS *DAVIS, S.C., DERRICK, P.J. AND GREATHEAD, R.J.	1246
A SIMPLE DIFFERENTIALLY PUMPED EXTERNAL SECONDARY ION SOURCE FOR FTMS *IJAMES, C.F. AND WILKINS, C.L.	1248
NEGATIVE IONIZATION IN QUADRUPOLE ION TRAP MASS SPECTROMETRY: EFFECTS OF APPLIED VOLTAGES AND REACTION TIMES *BERBERICH, D.W. AND YOST, R.A.	1250
CALIBRATION AND ACCURATE MASS MEASUREMENT WITH GC-FTMS USING AN EXTERNAL ION SOURCE *GROSSMANN, P. AND KELLERHALS, H.P.	1252
MULTIPHOTON IONIZATION ON A FOURIER TRANSFORM MASS SPECTROMETER WITH AN EXTERNAL ION SOURCE *GROSSMANN, P., ALLEMAN, M., LOEPFE, M. AND KELLERHALS, H.P.	1254
USE OF HIGH VOLTAGE PULSES TO STUDY LASER DESORBED IONS IN A DUAL CELL FT/ICR *HONOVICH, J.P. AND MARKEY, S.P.	1256
LASER DESORPTION WITHIN THE ION TRAP CAVITY: OPTIMIZATION AND APPLICATION *HELLER, D.N., COTTER, R.J. AND UY, O.M.	1258
APPLICATIONS OF LASER DESORPTION IN AN ION TRAP MASS SPECTROMETER *GOERINGER, D.E., GLISH, G.L., ASANO, K.G. AND MCLUCKEY, S.A.	1260
AMMONIUM HYDROXIDE CHEMICAL IONIZATION MASS SPECTROMETRY *CODY, R.B.	1262
ION TRAP GC/MS/MS DETERMINATION OF EICOSANOIDS STRIFE, R.J. AND *SIMMS, J.R.	1264

ION TRAP QUANTITATIVE MS/MS ASSAYS USING RF/DC *STRIFE, R.J., SIMMS, J.R. AND LACEY, M.P.	1266
PHASE ANGLE SYNCHRONIZATION BY FREQUENCY SWEEP EXCITATION IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE *HANSON, C.D., CASTRO, M.E. AND RUSSELL, D.H.	1268
THE EFFECT OF MAGNETIC INHOMOGENEITIES ON ION PARTITIONING IN A TWO-SECTION FT-ICR CELL *KERLEY, E.L., HANSON, C.D., CASTRO, M.E. AND RUSSELL, D.H.	1270
MEASUREMENTS OF KINETIC ENERGIES OF ION MOLECULE REACTION PRODUCT IONS BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE *CASTRO, M.E., KERLEY, E.L., HANSON, C.D. AND RUSSELL, D.H.	1272
A FIELD CORRECTED ION CELL FOR INCREASED PERFORMANCE IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE *HANSON, C.D., CASTRO, M.E. AND RUSSELL, D.H.	1274
PULSED ION INJECTION WITH AN EXTERNAL SOURCE TIME-OF-FLIGHT FTMS *BEU, S.C., LAUDE, D.A. AND HOGAN, J.D.	1276
ANALYTICAL APPLICATIONS OF LASER DESORPTION AND PHOTODISSOCIATION FOURIER TRANSFORM MASS SPECTROMETRY *NUWAYSIR, L.M. AND WILKINS, C.L.	1278

Metabolism and Toxicology

CONFIRMATION OF NEWLY IDENTIFIED AMPICILLIN METABOLITES *SUWANRUMPHA, S. AND FREAS, R.B.	1280
NOVEL PATHWAYS FOR THE OXIDATIVE DEGRADATION OF ALIPHATIC CHAINS IN CANNABINOID METABOLISM *HARVEY, D.J.	1282
A METHOD FOR THE IDENTIFICATION OF TETRAHYDROCANNABINOL METABOLITES BY GC/MS USING CATALYTIC HYDROGENATION *HARVEY, D.J. AND BROWN, N.K.	1284
CANNABICHROMENE METABOLISM IN THE RABBIT: A NEW METHOD FOR METABOLITE IDENTIFICATION AND THE CHARACTERIZATION OF EIGHT NEW DIHYDROXY METABOLITES *HARVEY, D.J. AND BROWN, N.K.	1286
METABOLISM OF CANNABIDIOL BY THE DOG: IDENTIFICATION OF GLUCOSE CONJUGATES AS THE MAJOR URINARY METABOLITES *HARVEY, D.J. AND SAMARA, E.	1288
TANDEM (MS/MS) MASS SPECTROMETRY UTILIZING ELECTRON IMPACT IONIZATION AND MULTIPLE REACTION MONITORING FOR THE RAPID, SENSITIVE, AND SPECIFIC IDENTIFICATION AND QUANTITATION OF MORPHINE IN WHOLE BLOOD WITH 5 METER CAPILLARY COLUMN CHROMATOGRAPHY *PHILLIPS, W.H., WADE, N., OTA, K., KITAGAWA, R., WARDEN, D., ZEHNDER, S., APPEL, T. AND DAVIS, G.	1290
AN IMPROVED METHOD FOR EXTRACTING OPIATES FROM URINE SAMPLES PRIOR TO FULL SCAN GC/MS ANALYSIS *DE KANEL, J. AND HALL, T.	1292
TRIPLE QUADRUPOLE AND QUADRUPOLE ION TRAP TANDEM MASS SPECTROMETRY (MS/MS) FOR THE DETERMINATION OF MORPHINE ALKALOIDS *JOHNSON, J.V. AND YOST, R.A.	1294

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

IDENTIFICATION OF FOUR NEW METABOLITES OF COCAINE IN HUMAN URINE SPECIMENS *ZHANG, J.Y. AND FOLTZ, R.L.	1296
CHARACTERIZATION OF URINARY METABOLITES OF NAPAMEZOLE HCl IN RATS, DOGS, MONKEYS AND HUMANS *JOHNSON, J.A., SEGER, N.A., STACK, R.F. AND BENZIGER, D.P.	1298
IDENTIFICATION OF PHASE II METABOLITES OF ANTIPSYCHOTIC DRUGS USING HPLC COMBINED WITH FAB-MS *MCKAY, G., JACKSON, C.J., SHU, Y.-Z., HUBBARD, J.W., HAWES, E.M. AND MIDHA, K.K.	1300
UTILITY OF LC-MS AND TANDEM MS FOR THE ANALYSIS OF THE RAT URINARY METABOLITES OF PYRILAMINE KORFMACHER, W.A., FREEMAN, J.P., *BLOOM, J., GETEK, T.A. AND HOLDER, C.L.	1302
CHARACTERIZATION OF THE URINARY METABOLITES OF TIBENELAST FROM MALE HUMANS USING LIQUID SECONDARY ION (LSIMS), DIRECT PROBE CHEMICAL IONIZATION (DCI) AND THERMOSPRAY (TSP) MASS SPECTROMETRY PEYTON, A.L., ZIEGE, E.A., GOODWIN, R.M. AND *BREAU, A.P.	1304
APPLICATION OF THERMOSPRAY LC-MS FOR THE ANALYSIS OF THENYLDIAMINE AND TRIPELENNAMINE AND THEIR MICROBIAL METABOLITES *KORFMACHER, W.A., GETEK, T.A., HANSEN, E.B. AND CERNIGLIA, C.E.	1306
THE DETERMINATION OF SERUM URIC ACID BY ISOTOPE DILUTION LC/MS AND LC/MS/MS *WELCH, M.J., CHRISTENSEN, R.G. AND ELLERBE, P.	1308
QUANTITATIVE DETERMINATION OF LEUCINE TRANSAMINATION IN THE FETUS FOLLOWING TRACER INFUSION OF 13-C LEUCINE *BROWN, K.A., EVANS, M.A., SCHAEFER, M.S. AND LIECHTY, E.A.	1310
ISOTOPE DILUTION METHOD FOR QUANTITATIVE GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF HYDROXY AND KETO ACIDS IN URINE *OSEI-TWUM, E.Y. AND MAMER, O.A.	1312
DETERMINATION OF 13C-, 15N-, AND 2H- ENRICHMENT USING CAPILLARY GAS CHROMATOGRAPHY - REACTION INTERFACE / MASS SPECTROMETRY (GC-RIMS) CHACE, D.H. AND *ABRAMSON, F.P.	1314
QUANTIFICATION OF THIODIGLYCOL IN RAT URINE BY CAPILLARY COLUMN CHROMATOGRAPHY USING ELECTRON IONIZATION MASS SPECTROMETRY *JAKUBOWSKI, E.M., WOODARD, C.L. AND DOLZINE, T.W.	1316
APPLICATION OF FAST ATOM BOMBARDMENT AND THERMOSPRAY MASS SPECTROMETRY TO THE IDENTIFICATION OF TWO GLUCURONIDE METABOLITES OF METHAPYRILENE *LAY, J.O., GETEK, T.A., KELLY, D.W., SLIKKER, W. AND KORFMACHER, W.A.	1318
BIOACTIVATION OF 1,2-DIBROMO-3-CHLOROPROPANE TO FIVE GLUTATHIONE CONJUGATES IN VIVO: MECHANISM OF FORMATION AND DETECTION BY TANDEM MASS SPECTROMETRY *PEARSON, P.G., HOWALD, W.N. AND NELSON, S.D.	1320
A SCREENING STRATEGY FOR THE DETECTION OF DERIVATIZED GLUTATHIONE CONJUGATES BY TANDEM MASS SPECTROMETRY (MS/MS) *PEARSON, P.G., HOWALD, W.N., NELSON, S.D. AND BAILLIE, T.A.	1322
ANALYSIS OF BENZO(a)PYRENE METABOLITES BY CONTINUOUS-FLOW FAB/MS *TEFFERA, Y., BAIRD, W.M. AND SMITH, D.L.	1324
CHARACTERIZATION OF THE BILIARY METABOLITES OF THE HEPATOTOXIC MONOTERPENE PULEGONE BY TANDEM MASS SPECTROMETRY (MS/MS) *THOMASSEN, D., PEARSON, P.G. AND NELSON, S.D.	1326

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

RAPID SCREENING OF NEONATAL WHOLE BLOOD FOR VARIOUS METABOLIC DISORDERS BY FAB/MS/MS *KODO, N., NORWOOD, D.L. AND MILLINGTON, D.S.	1328
URINARY EXCRETION PATTERN OF DICARBOXYLIC ACID AND ACYL-CARNITINES IN THE PATIENTS TREATED WITH CALCIUM HOPANTENATE *MATSUMOTO, I., MATSUMOTO, M., KUHARA, T., SHINKA, T., INOUE, Y. AND OHKURA, T.	1330
CHARACTERIZATION OF ORGANIC NITROXIDE RADICALS BY MASS SPECTROMETRY *SWEETMAN, B.J., ANJANEYULU, P.S.R., BETH, A.H. AND STAROS, J.V.	1332
DETERMINATION OF INTACT STYRENE-7,8-OXIDE IN BLOOD USING AUTOMATED GAS CHROMATOGRAPHY-MASS SPECTROMETRY *LANGVARDT, P.W. AND NOLAN, R.J.	1334
MASS SPECTROMETRIC CHARACTERIZATION OF A PROPOSED PENULTIMATE METABOLITE OF LANOSTEROL DEMETHYLASE METABOLISM MCKAY, R.G., *LARSEN, B.S., FISCHER, R.T., KO, S.S., BROSZ, C., MAGOLDA, R.L. AND TRZASKOS, J.M.	1336
DETERMINATION OF RADIATION INDUCED CHOLESTEROL OXIDES BY ON-COLUMN CAPILLARY GC-MS *HARTMAN, T.G., ROSEN, R.T., MAERKER, G. AND LAKRITZ, L.	1338
CONTINUOUS FLOW L-SIMS, GC/MS AND BIOASSAY IN THE ISOLATION AND IDENTIFICATION OF PLANT TOXINS *DUNCAN, M.W., MARINI, A.M., WANG, T-C.L., SHIH, M-C. AND MARKEY, S.P.	1339
DETERMINATION OF ETHYL CARBAMATE (URETHANE) IN COMMERCIAL PROTEIN BASED CONDIMENT SAUCES (SOY SAUCES) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY *HARTMAN, T.G., ROSEN, R.T. AND LECH, J.	1341
AMMONIA CHEMICAL IONIZATION GCMS ANALYSIS OF POLYHYDROXY AMINES FROM PLANT SOURCES *APLIN, R.T., COFFEY, D.P.M., FLEET, G.W.J. AND FELLOWS, L.E.	1342
DETERMINATION OF MONO (2-ETHYL-HEXYL) PHTHALATE IN HUMAN SERUM ALBUMIN *ZAMECNIK, J., ETHIER, J-C., BLACK, D.B. AND NEVILLE, G.A.	1344
ANALYSIS AND MASS SPECTROMETRIC IDENTIFICATION OF FOOD DYES AND THEIR SUBSIDARY COLORED MATERIAL *SUZUKI, M., MASUDA, K., OKA, H. AND HARADA, K-I.	1346
MASS SPECTROMETRIC ANALYSIS OF IN VITRO METABOLITES OF FLUORINATED HERBICIDES FUJIWARA, H., SOLSTEN, R.T., *CHOTT, R.C. AND LOGUSCH, E.W.	1348
ANALYSES OF ACIDIC AND NEUTRAL CATECHOLAMINE METABOLITES IN BRAIN TISSUES, CSF, PLASMA AND URINE *CHANG, S.Y., REINHARD, J., COOPER, B. AND TAYLOR, L.C.E.	1350
QUANTITATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND THREE OF ITS METABOLITES IN RAT BRAIN BY ION TRAP MASS SPECTROMETRY *LIM, H.K. AND FOLTZ, R.L.	1352
METABOLISM OF KETOXIME ANALOGS OF BETA-ADRENERGIC BLOCKERS: GC/MS AND THERMOSPRAY LC/MS STUDIES *PROKAI, L. AND BODOR, N.	1354
FAST ATOM BOMBARDMENT OF WARFARIN AND WARFARIN METABOLITES *HILKER, D.R., ALDOUS, K.M., SPINK, D. AND KAMINSKY, L.	1356
SIMULTANEOUS PREPARATION OF ACIDIC BASIC AND NEUTRAL URINARY CONSTITUENTS FOR GC/MS *SHOEMAKER, J.D. AND ELLIOTT, W.H.	1358

SIMULTANEOUS DETERMINATION OF ACIDS AND AMINES BY GC-NCIMS *AVERY, M. AND JUNK, G.	1359
IDENTIFICATION OF MERCAPTURIC ACID METABOLITES OF ACRYLONITRILE BY TANDEM MASS SPECTROMETRY *TURNER, M.J., HELD, S.D. AND KEDDERIS, G.L.	1361

Ion Molecule Reactions II

VINYL ANION FORMATION AND REACTIVITY ANDERSON, K. AND *KASS, S.	1363
MULTIPLE, SITE-SPECIFIC REARRANGEMENTS IN THE ATMOSPHERIC PRESSURE IONIZATION MASS SPECTRA OF ALKYL PHOSPHONATE AND ALKYL PHOSPHATE ESTERS *SNYDER, A.P. AND HARDEN, C.S.	1365
A COMPARATIVE STUDY OF ISOELECTRONIC AND ISOGYRIC REACTIONS: MOLECULAR ORBITAL CALCULATIONS OF DIATOMIC HYDRIDES AND HALIDES *DEAKYNE, C.A., LIEBMAN, J.F., FRENKING, G. AND KOCH, W.	1367
LOW ENERGY CROSSED BEAM STUDIES OF THE CHARGE TRANSFER REACTION DYNAMICS OF $Ar+(2P_{3/2})$ WITH $O_2(X^3 \text{ } g^-)$ *BIGGERSTAFF, J., BIRKINSHAW, K., WANG, J., SHUKLA, A., HOWARD, S. AND FUTRELL, J.	1369
CROSS-SECTION FOR PROTON TRANSFER FROM LARGE BIOLOGICAL IONS TO SMALL MOLECULES IN THE GAS PHASE *DAVIS, S.C., DERRICK, P.J. AND GREATHEAD, R.J.	1371
FAB INDUCED CONDENSATION OF GLYCEROL WITH TETRAALKYL AMMONIUM SURFACTANTS *TUINMAN, A. AND COOK, K.	1373
FT-ICR DETERMINATION OF THE IONIZATION POTENTIALS OF CARBON CLUSTERS VIA CHARGE TRANSFER *BACH, S.B.H. AND EYLER, J.R.	1375
A CHARGE TRANSFER REACTION OF EXCITED CH_5^+ HERROLD, D., *LIN, H.Y. AND RIDGE, D.P.	1377
ON THE USE OF NEGATIVE ION CLUSTERING IN THE ANALYSIS OF SUBSTITUTED NITROBENZENES BY APIMS *ZOOK, D.R. AND GRIMSRUD, E.P.	1379
PROTON TRANSFER AND HYDROGEN HALIDE ELIMINATION REACTIONS OF HALOGENATED BENZENES FALCINI, M.R.A. AND *JENNINGS, K.R.	1381
DIFFERENTIATION OF OLEFINS AND NAPHTHENES USING ION-MOLECULE REACTIONS IN THE COLLISION CELL OF A HYBRID MASS SPECTROMETER *MISSLER, S.R. AND VASSILIADOU, M.	1383
GAS PHASE ION-MOLECULE REACTIONS OF CARBOCATIONS: RADIATIVE ASSOCIATION REACTIONS *PENTECOST, T.C. AND BABCOCK, L.M.	1385
NEUTRALIZATION-REIONIZATION STUDIES OF ISOMERIC IONS AND NEUTRALS *ZHANG, M.-Y., WESEMIOTIS, C., DRINKWATER, D.E., TURECEK, F. AND MCLAFFERTY, F.W.	1387
A PHOTOIONIZATION STUDY OF $C_4H_7^+$ IONS IN THE GAS PHASE *TRAEGER, J.C.	1389

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

DETERMINATION OF RADIATIVE ASSOCIATION RATE CONSTANTS BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY *FISHER, J.J. AND MCMAHON, T.B.	1391
DOUBLE IONIZATION ENERGIES OF FLUORETHANE MOLECULES MEASURED BY DOUBLE-CHARGE - TRANSFER-SPECTROSCOPY *GRIFFITHS, W.J. AND HARRIS, F.M.	1393
GAS-PHASE DEHYDROGENATION OF CYCLOALKANES TO AROMATIC HYDROCARBONS BY Os ⁺ , Os ²⁺ , Os ³⁺ TOLLENS, F.R. AND *MARSHALL, A.G.	1395
DIAMINE BASICITIES: A NEW MAXIMUM GAS-PHASE BASICITY TOLLENS, F.R., BALASUBRAMANIAN, V., FRAENKEL, G. AND *MARSHALL, A.G.	1397
THE MEASUREMENT OF ONSET POTENTIALS FOR EXOTHERMIC AS WELL AS ENDOTHERMIC PROTON TRANSFER REACTIONS WITH A TRIPLE QUADRUPOLE INSTRUMENT BURSEY, M.M., BLACKBURN, T.R., MEYERHOFFER, W.J. AND *MATTSON, W.A.	1399
HYDROGEN-DEUTERIUM EXCHANGE PROCESSES OCCURING DURING THE RETRO-DIELS-ALDER REACTION OF BETA-LACTAM ANTIBIOTICS IN A THERMOSPRAY MASS SPECTROMETER INTERFACE *SIEGEL, M.M., COLTHUP, N.B. AND KENION, G.	1400

Environmental

RAPID DETECTION AND CHARACTERIZATION OF FOSSIL-DERIVED FUELS IN WATER AND SOIL SAMPLES USING DIRECT PURGE ION TRAP MASS SPECTROMETRY WISE, M.B., *BUCHANAN, M.V. AND GUERIN, M.R.	1402
PARALLEL COLUMN CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE ANALYSIS OF COMPLEX MIXTURES *QUILLIAM, M.A.	1404
THE ROLE OF MASS SPECTROMETRY IN THE CLEAN-UP OF THE SYDNEY TAR PONDS *SIM, P.G., QUILLIAM, M.A., PERREAU, H. AND ELSON, C.M.	1406
CAPILLARY GC/MS CHARACTERIZATION OF CRESOTE CONTAMINATION WITH COMPARISON TO GC/FTIR AND GC/MIP RESULTS *BRILIS, G., TETTA, D., CUMMINGS, R., POTTER, B.B., BRUMLEY, W.C., FARNHAM, I. AND PYLE, S.	1408
ANALYSIS OF FIVE- AND SIX-RING NITRO-PAHs: A COMPARISON OF EI, API AND METHANE NICI MASS SPECTROMETRY *LOPEZ-MIRANDA, A.T., KORFMACHER, W.A., ROSARIO, O. AND FU, P.P.	1410
ENVIRONMENTALLY SIGNIFICANT HALOGENATED COMPOUNDS IN FLUFF *GRANGE, A.H., DONNELLY, J.R., NUNN, N.J. AND SOVOCOL, G.W.	1412
COMPARISON OF MS/MS AND SELECTED ION RECORDING (SIR) IN THE ANALYSIS OF TCDD AND TCDF TONDEUR, Y., *WEIR, O., WOOD, D. AND CLENCH, M.	1414
ANALYSIS OF MONOBROMINATED PCDD AND PCDF IN MUNICIPAL INCINERATOR FLYASH *TONG, H.Y., MONSON, S.J. AND GROSS, M.L.	1416
EI AND NEGATIVE ION CIMS OF POLYBROMINATED FURANS IN PLASTICS *BRUMLEY, W.C., GRANGE, A., NUNN, N., DONNELLY, J., BRILIS, G.M. AND SOVOCOL, W.	1418
GC/MS METHODS UTILIZED IN A SURVEY OF SELECTED XENOBIOTIC CONTAMINANTS IN FISH FROM SITES ACROSS THE UNITED STATES *BUTTERWORTH, B.C., KUEHL, D.W., MARQUIS, P.J., LARSEN, M.L., MOZOLL, A.E. AND HOLLAND, L.G.	1420

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

GC/MS ANALYSIS OF HALOGENATED ORGANIC COMPOUNDS IN WATER, FISH, AND SEDIMENT SAMPLES COLLECTED NEAR AN INDUSTRIAL OUTFALL IN THE CALCASIEU RIVER ESTUARY, LOUISIANA *LEIKER, T.J., PEREIRA, W.E. AND ROSTAD, C.E.	1421
GC/MS IDENTIFICATION OF TRICHLOROPHENYL ETHANOL IN IMPORTED PEPPERS: POTENTIAL MISIDENTIFICATION AS A TRICHLOROPHENYL METHYL METHOXY BENZENE *BARRY, T.L., PETZINGER, G., CAIRNS, T. AND SIEGMUND, E.	1423
DIRECT ON-LINE VAPOR MONITORING BY TRANSFER LINE GC/MS *ARNOLD, N.S., ROBERTS, K.A., MCCLENNEN, W.H. AND MEUZELAAR, H.L.C.	1425
INLET SYSTEM FOR AUTOMATED GC/MS ANALYSIS OF COMPOUNDS IN AMBIENT AIR AS COLLECTED IN STEEL CANISTERS *CARDIN, D.B., LIN, C.C., JACOBS, D.B. AND LEV-ON, M.	1427
REAL-TIME, IN-SITU POINT MONITORING BY ION TRAP MASS SPECTROMETRY HEMBERGER, P.H., *LEIBMAN, C.P., CANNON, T.M., WOLF, M.A. AND KAISER, R.E.	1429
APPLICATION OF MASS SPECTROMETRY TO INDUSTRIAL CENTRAL ATMOSPHERIC MONITORING *KOSLIN, M.E., ROTHERAM, M. AND HEPPNER, R.A.	1431
MASS SPECTROMETRIC ANALYSIS OF SOME CHLOROALKYL PHOSPHATE FLAME RETARDANTS *ROSTAD, C.E., PEREIRA, W.E. AND LEIKER, T.J.	1433
DETECTION OF TRACE ORGANICS IN AIR USING THERMAL DESORPTION ITMS WISE, M.B., *BUCHANAN, M.V. AND ILGNER, R.H.	1435
IDENTIFICATION OF O-(2-DIISOPROPYLAMINOETHYL) O'-ETHYL METHYLPHOSPHONITE DEGRADATION PRODUCTS BY CHEMICAL IONIZATION MASS SPECTROMETRY *ROHRBAUGH, D.K.	1437
ANALYSIS OF CHEMICAL WARFARE AGENTS IN A COMPLEX MATRIX BY CAPILLARY COLUMN GC-MS AND GC-MS/MS *D'AGOSTINO, P.A., PROVOST, L.R. AND ANACLETO, J.	1439
USE OF BENZENE CHARGE EXCHANGE AT ATMOSPHERIC PRESSURE FOR LOW LEVEL DETECTION OF POLLUTANTS IN AIR *KETKAR, S.N., DULAK, J.G., DHEANDHANOO, S. AND FITE, W.L.	1441
FORMATION OF ADDUCT IONS IN THE METHANE ENHANCED NEGATIVE ION MASS SPECTROMETRY OF ARYL-N-METHYLCARBAMATES *MATTINA, M.J.I. AND HUANG, L.Q.	1443
CONFIRMATION OF ETHYLENETHIOUREA (ETU) RESIDUES IN FOODS BY CAPILLARY GC/TANDEM MASS SPECTROMETRY *ROACH, J.A.G., ANDRZEJEWSKI, D. AND KRAUSE, R.T.	1445
MASS SPECTROMETRY OF TRIAZINES UNDER METHANE ENHANCED NEGATIVE CHEMICAL IONIZATION CONDITIONS *HUANG, L.Q. AND MATTINA, M.J.I.	1447
ESTIMATION OF MUTAGENIC/CARCINOGENIC POTENTIAL OF ENVIRONMENTAL CONTAMINANTS BY ION-MOLECULE REACTIONS AND TANDEM MASS SPECTROMETRY *FREEMAN, J.A., YOST, R.A., JOHNSON, J.V., HAIL, M.E. AND KUEHL, D.W.	1449
APPLICATION OF PURGE AND TRAP GC/MS FOR THE ANALYSIS OF OCTAMETHYLCYCLOTETRASILOXANE IN WATER AT SUB-PART PER BILLION LEVELS *TEWS, E.C., BUJANOWSKI, V.J. AND MOORE, J.A.	1451
DETERMINATION OF THE PHOTODEGRADATION PRODUCTS OF DISPERSE ORANGE 29 BY GC/MS AND HPLC/MS *HANEY, C.A., FREEMAN, H.S., MCINTOSH, S.A. AND VOYKSNER, R.D.	1453

DEVELOPMENT AND TESTING OF ICP/MS METHOD 6020 FOR ENVIRONMENTAL ANALYSIS DOBB, D.E., LAING, G.A., *DONNELLY, J.R., HURD, M.L. AND BUTLER, L.C.	1455
FACTORS CONTRIBUTING TO THE EFFICIENCY AND LINEARITY OF COLD-ON-COLUMN INJECTORS FOR USE IN THE GC/MS EPA METHOD 625 *GRINDSTAFF, Q.G., SLAGLE, R.E. AND SHEEN, J.T.	1457
RESPONSE FACTOR ALIGNMENT *SAUTER, A.D., DOWNS, J.J., ISAACSON, P., MCCARTY, H., BOTTRELL, D. AND FISK, J.	1459
ANALYSIS OF HAZARDOUS SUBSTANCES IN FISH TISSUE BY GC/MS *YANG, L., BEN-HUR, D., FULLERTON, M., OSTERMAN, T.T. AND O'NEAL, J.M.	1461
CHARGE EXCHANGE CI/ELECTRON ATTACHMENT NCI AS AN ALTERNATIVE IONIZATION MODE FOR CHARACTERIZATION OF ENVIRONMENTAL SAMPLES: APPLICATION TO MARINE MAMMALS *KEUHL, D.W., BUTTERWORTH, B.C., LUNDMARK, E. AND MARQUIS, P.	1463
DIRECT TRACE ANALYSIS OF VOLATILE ORGANICS IN AIR AND WATER BY MEMBRANE/MASS SPECTROMETRY *LAPACK, M.A., TOU, J.C. AND ENKE, C.G.	1465
DETERMINATION OF SELECTED PRINCIPAL ORGANIC HAZARDOUS CONSTITUENTS FROM INCINERATORS USING CAPILLARY COLUMN GC/MS/DS INFANTE, R. AND *TORRES, A.	1467
DETERMINATION OF ORGANICS IN PUBLICLY OWNED TREATMENT WORKS (POTWS) BY ON-COLUMN GC/MS: A COMPARISON OF THREE FACILITIES *CLARK, L.B., ROSEN, R.T., HARTMAN, T.G., LECH, J. AND ROSEN, J.D.	1469
ISOTOPIC COMPOSITION OF LEAD IN MAN AND THE ENVIRONMENT IN FINLAND *KEINONEN, M	1470

FRIDAY ORAL SESSIONS

Symposium: Separations and Peptides

COMPARISON OF LC/MS AND CZE/MS FOR THE ANALYSIS OF ENZYMATIC DIGESTS AND RELATED BIOLOGICAL SAMPLES *HENION, J. AND HUANG, E.	1472
COMBINING CF-FAB WITH SEPARATIONS TECHNIQUES *CAPRIOLI, R.M.	1474
CARBOHYDRATE STRUCTURAL DETAIL BY SELECTIVE OXIDATION, DERIVATIZATION AND SFC-MS *REINHOLD, V.N., CHAN, S.Y. AND JOHNSON, R.S.	1476

Environmental Applications

SAMPLING AND ORGANIC ANALYSIS OF ODOURIFEROUS EMISSIONS FROM AN OIL-SANDS PLANT THANAWALA, P., ROBERTS, J., KLYM, D., BRASSARD, B., MACHIN, J., MAXWELL, D. AND *HEADLEY, J.V.	1478
THE GAS CHROMATOGRAPHIC SEPARATION AND MASS SPECTROMETRY OF NEUTRAL AND PHENOLIC POLYMERIC CHLORINATED AROMATIC COMPOUNDS FOUND IN TECHNICAL PENTACHLOROPHENOL *GRIFFIN, D.A. AND DEINZER, M.L.	1480

CHARACTERIZATION OF POLYAROMATIC HYDROCARBONS BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY *DASS, C.	1482
RAPID ANALYSIS OF VOLATILE ORGANICS IN WATER AND SOIL USING DIRECT PURGE INTO AN ITMS WISE, M.B., *BUCHANAN, M.V. AND GUERIN, M.R.	1484
COMPARISON OF TWO MAGNETIC INSTRUMENTS IN OBTAINING NEGATIVE CHEMICAL IONIZATION MASS SPECTRA *ROSEN, R.T., HARTMAN, T.G., LECH, J. AND ROSEN, J.D.	1486
IDENTIFICATION OF A DERIVATIVE OF KEPONE IN SEDIMENT BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY *GREAVES, J. AND EDSTROM, R.	1488
POLYCHLORINATED DIBENZO-P-DIOXIN (PCDD) AND DIBENZOFURAN (PCDF) LEVELS IN HUMANS: VIETNAM VETERANS, OCCUPATIONAL (PRODUCTION) & ENVIRONMENTALLY EXPOSED (RESIDENTIAL & RECREATIONAL) INDIVIDUALS *PATTERSON, D.G., ALEXANDER, L.R., TURNER, W.E., NEEDHAM, L.L., PIRKLE, J.L. AND SAMPSON, E.J.	1489
ANALYSIS OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS BY HYBRID MASS SPECTROMETRY *CHARLES, M.J., GREEN, B.N., TONDEUR, Y. AND HASS, J.R.	1491
HEXACHLOROCYCLOPENTADIENE TRIMERS IN TECHNICAL CHLORDANE AND HUMAN TISSUE *DEARTH, M.A. AND HITES, R.A.	1493
EVALUATION OF A HOLLOW FIBER MEMBRANE FOR THE DETERMINATION OF VOLATILE ORGANICS IN WATER *BAUER, M.R., SLIVON, L.E., HO, J.S. AND BUDE, W.L.	1495
A COMPARISON OF PART PER TRILLION LEVEL ANALYSIS OF POLYNUCLEAR AROMATIC COMPOUNDS IN DRINKING WATER USING PREVIOUS GC/MS FULL-SCAN & NEW SELECTED ION MONITORING METHODS *SCHRYNEMECKERS, P.J. AND CLARK, M.S.	1497

Fundamental Ion Chemistry Clusters and Surfaces

VERY LOW ENERGY CHEMISTRY OF GAS PHASE IONS AND ELECTRONS *SMITH, M.A., HAWLEY, M. AND RANDENIYA, L.	1498
TIME-RESOLVED PHOTODISSOCIATION AND ENERGY RELAXATION PROCESSES IN STYRENE IONS *DUNBAR, R.C.	1500
GAS-PHASE CHEMISTRY OF CH ₃ SOH, -CH ₂ +SHOH, CH ₃ SO AND CH ₂ SOH BY NEUTRALIZATION-REIONIZATION MASS SPECTROMETRY *TURECEK, F., DRINKWATER, D.E. AND MCLAFFERTY, F.W.	1502
OBSERVATION OF DISSOCIATIVE AND RADIATIVE STATES OF N ₂ H BY NEUTRALIZED ION BEAM TECHNIQUES SELGREN, S.F., MCLOUGHLIN, P.W. AND *GELLENE, G.I.	1504
THE LASER MICROPROBE FTMS PELLETIER, M., KRIER, G., *MULLER, J.F., WEIL, D. AND CAMPANA, J.	1506
PROPERTIES OF CARBON CLUSTER IONS, C _n , FORMED BY DISSOCIATIVE IONIZATION *LIFSHTITZ, C., PERES, T. AND AGRANAT, I.	1508
STRUCTURAL EXAMINATION OF ALUMINUM CLUSTERS IONS, Al _n -FOR n= 3-50 *HETTICH, R.L.	1510

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

- THE FORMATION, DISSOCIATION, AND REACTION OF INDIUM CLUSTER IONS, IN X^+ 1512
($X = 2-9$)
*KING, F.L., ROSS, M.M. AND WYSOCKI, V.H.
- PROBING THE DEHYDROGENATION OF CYCLOHEXANE ON PT(111) WITH LITD/FTMS 1514
*LAND, D.P., PETTINETTE-HALL, C.L., HEMMINGER, J.C. AND MCIVER, R.T.

FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY: PRECEPTS AND PROSPECTS

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Perhaps more than for any other type of mass spectrometry, progress in FT/ICR¹ has been closely associated with an ever-deepening understanding of the fundamental motions of ions stimulated by an rf electric field in a static electromagnetic ion trap. In this abstract, I shall try to show how that understanding has led to an ever-improving series of excitation waveforms, detection modes, ion trap geometries, and a host of techniques for transformation and analysis of digitized data. When combined with the extensive arsenal of previously developed tools for making ions and manipulating neutrals, FT/ICR offers a family of hybrid methods spanning an extraordinary range of academic and practical applications. Experiments formerly requiring a series of collision chambers and mass analyzers can now be performed by a single mass analyzer with a sequence of events separated in *time* rather than in *space*.

The circular trajectory of an ion moving in a fixed magnetic field was well understood (in the design of magnetic sector mass spectrometers) long before Lawrence and Livingston exploited resonant excitation of that motion in their original "cyclotron".² For the next twenty years, cyclotrons were used principally for producing high-energy charged particles. In 1949, Sommer and Hipple introduced the "omegatron", the first of three basic methods for detecting ion cyclotron resonance frequency. In the omegatron,³ ions of a given m/z were coherently driven to larger ICR orbital radius by continuous application of a resonant radiofrequency electric field until the ions struck an electrode plate to produce a detectable current. Much later, Wobschall used a resonant circuit to detect the power absorbed by ions *during* continuous resonant excitation.⁴ More than 40 ICR instruments based on Llewelyn's commercial design⁵ were in use by 1974. Finally, Comisarow and Marshall⁶ separated the ICR excitation and detection events, by exciting ions to large orbits and then detecting the charge induced in detector electrodes *after* the rf excitation had been turned off. Discrete Fourier transformation of a digitized time-domain transient then yielded a broad-band spectrum of ICR frequencies (which could easily be converted to an m/z scale). More than 90 FT/ICR instruments based on that idea are now in use worldwide.

Subsequent technique development in FT/ICR mass spectrometry can be classified into several areas: (a) ionization/ion introduction methods (i.e., how to form and introduce ions into the trap); (b) ion trap improvements; (c) excitation methods; (d) detection modes; (e) data reduction methods; and (f) experimental event sequences for MS/MS and other studies of ion-molecule reaction pathways, kinetics, and equilibria. I shall now describe some of our recent contributions to many of those areas, with emphasis on the fundamentals of ion behavior in electric and magnetic fields.

Ionization techniques

Many previously developed ionization techniques (electron ionization, chemical ionization, fast atom bombardment, plasma desorption, laser desorption, photoionization, fast neutral beam, secondary ionization) have been adapted to ICR. Perhaps the most novel was "self chemical ionization"⁷ in which chemical ionization is

achieved at low pressure, simply by "incubating" the ions with their parent neutrals, with the result that protons are commonly transferred from ionized daughter fragments to neutral parent molecules to produce abundant $(M+H)^+$ ions without the addition of a chemical ionization reagent gas. In terms of technique development, our principal contributions in this area have been the interface of a fast neutral beam SF_6 source to an FT/ICR instrument,⁸ and the extension of laser desorption/ionization from the original CO_2 laser (1.06 μ) to a Nd/YAG laser at fundamental and harmonic frequencies (1064, 532, and 266 nm) for applications to analysis of drugs and polymers.⁹

Ion trap improvements

McIver's "trapped-ion cell"¹⁰ provided the first means for confining ions for extended periods (a few milliseconds to seconds) to provide for ion-molecule reactions and/or high-resolution detection. Comisarow's cubic trap¹¹ provided a good approximation to a quadrupolar electrostatic potential, and has been widely used. However, the cubic (or orthorhombic) trap configuration generates *curved* isopotential contours, with two undesirable effects. First, attempts to apply a static trapping potential along the magnetic field (z-) direction produce an outward-directed *radial* static field which shifts ICR frequencies and reduces the upper m/z limit. Even a trap with hyperbolically curved electrodes (which generate near-perfectly quadrupolar electric potential) reduces the ICR upper mass limit.¹² Second, an attempt to apply a transverse radiofrequency electric field excitation also generates a z-field which excites z-motion and can result in mass-dependent ion ejection (so that ICR relative peak areas no longer accurately represent the relative numbers of ions of those m/z-values). As reported elsewhere at this meeting, we have largely solved these problems by the use of grounded screens (to shield the trapped ions from the static trapping potential throughout most of the ion trap volume)¹³ and by rf "shimming" to reduce the z-component of the rf electric field during excitation.¹⁴ FT/ICR mass spectral peak frequencies and areas should now be essentially independent of ICR orbital radius, a major advantage for mass measurement and quantitation of relative m/z abundances.

Signal generation

The very first FT/ICR signals were excited with an rf pulse of approximately rectangular envelope.⁶ That waveform offers broad-band frequency-domain magnitude, but requires high time-domain amplitude and is non-selective over the excitation bandwidth. The d.c. or single-frequency rf-pulse idea has since been revived twice in slightly modified form as "burst"¹⁵ or "impulse"¹⁶ waveforms with essentially the same frequency-domain magnitude profiles. Frequency-sweep ("chirp") excitation,^{17,18} now employed in virtually all FT/ICR instruments, can produce power over a broad frequency range from a relatively low-amplitude time-domain waveform, but the excitation frequency-domain magnitude is neither flat nor sharply selective. In 1985, we introduced stored waveform inverse Fourier transform ("SWIFT") excitation as a means for producing optimally flat excitation power with optimally high selectivity and optimally low time-domain amplitude.¹⁹ As detailed in a recent review,²⁰ SWIFT excitation may be used for: improved quantitation of relative ion abundances; MS/MS with ultrahigh mass resolution in the first MS stage; the first truly simultaneous multiple-ion monitoring; multiple-ion ejection for enhancement of dynamic range; and all sorts of applications in which uniform selective excitation is required.

In 1984, we showed that a 180° phase shift at the midpoint of a time-domain single-frequency excitation waveform could effectively "de-excite" ions back to their starting points.²¹ That principle has since been applied by Gäumann *et al.* to develop a two-dimensional FT/ICR MS/MS technique,²² and by Russell *et al.* to produce mass-selective excitation by use of non-selective radiofrequency excitation.²³

Signal detection

For *infinitely extended* electrodes, the oscillating charge induced in the detector electrodes would be purely sinusoidal. However, for detector electrodes of *finite* dimensions, signals at odd multiples of the ICR orbital frequency are observed.²⁴ Recently, Wanczek (for a cylindrical trap)²⁵ and we (for a cubic trap)²⁶ have shown that the harmonic signal magnitudes increase with ICR orbital radius, but are typically quite small (<2%) under typical operating conditions.

All present FT/ICR instruments detect the ICR signal induced on a *single* pair of opposed electrodes. We have recently shown that a potential increase by a factor of $\sqrt{2}$ in signal-to-noise ratio results from *quadrature* detection from *two* orthogonal pairs of electrodes.²⁷ We have suggested several other applications for quadrature detection: e.g., halving the lower mass limit for a given maximum digitizer speed; and dual-range detection (say, simultaneous wide-range low-resolution, and narrow-range high-resolution).

Data reduction

FT spectroscopy differs fundamentally from other types of spectroscopy by its inherent need for *discrete* data.²⁸ As a result, the quality of an FT/ICR mass spectrum depends in detail upon how the "raw" time-domain *analog* signal is converted ("sampled") into digital data. For example, sampling at a rate less than twice per cycle of the highest frequency in the spectral bandwidth results in "foldover" ("aliasing"), so that the apparent ICR frequency (and thus the apparent m/z) can be shifted to an incorrect value. Recently, we showed how the interleaving of successive time-delayed acquisitions can be used to extend this "Nyquist" limit, thereby lowering the lower mass limit for FT/ICR.²⁹ Conversely, we have shown that intentional *undersampling* may be used to provide *simultaneous ultrahigh mass resolution* for ions of widely separated m/z -values.³⁰ In work reported elsewhere in this volume,³¹ we discuss the advantages of intentional *oversampling* (i.e., sampling more frequently than required by the Nyquist limit). Finally, the quality of an FT spectrum is determined by the *vertical* as well as the *horizontal* resolution in time-domain sampling; in the extreme limit of 1-bit vertical analog-to-digital conversion (ADC) resolution, we showed that such extremely "clipped" data can provide almost as much information as from more conventional 12-bit ADC's.³²

There is considerable current interest in *non-FT* methods for obtaining a (frequency-domain) spectrum from a discrete time-domain signal. We recently showed that the Hartley transform offers all of the advantages of fast FT data reduction without the use of mathematically complex algebra.³³ More recently, we have compared Bayesian and maximum-entropy analysis with FT data reduction, as discussed elsewhere in this volume.³⁴

The Fourier transform produces mathematically "real" and "imaginary" data sets. However, extraction of the desired absorption-mode spectrum requires accurate *phasing* of the complex spectrum. We provided the first method for broad-band phasing of FT/ICR data,³⁵ based on plots of imaginary vs. real FT spectral data.³⁶ We also pointed out that the negative-frequency spectral peaks (which themselves have no physical significance) can shift the (observed) positive-frequency peaks in an FT spectrum, thereby affecting mass calibration (in a calculable way).³⁷

Perhaps the most important issue in data reduction is the *precision* of the determined FT/ICR spectral parameters: peak frequency (from which m/z is computed); peak width (which offers a direct measure of reduced ion-molecule collision frequency); peak height (or preferably peak area, which offers a direct measure of

relative ion abundance). Under very general conditions, we have shown that frequency-domain precision is proportional to signal-to-noise ratio (as for analog data) and to the (square root of the) number of data points per peak width.^{38,39} Finally, we have used a three-point fit⁴⁰ to each magnitude-mode spectral peak to evaluate the effects of noise, zero-filling, time-domain signal truncation, and the round-off errors of the FFT algorithm itself on spectral precision.⁴¹

This retrospective survey has been devoted mainly to *technique development*. Analytical and chemical problem-solving applications based on those techniques may be found in abundance elsewhere in this volume, from a host of FT/ICR practitioners.

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Molecular Ion Spectroscopy: The Third Generation

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Within the obvious limitations deriving from any particular system of classification, it seems reasonable to characterize the evolution of high resolution (i.e. rotationally resolved) molecular ion spectroscopy into three generations; the basis of this classification is the degree of complexity of the ions studied, which, in turn, serves to characterize the technical sophistication of the methods used to study them.

The first generation principally involved the study of diatomic ions by optical emission spectroscopy of both electrical discharges in the laboratory and of comet tails. The "Diatomic Era" yielded detailed information on the electronic and geometrical structures of approximately 50 diatomic ions as well as similar information for a few polyatomic species. The chemical information derived from seven decades of investigation is compiled in the monograph by Huber and Herzberg (1) and is described in several reviews.

The second generation of molecular ion spectroscopy, the "Polyatomic Age", was initiated by the discovery of the molecular ions HCO^+ and HNN^+ in interstellar dust clouds by microwave astronomy and by the subsequent development of laboratory microwave spectroscopy of molecular ions in electrical discharges (2-7). The quest to understand the high abundance of molecular ions in the interstellar medium provided strong motivation for the development of laboratory spectroscopy efforts. Following the pioneering microwave work of Woods and coworkers, infrared spectra of a few simple ions (HD^+ , HeH^+ , H_3^+) were obtained with laser ion beam experiments employing indirect (mass spectrometric) detection of the effects of photon absorption. The rather severe dynamical constraints involved in this approach restricted the generality of these otherwise elegant methods. In 1980, Takeshi Oka demonstrated the first use of tunable infrared laser absorption spectroscopy for measuring vibration-rotation spectra of ions (H_3^+) generated in a glow discharge. While this straightforward approach was soon extended to NeH^+ , more general application was impeded by the overwhelming interference caused by the much more abundant neutral species present in chemically complicated discharges. A sensitive, ion-selective detection method was obviously required in order to genuinely advance infrared spectroscopy of ions.

Such a method (called "Velocity Modulation Laser Spectroscopy") was developed at Berkeley by Gudeman et al. (8) in 1983. By employing the Doppler shift resulting from ion drift motion in the positive column of a glow discharge, laser absorption spectra of ions could be separated from neutral absorption features. Moreover, the anticorrelated translational motions of positive and negative ions in a discharge make it possible to unambiguously determine the sign of the charge of the absorbing ion. As a result of the development of velocity modulation, as well as several other ion selective modulation methods, approximately 60 different molecular ions have been studied with infrared laser spectroscopy by some eight different spectroscopy groups. These include some of the most fundamental ions in chemistry (e.g. H_3O^+ , NH_4^+ , CH_3^+ , NH_2^+ , OH^- , NCO^-) (9-12).

The third generation of molecular ion spectroscopy might be called "The Cluster Era". The most sophisticated spectroscopy experiments that have been developed recently have the capability to measure rotationally resolved spectra of cluster ions, such as $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$, and complicated species like nonclassical carbonium ions. Rotationally resolved laser induced-fluorescence of ions produced by laser ionization in a supersonic jet has been demonstrated by Terry Miller (13). A new experiment developed by Yuan

Lee and coworkers (14) has produced rotationally resolved IR spectra of $\text{H}_2\text{O}^+(\text{H}_2\text{O})_n$ and $\text{NH}_4^+(\text{NH}_3)_n$. This approach features production of cold ($\sim 10\text{K}$) ions in a supersonic corona discharge, trapping mass-selected ions in an octopole ion trap, and using laser photofragmentation/double resonance methods to measure the IR absorptions with mass spectrometer detection. Recently, microwave spectroscopy of a hollow cathode discharge was employed by Bogey et al. (15) to study detailed spectra of the ArH_3^+ cluster ion. This excellent work may soon be extended to other ions.

Coe et al. (16) have developed (DIBLAS) a new method called Direct Ion Beam Laser Absorption Spectroscopy for measuring very high resolution spectra of mass identified ions. In this approach, ions are extracted from a suitable source into a fast (3kV) ion beam operating near the space charge limit of current ($\sim 10^9$ ions/cm²). Infrared transitions are measured in direct absorption. Kinematic compression of the nascent velocity distributions results in linewidths which are ~ 10 times narrower than the room temperature Doppler width. Each absorption feature is mass identified with a precision of ~ 0.1 amu by the observed Doppler shift. Because the absolute densities of all ions can be determined quite readily, absolute infrared absorption strengths can be determined. Initial results have been demonstrated for HF^+ , HCO^+ , HNN^+ , H_2O^+ , and NH_4^+ . With the use of supersonic coronas as ion sources, we expect this method to be successfully applied to the study of vibration-rotation-tunnelling spectra of cluster ions like H_3O^+ . In this work, the hydrogen tunnelling migration dynamics will be studied, in addition to determining the structures and other properties of such clusters.

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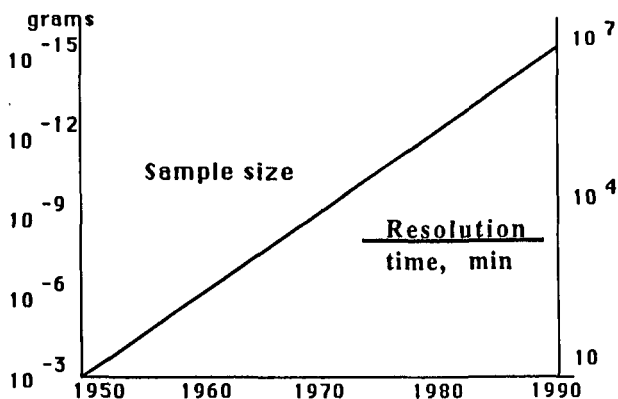
MASS SPECTROMETRY: HOW DID WE GET HERE
AND WHERE ARE WE GOING?

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Mass spectrometry has undergone an amazing growth over the last four decades. Possibly a perspective examination of this can provide guidance to make its future even more amazing. Certainly a hallmark of this progress has been the high creativity in, and unusual communication between, highly diverse scientific areas doing both basic and applied research in academic, government, and industrial laboratories. Breakthroughs on fundamentals of ion optics, kinetics, ionization processes, and mechanisms have depended on developments in reference data, instrumentation, and computer utilization, all of which have inspired unique applications across physics, geology, chemistry, biology, medicine, and environmental sciences. The basic chemistry found for small gaseous ions has led to mechanistic rationalizations for larger species, in turn sparking exciting developments in instrumentation to ionize increasingly large molecules, even those above 100,000 daltons. Coupling to chromatographic separation methods now accounts for the majority of analytical applications, while tandem mass spectrometry makes possible further new dimensions of information. As with GC/MS interfacing, MS/MS requires new methods to convert the mass-separated ion species into characteristic new masses for the next MS separation, while a multiplicity of such steps (MS^n) gives promise for extended characterization of the unusually large molecules that can now be ionized. Finally, automating the analytical process to take advantage of the unusually high information density of MS and its comprehensive collections of reference data has directly utilized the astonishing new capabilities of the computer revolution.

Why Is MS So Wonderful? Two basic reasons are proposed for the tremendous success and future promise of mass spectrometry: (1) the unusually high volume of data obtainable from unusually small samples, and (2) the success in converting these data into structural and analytical information, mainly through understanding of the chemistry involved and the application of modern computer technology. For (1), picogram sensitivities are now commonplace in molecular mass spectrometry, while modern carbon-14 dating of 100,000 year old samples in milligram quantities requires measurements at the attogram (10^{-18} g) level. The unusual data volume also results from the multiple dimensions of possible data. The high response per molecule and dynamic range of mass spectrometry represent its first dimension, while the number of individual masses for which such measurements can be made provide the second dimension. These latter "resolution increments" depend on both mass range and resolution. Miraculously, several research groups have now achieved ionization of molecules of larger than 100,000 molecular weight. The best resolution of a commercial instrument is provided by the Fourier-transform mass spectrometer, with values of 3×10^6 at m/z 100 and 3×10^5 at m/z 1,000 with heterodyne measurement over a limited mass range. Of course, the utility of this resolution is limited by the exact masses possible from combinations of the component isotopes of the molecular and fragment ions in the mass spectrum; for ordinary samples, this is probably around 10^6 , as our current database of 118,000 different compounds contains 35,734 different molecular formulae. The figure shows the dramatic improvement in instrumental capabilities to measure these first two dimensions over the last forty years.

ANALYTICAL MASS SPECTROMETRY



Multiple Dimensions Systems. One of the most exciting areas of mass spectrometry expansion has been into three and more dimensions of analytical data. The pioneering effort of this was gas chromatography/mass spectrometry, first made into a usable system by Roland S. Gohlke, one of the first persons I hired in the Spectroscopy Laboratory of the Dow Chemical Company. I first heard of gas chromatography at a 1954 Gordon Conference (chaired by my Cornell colleague, Don Cooke) from Drs. Wilson of ICI and DalNogare of DuPont. Gohlke soon had a prototype GC working in the laboratory, and went on to build some 200 for various parts of Dow before they were available commercially in the US. I first heard of the Bendix time-of-flight instrument from Bill Wiley at a Pittsburgh Conference, and in 1955 Gohlke and I went to Bendix in Detroit to see this amazing device that scanned 10,000 mass spectra per second; our pen and ink recorders required 30 minutes per spectrum at that time. Dow took delivery in 1956 of the third commercial instrument built by Bendix, and Gohlke had a successful GC/MS solving problems in less than a year; never mind that no one else seemed to be able to get useful data from supposed copies of his instrumentation for several years. Of course, the speed and sensitivity of MS were keys to the ultimate success of GC/MS, but other critical elements to its explosive growth in the 1970's were the unusually clever interface designs of Watson and Biemann and of Ryhage and the Stenhagens (for the latter jet separator, I believe the first all glass model was also invented by Gohlke when he went to Finnigan) and the computer acquisition and reduction of the resulting volumes of data.

The high performance liquid chromatograph burst upon the scene in this period. Here I was fortunate to become well acquainted with Jim Waters while running Dow's basic research lab in Framingham, MA, 1956-1964, as Waters had a small company making differential refractometers in the basement of the Framingham Police Station. John Brown of Dow/Texas invented gel permeation chromatography, for which the Waters RI detector was a key, and the Waters development of this technology led to their early preeminence in high-performance liquid chromatography. The first commercial LC/MS instrument employed a moving belt interface, while Mike Baldwin, Patrick Arpino, and Bob Dawkins at Cornell developed our "direct solution introduction" interface first reported in 1973. This was done with Waters as well as NIH support and first introduced commercially by Hewlett-Packard. Certainly the plethora of far better interfaces developed in the last few years, such as thermospray, flowing FAB,

particle beam (MAGIC), and electrospray should soon bring LC/MS to the importance level of GC/MS

Tandem mass spectrometry in many ways has the highest potential of all multidimensional methods, and of course can also be used in conjunction with separation methods like GC and LC. Here, however, the history is really one of the extremely slow recognition of the analytical possibilities of MS/MS observations made over many years, starting with Sir J.J. Thomson himself. In our laboratory the recognition of the

TANDEM MS; MSⁿ

J.J. Thomson (1912): "secondary rays" from collisions
in the fields; "Aston bands" (CAD).

Hipple & Condon (1945): metastable ions (MI)

MI: ion structures, T. Shannon (1966); mixture analysis,
Bryce; Wanless (1967).

CAD: Jennings; Haddon (1968); peptide mixtures (1973)

"MS/MS" (1977): Cooks, Bockhoff, Enke & Yost, Gross,
Boerboom, Derrick, Nibbering, etc.

possible utility for ion structure characterization by metastable ion spectra only started with Tom Shannon in 1966 and by collisionally activated dissociation by Bill Haddon in 1968, while the utility of MI spectra for mixture analysis was reported by Tom Bryce in 1967 and of both MI and CAD spectra for sequencing of peptide mixtures by Hans Kasper-wipf, Phil Irving, and Malcolm McCamish in 1973. The true recognition of the analytical scope of MS/MS came a few years later through the pioneering efforts of Graham Cooks, as well as Frank Bockhoff in our laboratory. Cooks and Mike Gross recognized early on that further dimensions of mass spectrometry (MSⁿ) should have similarly expanded analytical utility, and he and Chris Enke have pioneered both instrumental and conceptual methods of exploiting MSⁿ. Ben Freiser and others have similarly showed MSⁿ to be a powerful tool in studying ion-molecule reactions.

Instrumentation. Instrumental advances have spearheaded much of the growth of the field since Al Nier led the World War II development yielding literally hundreds of mass spectrometers at Oak Ridge alone for isotopic assay of samples, production control of the gaseous diffusion plant, plus the 72 3-story "calutrons" that produced the ²³⁵U for the first New Mexico atomic bomb.

The development of modern computer hardware has been a tremendous boon to mass spectrometry for data acquisition and reduction, as outlined in the table. Dr. Rengachari (Babu) Venkataraghavan led our research

DATA ACQUISITION & REDUCTION

Analog: 12 simultaneous equations	(1946)
Visicorder with 5 galvanometers	(1955)
Analog magnetic tape: Merritt, McMurry	(1965)
Photodensitometer: Biemann, Burlingame, Venkataraghavan	(1966)
Peptide sequencing: Senn, Biemann	(1966)
MS/MS: Shannon, Kiser, Coutant, Brunno, Bonto, Lacey & MacDonald	(1967)
On-line computer: Venkataraghavan, Burlingame, Klimowski, Biemann, Varian	(1968)

efforts on this from 1965 to 1977, with great contributions from Rick Klimowski, John Coutant, Paul Bente, Tim Wachs, John Michnowicz, Pete Rogerson, and others.

Focusing our attention on MS/MS instrumentation, it was just ten years ago that Peter Todd built our first tandem double focusing mass spectrometer for his Ph.D. thesis, with tremendous inputs from Mike Baldwin and Don McGilvery. For 8 kV ions, the magnets had upper mass limits of 12,500 and 5,000, but we soon found that CAD of even a 2,100 molecular weight peptide gave very poor fragmentation, that it was very difficult to obtain more than 1,000 resolution with MS-II because of the high energy spread of the collisionally produced ions, and that the required spectral scanning was wasteful of sample (luckily, the same instrument is ideal for our neutralization-reionization MS studies). However, the work of Comisarow, Marshall, Gross, Wilkins, Russell, McIver, Hunt, and others indicated that Fourier-transform is a highly promising alternative as an MSⁿ instrument. The Table summarizes key advantages of FTMS for MSⁿ studies of large molecules. For example, Evan Williams,

MSⁿ by Fourier -Transform MS

ICR + Comisarow/Marshall

Energy independent: resol. >200,000 at m/z 1300

Repetitive mass analysis: MSⁿ

Multichannel detection: e.g., m/z 90-16,000

Multichannel MS/MS: + Hadamard transform

Remeasurement of large ions: >97% efficiency

Ion photodissociation, SID, e⁻ + Mⁿ⁺

Bing Wang, and Kent Henry in our laboratory have found that large ions can be remeasured with nearly 100% efficiencies in the "quench off" mode, as the short measurement transients apparently result from loss of translational energy with minimal scattering, causing the ions to wind back to the center of the cell where they can be re-excited and remeasured. Further, Evan Williams and Stanton Loh here have developed a Hadamard-transform technique in which the secondary mass spectra of many precursor ions can be collected simultaneously, greatly improving the signal/noise ratio. Selecting half the precursor ions with Marshall's SWIFT technique and measuring their collective MS-II spectrum, from which is subtracted the MS-II spectrum of the saved remaining half of these ions, is then done for all possible combinations of precursors. The individual MS-II spectra are then calculated from the appropriate simultaneous equations, with the resulting signal-to-noise equivalent to that for one spectrum measured individually with the same amount of sample used for all MS-II spectra. Also critical for MSⁿ has been the development of methods for ion dissociation. Excimer laser photo-

MS/MS PRIMARY ION DISSOCIATION METHODS

Collisionally Activated Dissociation. Ineffective for m/z >2500

Electron (EIEIO, Freiser & Cody, + ions), Ion bombardment (- ions)

Surface-induced dissociation (Cooks, upper mass limit?)

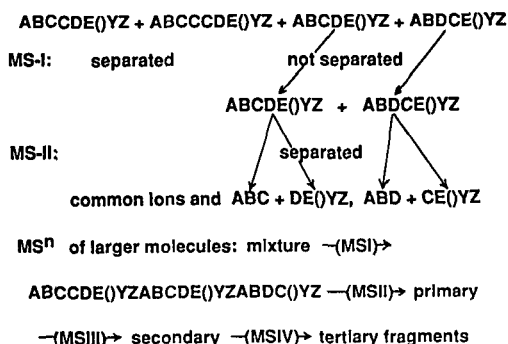
193 nm laser photodissociation (McIver, Hunt) non-ergodic?

Neutralization of multiply protonated ions to form unstable hypervalent ions yielding one or two fragment ions

dissociation appears especially promising, as this may cause dissociation in times short compared to that for energy randomization through the large molecule. Cooks' method of surface induced dissociation is highly promising for FTMS in preliminary experiments at Cornell and in collaboration with Hunt and Shabanowitz for singly charged ions up to mass 3,000. For multiply-charged larger ions from electrospray, both Smith and Loo, and Henion, have shown that CAD is promising, as also indicated by our experiments for SID.

The recent success of Evan Williams and Kent Henry, in collaboration with Jeff Shabanowitz and Don Hunt, in obtaining electrospray spectra of peptides of molecular weights up to 17,000 is very promising for MS^n sequencing of even larger peptides. Smith and Loo have obtained electrospray spectra of a 130,000 molecular weight peptide, but with more than a hundred charges; for the resulting m/z 800 - 1400 ions the FTMS instrument should have a resolving power of $>130,000$. Quite frankly, I am

MS^n MIXTURE SEQUENCING soft ionization



as excited about the potential of this approach to MS^n of large molecules as I have been of any new breakthrough in mass spectrometry since 1950.

MS Data $\rightarrow$ Structural and Analytical Information. Volumes and volumes of data are useless if they cannot be converted into desired information. As a chemist, one of the most rewarding aspects of mass spectrometry has always been that the most critical factor for this conversion to information is a basic understanding of the chemistry involved. Key to this is the unimolecular dissociation of singly charged cations, with exploitation of the bimolecular chemistry of cations and the chemistry of anions, multiply charged ions, and neutrals of high importance for the future. A second fortunate difference with conventional chemistry is that most of the basic data concerning unimolecular cation reactions is numerical, not bibliographic; thus, modern computer science is a powerful ally in the development of this chemistry. With the untimely deaths of my former collaborators Einar Stenhagen and Sixten Abrahamsson, the great majority of our current database of 140,000 different mass spectra of 118,000 different compounds has been collected at Cornell. Gerard Salton, Cornell's Professor of Computer Science, has been a key figure in developing statistical methods for document retrieval from libraries. We have tried to use all of his principles in our Probability Based Matching algorithm, which has undergone many improvements in the last fifteen years.

PROBABILITY BASED MATCHING

Reverse Search

Occurrence Statistics: Mass, Abundance	(1974)
Flagged peaks, M ⁺ , etc.; Reliability Ranking	(1977)
Tilting, quadratic scaling	(1980)
Spectrum subtraction (1979), forward search	(1985)
Weighted file order: 2 s search (PC, 9 s)	(1981)
Exact mass unknown, unit resol. reference	(1989)
Compensate other Markovian statistics	(1990?)

Salton has shown that reliable evaluations of retrieval performance must be two dimensional; such "recall/reliability" plots show that even early versions of PBM were superior to any other available retrieval algorithm. However, if the unknown compound is not in the reference file, of course it is hoped that PBM will show no promising retrievals; this requires an interpretive scheme, for which we have developed the Self-Training Interpretive and Retrieval System. This is cited as one of the first Artificial Intelligence systems of broad applicability in a scientific field.

ARTIFICIAL INTELLIGENCE (1973)

Self-Training INTERPRETIVE & Retrieval System

Supervised learning: 26 data classes	
Parametric: large data base (fast learner)	
Molecular Wt.: 92% first, 95% 1 st or 2 nd	(1980)
587 Substructures, reliability value	(1981)
Maximal Common Substructure	(1977)
(slow; fast processor, 1990?)	

Understanding Ion Chemistry. In its early years mass spectrometry was plagued with a terrible reputation concerning its capabilities for molecular structure elucidation. The quasiequilibrium theory developed by Eyring and his students demonstrated that mass spectra followed basic principles of chemical kinetics, but the conversion of organic chemists probably only began by showing that unimolecular ion reactions were

CATION UNIMOLECULAR CHEMISTRY

Aston: CH ₃ Br, SiF ₄ , BF ₃ , SO ₂ , AsH ₃	(1922)
Cornell, Δ -C ₄ H ₁₀ , J. Am. Chem. Soc.	(1933)
Eyring, et al.: Quasiequilibrium Theory	(1952)
Correlations; Rearrangements	(1956)
Meyerson, tropylium: product ion stability	(1957)
Friedel, Sharkey; <u>Baynon</u> ; <u>Biemann</u> ; Stenhagens, Ryhage;	
Reed; Shannon; <u>Budzikiewicz</u> ; <u>Djerassi</u> ; <u>Williams</u> ; etcl	
Radical vs. Charge site reactions	(1966)
RRKM; non-ergodic; stepwise vs. concerted	

separate papers by Wesdemiotis, Turecek, Zhang, Drinkwater, Leyh, and Fura. A special bonus of these NRMS studies has been in the formation and characterization of unstable, exotic neutral species such as antiaromatic molecules, radicals, carbenes, enols, zwitterions, ylides, and hypervalent species. To prepare such neutrals it is only necessary that the corresponding cation or anion is stable. An especially promising technique is the MS/MS/MS characterization of the relative free energies of their dissociations by CAD of the neutrals, followed by reionization and isomeric characterization of the ionic products by further CAD.

Future. Thus, it would appear that in a generation or so of mass spectrometry we have gone from the reputation of "no real chemistry there" to a vital source of uniquely new chemistry. With the explosive expansion of mass spectrometry to the study of really large molecules we can look forward to further understanding of their chemistry, hopefully again to make a further contribution of real value to other areas such as biology and medicine.

One Man's Journey in Mass Spectrometry

by J.H.Beynon, Chemistry Building, University College of Swansea, Swansea SA2 8PP, U.K.

My career did not begin in mass spectrometry. When I graduated, in 1943 in the middle of the Second World War, I was immediately drafted into an Army post in which I was concerned with the design of gyroscopic stabilizers for tank guns. When I was released from war service in 1947, I was fortunate to obtain a position in the Research Department of ICI Ltd in Manchester, U.K. This was the largest chemical research unit in the British Commonwealth at that time. The Department housed 750 qualified chemists. Part of it is shown in Figure 1. It formed part of the Dyestuffs Division of the Company and made and sold over 6000 different products - dyestuffs and pigments, polymers, pharmaceuticals and various speciality chemicals such as wetting and waterproofing agents, chemicals for rubber and other auxiliary chemicals.

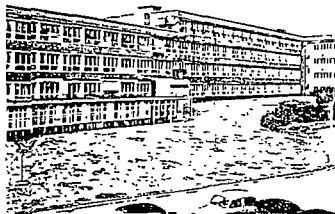


Fig. 1

effects they produced and not because of their chemical formulae. A dyestuff, for example, is sold on the basis of its colour, its fastness to light, its ability to bind to fabrics, etc. and it is these properties that have to be maintained constant in the commercial product sold over the years. Because impurities, sometimes even trace impurities, present in the products sold affected their properties (for example cause weakening of a polymer fibre) the analytical chemists were always looking for new, more sensitive and specific methods of analysis, infra-red absorption spectroscopy being the most versatile and up-to-date method at the time. But the wide analytical potential of mass spectrometry was not foreseen although it was as the result of one particular analytical problem that it was introduced in ICI. The Pharmaceutical group needed a method of tracking drug metabolites through the body and had decided that although radio-active labelling of drugs could be very useful analytically, it was too dangerous ever to contemplate using such compounds on human beings. Labelling with stable isotopes, on the other hand, using a mass spectrometer to make quantitative estimates of the amounts of various compounds present, could overcome the difficulty. This decision had been taken before I joined the Company and you can imagine my surprise when I arrived for work on my first day, to find on my desk a paper that read "Remit: Build a mass spectrometer."

I knew little or nothing about mass spectrometry and spent the next several weeks in the Library learning all that I could about the subject. In those days there was no computer-aided searching of the Literature so that references had to be found by laborious and painstaking search. And when a good paper was found, since photo-copying was not yet available, copious notes had to be made by hand. Although this process was slow, it meant that one read the papers thoroughly, and that by the time one's notes and sketches were finished, one understood it pretty well.

A design of mass spectrometer that appealed greatly to me had recently been published in the Journal of Scientific Instruments by Graham, Harkness and Thode and I decided to copy this design. Another Division of ICI had also decided to build a mass spectrometer and we decided to combine our resources and build two identical instruments based on the published design. We then discovered that Graham was temporarily in Britain and we engaged him as a consultant with whom we were able to have two or three meetings to obtain further details on the design. At this time Britain was still trying to recover from the effects of the Second World War. Many of the basic materials and components that we needed were in very short supply and no dollars were available to buy from the United States. A commercial mass spectrometer was already available in the United States, the famous 180° sector instrument made by CEC but there was no chance of obtaining the money to buy one of these. In Britain, Metropolitan Vickers had decided to build and sell a Nier-type instrument, but the fact that this was not expected to be ready for, perhaps, two years was a major factor in our decision to build our own machine. Another important consideration was that we felt we would be more able to maintain and develop the equipment if we succeeded in

building it ourselves.

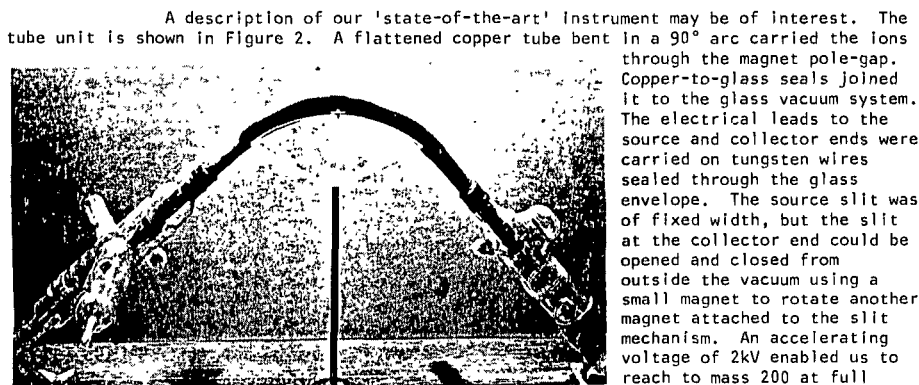


Fig. 2

A description of our 'state-of-the-art' instrument may be of interest. The tube unit is shown in Figure 2. A flattened copper tube bent in a 90° arc carried the ions through the magnet pole-gap. Copper-to-glass seals joined it to the glass vacuum system. The electrical leads to the source and collector ends were carried on tungsten wires sealed through the glass envelope. The source slit was of fixed width, but the slit at the collector end could be opened and closed from outside the vacuum using a small magnet to rotate another magnet attached to the slit mechanism. An accelerating voltage of 2kV enabled us to reach to mass 200 at full magnetic field and the mass resolution (10% valley) was just over 200. The high voltage was developed via a mains transformer and mercury-vapour rectifiers. It supplied 0.25A of current which would almost certainly have killed anyone who touched it. It had one tremendous advantage, however. Throughout the 20 years lifetime of the instrument, it was (very fortunately, because of the difficulty of doing so) never necessary to remove the source for cleaning. Any conducting material that built up on the quartz insulators and that caused electrical breakdown, was quickly burned away by the very large current available. Every few years, we did insert a miniature scraper into the source through the hole through which the filament was mounted, loosen some of the carbon deposit and suck it out using a little vacuum pipe. It was quite a difficult job to change a filament. This involved cutting the glass envelope removing the entire re-entrant tube on which the filament was mounted, re-welding a new tungsten ribbon filament in position then re-inserting the filament holder and glass-blowing it, while holding the filament exactly in position, so that it formed a vacuum-tight seal. As a result of the carbon coating on the inside of the source and the use of an all-glass sample-handling system, there was less degradation of sample than in metal systems at the same temperature.

The collector was a Faraday cup and this was connected to an 'acorn' vacuum tube with a 10^{11} ohm resistor in a feed-back circuit. Currents down to 10^{-15} A could be measured with a time constant of about 1s. Thus it required at least 10s to scan over a single peak. When the instrument was first built, pen recorders had not become available in Britain (although the famous Speedomax recorder was available in the USA) and so our output current was fed to a galvanometer having a lamp-and-scale unit with a scale of 2m radius. On this, currents could be measured very accurately and it proved of great value in later experiments when small isotopic enrichments had to be measured. Later, we acquired a very fast potentiometric pen recorder with a 10 inch scale. The pen carriage floated between two clutches that were rotating very rapidly in opposite directions. Any out-of-balance signal was amplified and used to pull the pen carriage magnetically to one or other of the clutches which moved the pen in a fraction of a second to its new position on the chart. Problems arose, however, if the gain of the amplifier was a little too high, because this caused the system to 'hunt' and the rapidly vibrating pen became a hazard to all in the vicinity because it sprayed out ink in all directions.

The vacuum system was based on a home made, single stage, glass diffusion pump using mercury as the fluid. It had to be used in conjunction with a liquid-nitrogen cold trap and was capable of reaching a vacuum of 10^{-6} Torr after baking. The glass sample system used greaseless glass stopcocks lubricated with graphite from a 2B pencil and sealed with mercury. A diagram of one of these taps is shown in Figure 3. Another sample system based on a 'break-off' system enabled samples immersed in a low-temperature bath to be connected directly to the ion source without an intervening leak. The sample pressure

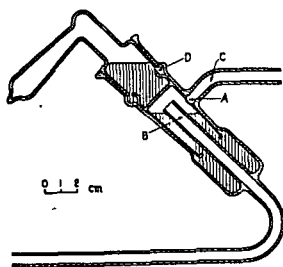


Fig. 3

was raised to the appropriate value for plotting a spectrum by carefully raising the bath temperature.

Mostly, spectra were plotted by hand. One operator would slowly rotate the knob of the 25 turn helical potentiometer that controlled the magnetic field and as each peak arrived, he would adjust the shunt that controlled the amplifier sensitivity to keep the peak on scale. He would call out the peak reading, continue scanning and then call out the zero reading following the peak. A second operator would record the readings and, using a mechanical hand calculator, normalise each peak to the base peak of the spectrum and plot the spectrum on graph paper. A complete spectrum up to, say, mass 200 would be ready in just over half an hour. The CEC instruments used in USA scanned the spectra by varying the accelerating voltage and this meant that sensitivity was reduced at the higher masses, making comparisons of standard spectra difficult on the two types of instrument.

As well as the difference in the method of scanning, there was one other major difference between the instruments used in Britain and USA. Many of those in USA had been built for one major application, the quantitative analysis of a mixture of hydrocarbons. These instruments were fitted with a computer which had a very poor performance indeed compared to modern computers, but which could nevertheless invert a large matrix much more rapidly than this could be done by hand. It is a sobering thought to all of us who earn our livings by mass spectrometry that if gas chromatography had been invented at that time, it could have analysed the mixture of hydrocarbons much more quickly and cheaply and the main commercial market for mass spectrometers would not have existed so that it is almost certain that they would not have been developed commercially and would have remained exclusively in Physics laboratories. From my own personal viewpoint the computers were responsible for one dreadful development; they needed a flat baseline to enable them to calculate abundances from the peak heights and so a 'metastable suppressor' was fitted so that no beautiful metastable peaks were seen. It is a very dangerous philosophy to try to eliminate features of a spectrum in this way because every feature adds to the sum total of information contained in the spectrum and aids in problem solving. My own motto has always been: If it wiggles, study it closely.

Accurate mass measurements could be made on these early instruments, though not many people realised the usefulness of such measurements at the time. Using an internal reference compound, measurements accurate to 50 ppm could readily be made even though the mass resolution was only of the order of 200. It is worth pointing out that this accuracy was not as high as Aston had achieved with comparable resolution some 25 years earlier, but we tended to be satisfied with it. Used in conjunction with isotopic abundance measurements it enabled the empirical formulae of many compounds sent in for analysis to be determined. Despite my feeling, held over many years that for some applications there would be great benefits to be gained by improving our accuracy of mass measurement to something closer to the 0.01 ppm that the physicists achieve, all is sacrificed at the altar of speed of production of spectra. Speed is necessary in many research problems but there are many problems in which the gain in sensitivity and precision achieved by plotting a spectrum more slowly are of vital importance. After all, a few minutes is a very short time indeed compared to say the time it takes to achieve a PhD degree.

It was clear to us that there were major advantages to be gained, in the types of problem in which we were mainly involved, if the mass resolution could be raised so as to separate the components of multiplet peaks. I achieved my first separation of a doublet in 1953 for which the value of $m/\Delta m$ was 1,000. The doublet was OH^+ and NH_3^+ and it is shown, plotted on the galvanometer lamp-and-scale unit in Figure 4. We also tried another method of obtaining good resolution which, although it worked well, has never been followed up. It is based on the fact that, in theory, a complete slit is not necessary to obtain good resolution, but only one edge of the slit. If the peak is oscillated across this slit edge, all the information contained in the ion beam can be extracted.

To do this and thereby obtain the first or second derivative of the peak shape, it is only necessary to apply a very small voltage $\sin \omega t$ to the accelerating voltage and to use a detector amplifier tuned to the frequencies ω or 2ω respectively. The

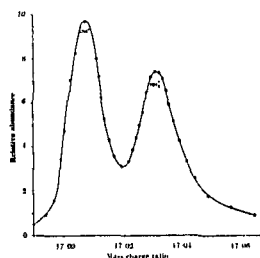


Fig. 4

were obtained in 1957; with modern electronics and phase-sensitive detection, I believe that a powerful method of overcoming the problems of opening and closing very narrow slits is available to us.

In order to increase our resolution still further, it was decided to approach Metropolitan Vickers (whose name later changed to GEC to AEI and then to Kratos) to ask if they would build a double focusing machine of Nier-Johnson geometry for it to be used to resolve the many doublet peaks being encountered in our organic chemical work. They agreed to aim for a resolution of 2,500 but would not guarantee this figure over a period of time because they expected that as soon as it became contaminated with organic material from the samples being examined, the resolution would fall. The machine, built with a 6 inch radius in the magnetic field was christened the MS8. It was delivered in 1955 and gave an immediate and sustainable resolution, after the slits had been narrowed of 13,500. It was immediately decided by the manufacturer not to build any more MS8's but to double the size and call the new instrument the MS9. The MS9 proved to be extremely successful and, in improved form, it is still in use in many laboratories worldwide. CEC had also decided to build a high resolution instrument, but of Mattauch-Herzog geometry. This was completed at almost exactly the same time as the MS8, but since this is meant to be my personal story, I will say no more about it other than that it, too, was an extremely successful and widely-used instrument.

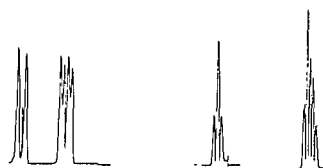


Fig. 5

(a)

(b)

Following this period, I was promoted within ICI, ultimately becoming the Research Manager controlling Physical Chemistry. No longer did I spend any time in the laboratories, though I did keep one assistant to indulge me in some mass spectrometry, which was now just a hobby. After 10 years of this, when I believed that my days in the front line of mass spectrometry were well and truly over, I received a tremendous surprise and a challenge. In 1968, I was approached by Purdue University and offered a Chair in their Chemistry Department to direct their mass spectrometry. ICI agreed to second me for a year and, filled with trepidation, for I had not done any practical work for a decade, I went to Purdue. In the event, I not only stayed for the year but commuted between Purdue and ICI for a further 6 years, becoming more and more of a Hoosier in the process. Throughout my time in USA I worked on yet another prototype instrument, the RMH-2 made by Hitachi. This was a very large machine, but in the event it never quite lived up to expectations as far as resolution was concerned. We modified it by putting a detector immediately after the electric sector and found that we had a new spectrum of metastable and collision-induced peaks that we called an IKE spectrum. This turned out to be a fortunate choice of name because we found that there were millions of buttons available all saying that they liked IKE. It was not long before we realised that if mass separation could precede production of the spectrum obtained by scanning the electric sector we would be able to produce complete 'fragmentation maps' which would be invaluable in the structural analysis of unknown compounds. In no time at all, we were building a reversed-geometry instrument using parts that had been donated to us by Kratos. We needed a name for the new spectra and decided to

call them MIKE spectra. Mass spectroscopists have always had a desire to invent special nomenclature and abbreviations for new techniques. So I was not at all surprised that, following the setting up of mass spectrometry nomenclature committees by IUPAC and by ASMS, both of which I chaired and both of which agreed that abbreviations should never be used unless they were defined first and both of which said that, in particular, the abbreviation MS should never be used (because it can mean so many things, such as mass spectrometer, mass spectrometry, metastable, manuscript, etc, etc) that in common usage today the MIKE technique that I have just described is lumped with others under the name MS/MS.

When I finally decided to leave the United States and return to Britain, it was to take up a Royal Society research Chair at Swansea. There, I took delivery of yet another prototype machine that we had persuaded VG to build for us, the reversed geometry ZAB-2F. This was my main instrument for the last decade of my working life and, as new attachments were invented, it soon bristled with extra sources, slits, sectors, collision cells, laser ports and so on. I had every bit as much fun on this machine (and every bit as much frustration) as on all the others. This period is only in the recent past, so that it is not appropriate to reminisce about it. Suffice it to say that I was also lucky enough to work again on a variety of topics, including building a hybrid instrument and a machine for high energy resolution, and to continue work on metastable ions on charge permutation and on the production of new ionic species. But I have already seen how the apparently quite *inexhaustible developments in mass spectrometry have quickly made much of this work obsolete.*

Throughout, I have talked about things that I have done, but I have always been part of a team and any ideas I may want to claim as my own will inevitably have been generated after constant discussions with my colleagues who will, consciously or unconsciously have helped to build up the idea in my mind. The teams to which I have belonged have included many clever and imaginative people to all of whom I owe a great deal. They are so many that I cannot list them all and I feel that it would be invidious to pick out just a few for special mention. I can only hope that while I learnt so much from them, they learnt something from me.

Looking back, it sometimes seems that whenever my research team came up with an idea, the more novel that idea was, the harder it was to get research support for it and the less likely it was that referees would recommend any ensuing paper for publication. It seems that the majority of scientists are very conservative and take a long time to accept new techniques and methods as being necessary or offering any advantages. This has been true in my own experience with such things as accurate mass measurement, high resolution and the study of metastable and collision-induced peaks. It seems hard to believe now, that when we combined gas chromatography's power as a separation technique with the mass spectrometer's power as an analytical device, in 1958 (in a very crude and unsophisticated way, I hasten to admit) we could get no support for developing the idea and a major manufacturer that we approached told us that the idea was crazy and had no commercial potential. Similarly, when we fitted an array detector to a mass spectrometer in 1975, we were told that this was an unnecessary refinement.

Fortunately, there exists a strong nucleus of innovative and adventurous scientists and they are often to be found in meetings such as this one. I can still remember my excitement when I attended my first meeting organized by ASTM Committee E-14 (the predecessor of ASMS) in San Francisco in 1955. From memory there were less than 100 people there and only about 50 papers were presented. It was possible to go to all the papers (there was no need for parallel sessions) and it was possible to get to know all the other delegates personally and to discuss their work with them. I was able to listen to talks outside my own area of knowledge and to broaden my outlook, but more importantly, it was the start of many research collaborations and of many personal friendships that have continued over the years. I also remember the hospitality suites which were in their heyday and how they helped in getting to know people. Many of you may not realise that if a suite was the focus of attention it was likely to stay open all night and to offer breakfast in the early hours of the morning.

I have never received the same stimulus from attending subsequent meetings as I got from attending my first. Indeed, I would suggest that support for young people to attend these meetings is of paramount importance even at the expense of rationing the visits by older and more experienced scientists. And to the younger people I would like to offer a few words of advice: Take the opportunity at this meeting of going to talks outside your own area and when you come across something that you don't understand or that you think is wrong,

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don't be afraid to ask questions. But at the same time, remember that just as you expect your own work to be appreciated, you should always look first for the good points in other people's work and be as constructive as possible in your criticisms. Remember, too to ask yourself questions about your own work and don't ever play down the unexpected (or un-wished-for) result. It may open up a whole new research area. Above all never get discouraged when things go wrong; misfortunes can provide you with stories to tell in your old age. I would like to end by telling you of the worst accident to befall my equipment. It happened with my old glass and metal machine when a leak appeared between the cooling coil on the diffusion pump and the main vacuum and the mass spectrometer filled up completely with water. I must confess that it took some time for us to accept that it was just one of those things that can happen when you tread the slippery path of mass spectrometry.

**IONIZATION BY POSITRONS:
WHAT'S THE MATTER WITH ANTIMATTER?**

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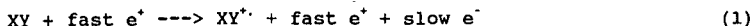
We are currently investigating the ionization of polyatomic molecules by positrons. The positron, the electron anti-particle, has been known since the 1930s and has been studied in a variety of media [1]. The ultimate fate of the positron in matter is annihilation with an electron yielding gamma photons. The vast majority of studies of the interaction of positrons with gases has involved the detection of the gamma photons following annihilation or the detection of unscattered positrons. It is recognized that ions are formed in the interaction of positrons with gases but only very recently have the ions that are formed been studied [2,3]. This paper briefly reviews what is known about ionization by positrons and what might be expected based on positron lifetime and positron scattering cross-section studies.

The likely mechanisms for ionization by positrons are categorized below along with the positron kinetic energy region in which the cross-section for the mechanism is expected to be maximized:

1. impact ionization- $K.E. (e^+) > IP(M)$
2. positronium (Ps) formation- Ore gap (see below)
3. complex formation (positron capture)- $K.E. (e^+) < IP(M) - 6.8 \text{ eV}$
4. free annihilation- thermal energies and below

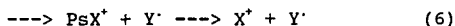
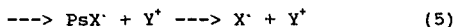
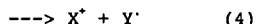
These mechanisms, the evidence for them, and the kinds of ions that may be formed from them are discussed below in turn.

Positrons with kinetic energies in excess of the ionization potential of the molecule can ionize the molecule in a fashion very similar to that for fast electrons. The field created by the fast moving positron can excite an electron sufficiently to liberate it from the molecule. This mechanism is depicted as:



We have ionized a number of compounds with 3000 eV positrons and find, as expected, that the spectra obtained from this approach are very similar to the analogous electron impact spectra. For example, Figure 1 compares mass spectra of toluene obtained by 3000 eV positrons, 2700 eV electrons, and 70 eV electrons. The only major differences between positron impact ionization and electron impact ionization are expected to appear near the threshold for ionization.

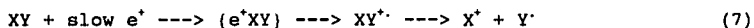
Ionization can occur below the IP of the molecule via the formation of positronium, a bound positron-electron, which has a binding energy of 6.8 eV. This process, depicted as:



is maximized when the positron kinetic energy falls within the so-called Ore

gap. The lower limit is the difference between the ionization potential of the molecule (IP(M)) and 6.8 eV. The upper limit is not well-defined. In practice it is reached when other processes, such as impact ionization, become more competitive. No mass spectra have yet been acquired that can clearly be identified as resulting from ionization via Ps formation although lifetime studies and scattering cross-section measurements clearly show that it occurs. For most organics, IP(M) is greater than 6.8 eV so that Ps formation is an endoergic charge transfer reaction. Note that equations (5) and (6) indicate fragmentation of the intermediate PsXY^+ prior to the annihilation of Ps. If the complex is sufficiently long-lived, this process can compete with annihilation.

Below the Ore gap, positron capture to form a long-lived complex (on the order of 1 ns) can occur for some molecules and is depicted as:



Schrader has predicted that some molecules can bind a positron [4]. Recently, Passner et al. have published mass spectra resulting from positron capture [3]. We have also recently begun to obtain this type of data. Figure 2, for example shows the mass spectrum obtained for toluene with < 3 eV positrons. We note no fragmentation in this data and find that the cross-section for this process is as least as great as that for ionization by 3000 eV positrons (which is on the order of a few Å²). We are intrigued by these data and intend to further explore the phenomena associated with and the potential selectivity of slow positron capture. Note again, as with the complex involving Ps, fragmentation of the e^+XY complex may compete with annihilation possibly leading to ions different from those formed from excited XY^+ .

A final mechanism of ionization by positrons involves so-called free annihilation of a positron by an electron in a molecule. This process is not preceded by Ps formation or the formation of a bound complex. The cross-section for this process is given by

$$\sigma = \pi r_0^2 c Z_{\text{eff}} v^{-1} \quad (9)$$

where r_0 is the classical radius of the electron, c is the velocity of light, v is the velocity of the positron, and Z_{eff} is the "effective" number of electrons in the molecule. Due to polarization of the electron density by the positron, Z_{eff} is larger than the actual number of electrons in the molecule. However, for molecules that do not bind the positron, the cross-section for free annihilation is expected to become significant only near thermal energies and below.

We have now begun to explore these processes. A description of the instrumentation and a longer discussion of these processes along with the presentation of the positron impact data will appear [5,6]. Our focus in the near future will be the study of the interaction of positrons of 0.1-10 eV with molecules to characterize the ions that are formed, the energetics involved, and the selectivity of this approach.

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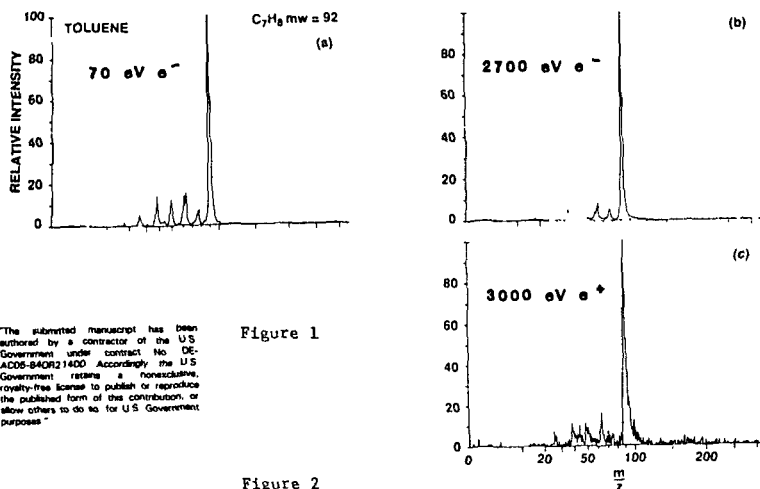
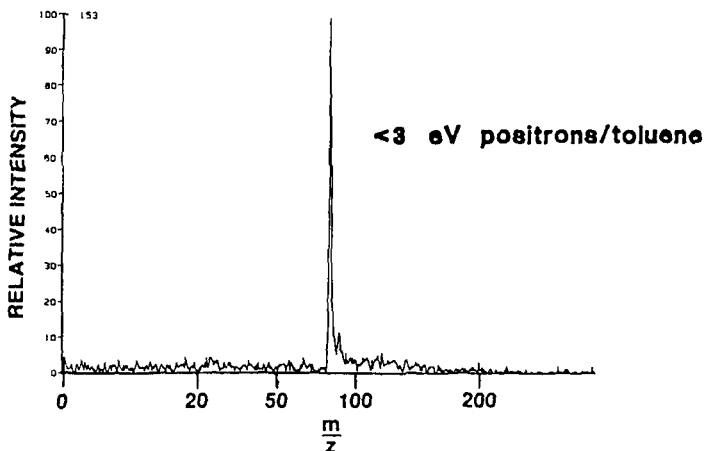


Figure 1

Figure 2



ION/SURFACE COLLISIONS: INSTRUMENTATION AND APPLICATIONS

R.G. Cooks, S. Habibi-Goudarzi, K.L. Morand, K.L. Schey and J.C. Schwartz
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Collisions of polyatomic ions with surfaces have become a subject of growing interest.¹⁻³ Surface-induced dissociation (SID), is a possible alternative to collision activated dissociation (CAD) or photodissociation (PD) in MS/MS experiments. Table 1 compares some characteristics of these three methods. Particular features of SID are control over the energy deposition, the relatively narrow distribution of internal energies deposited, and the modest dissociation efficiency. SID has been implemented using a hybrid BQ instrument,¹ a tandem quadrupole⁴ and a tandem time-of-flight instrument,⁵ all operated with large scattering angles (typically 90°) and energies up to several hundred eV. These experiments have demonstrated the usefulness of SID in ion structural work (Table 2). In almost all these cases SID provides better structural distinctions than CAD. Energy resolved measurements (ERMS) increase the value of ion/surface collisions for studies in ion chemistry.⁶

In addition to SID, several other reactions occur when polyatomic ions collide with surfaces (Table 3). These reactions have been investigated in some detail⁷⁻¹¹ and a specific example, the reflection spectrum of limonene, is illustrated in Figure 1. Among the interesting points evident in this spectrum is the ease with which the ion $C_8H_7^+$ undergoes reflection in competition with loss by neutralization and dissociation. By contrast $C_8H_6^+$ is readily neutralized.

Most polyatomic ion/surface collisions have been investigated using metal surfaces at 10^{-6} - 10^{-7} torr (i.e. covered by hydrocarbons) but some data on cleaner surfaces is available. Figure 2 illustrates typical results, showing large surface effects. Clearly aluminum is much less efficient than the steel target at causing dissociation. Other experiments established that clean graphite is relatively inert in terms of ion/surface reactions but effective at activation. This dimension of information remains to be explored, but fortunately for analytical purposes daughter spectra recorded by SID are highly reproducible in the usual mass spectrometer environment.

Wider use of surface-induced dissociation requires its implementation on conventional mass spectrometers, rather than special instruments with analyzers arranged at 90° to each other. To achieve this, a device can be inserted in-line into an ion beam and yet give large-angle collisions at a surface (Figure 3).¹² The performance of the in-line device, when fitted to a BEQ hybrid instrument, is illustrated in Figure 4. This example also demonstrates that SID is an attractive method of dissociating smaller biological compounds in order to obtain structural information.

Finally, it should be noted that ion/surface collisions are readily observed in a new hybrid mass spectrometer of BE/ion trap configuration. Figure 5 illustrates the daughter spectrum of the pyrene molecular ion selected with the BE portion of the instrument. The ions undergo chemical sputtering yielding alkyl fragments in competition with highly efficient SID to yield unsaturated fragments. The products of both these reactions are mass analyzed using the conventional mass-selective instability scan of the ion trap.

Table 1. Comparisons of Activation Methods

Parameter	SID (ev)	CAD (keV)	PD (uv)
Cross-section (cm ² /particle)	10 ⁻⁴	10 ⁻¹⁸	10 ⁻¹⁹
Energy Deposited	≤15 eV	≤3 eV	≤6 eV
Distribution P(E)	Narrow	Wide	Single-valued
Efficiency* (small ions)	2-10%	1%	<<1%
(biomolecules)	~20%	<1%	<<1%

*Beam experiments

Table 2. Ion Structure by Ion/surface Reactions

$C_2H_3O^+$	Mabud et al., J. Am. Chem. Soc., 109 7597 (1987)
$C_8H_{12}^+$	Vainotalo et al., J. Am. Chem. Soc., 109 3187 (1987)
$C_8H_6^+$	Mabud et al., Org. Mass Spectrom., 22 418 (1987)
$C_8H_6^+$, $C_8H_6^{2+}$	Hayward et al., J. Am. Chem. Soc., 110 1343 (1988)
$C_{10}H_{16}^+$	Vincenti et al., Org. Mass Spectrom., 23 585 (1988)
$C_8H_7S^+$	Cooks et al., J. Am. Chem. Soc., 111 859 (1989)
$C_8H_{10}O^+$	Kentamaa et al., J. Am. Chem. Soc., 111 1654 (1989)

Figure 1. Spectrum recorded by linking B and Q and observing (a) reflection and (b) deflection of 35 eV ions.

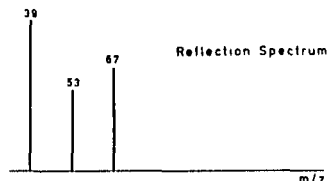
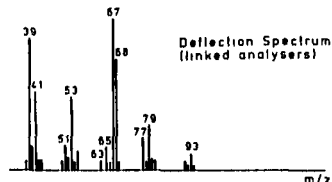


Table 3. Some Ion/surface Reactions

Charge exchange	$C_7H_6^{++} \xrightarrow{A} C_7H_6^+$
Dissociative charge exchange	$C_8H_6^{++} \xrightarrow{A} [C_8H_6]^+ \rightarrow C_3H_3^+ + C_5H_3$
Dissociative charge inversion	$C_8H_6CO_2 \xrightarrow{A} [C_8H_6CO_2]^+ \rightarrow C_8H_6^+ + CO_2$
Methyl abstraction	$C_8H_6N^+ \xrightarrow{A} C_8H_5N^+CH_3$
Chemical sputtering	$CF_3^+ \xrightarrow{A} C_3H_7^+ + [CF_3]$

A = adsorbate



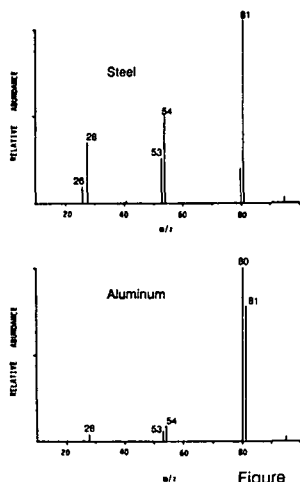


Figure 2. Daughter spectra of pyrazine, M^+ (m/z 80) after 25 eV collisions at steel and aluminum surfaces.

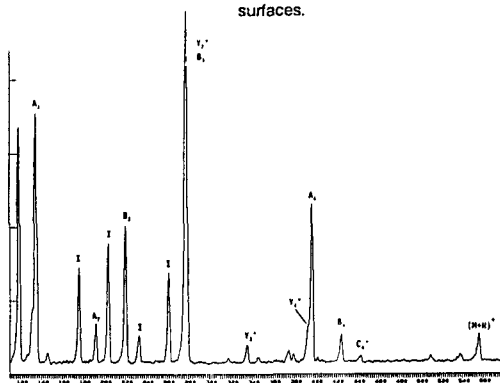


Figure 4. Daughter spectrum of protonated leu-enkephalin, 20 eV collisions, using in-line device in a BEQ hybrid.

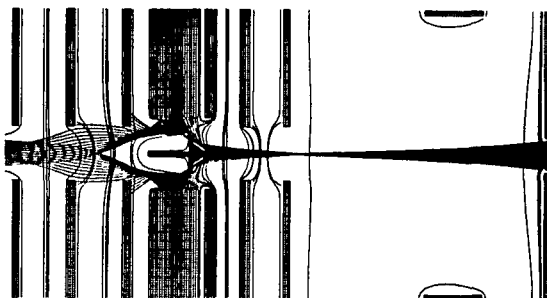


Figure 3. Simulated ion trajectories for SID in in-line device.

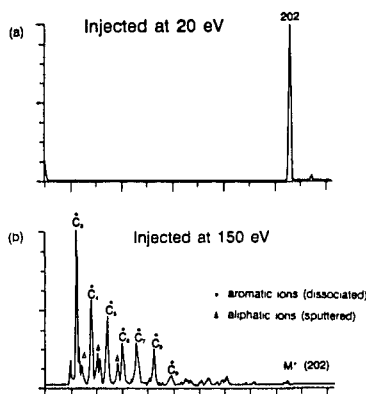


Figure 5. Daughter spectra for SID of pyrene M^+ , m/z 202 at 20 eV and 150 eV recorded in a new BE/Trap hybrid.

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ARRAY DETECTORS FOR MAGNETIC MASS SPECTROMETERS

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Historically, photographic plates have been used to simultaneously record a complete mass spectrum, even at high resolution. For this purpose, the plate was placed in the focal plane of a mass spectrometer of the Mattauch-Herzog geometry which had the unique feature of focusing ions of a wide mass range along a plane. The first commercial instrument of that type was the CEC 21-110 which had a focal range of 1:36 (for example, from m/z 20-760, depending on the accelerating voltage and magnetic field). With the advances in electronics and imaging devices, it became desirable to record the ion signals electronically and avoid the wet chemical treatment of photoplates. In the early 1970s, microchannel plates (MCP) consisting of over 1000 capillary electron multipliers per cm^2 became available in a configuration ($1/4" \times 1"$ active area) that lent itself for the recording of ion beams. Giffen *et al.* (1) at the Jet Propulsion Laboratory, Pasadena, CA, assembled 14 of these into a strip that was placed into the focal plane of a CEC 21-110 spectrometer. The burst of electrons emerging from the back of the plate whenever an ion struck the front was converted to photons, via a phosphor layer on the front of a 14" piece of fiber optics. The latter was folded in a way to generate a square field that was viewed through a lens system of a video camera, which allowed reading out the position of the illuminated lines in digital form (EOID, electro-optical ion detector). At about the same time, Tuithof *et al.* (2) placed a 1" MCP in the short focal plane of a single focusing magnetic instrument to simultaneously record a relatively narrow mass range (1:1.16). Shortly thereafter, Beynon *et al.* (3) accomplished the same for the recording of fast events utilizing 1" of the focal plane of a CEC 21-110.

The lens/video system was very inefficient and this shortcoming was eliminated by the emergence of photodiode arrays which could be mounted on the back of the fiber optics block (Fig. 1). The JPL group (4) incorporated this principle into the 14" device, which required an interdigitated fiber optics design to accommodate the physically larger photodiode arrays. This general principle of MCP, fiber optics and photodiode array has been incorporated into most array detectors used for single or double focusing (Nier-Johnson geometry) magnetic mass spectrometers. The exception is the detector employed by Owerkirk *et al.* (5) at the FOM-Institute, which senses the position of the electron burst emerging from the MCP and impacting a 45 mm array of 45 metal strips connected to a chain of resistors and capacitors. The charge differential developing at the extreme ends of the chain is related to the position of the electron beam and, therefore, that of the incident ion. This is an ion counting device, in contrast to the photodiode array. Such a device (covering a range of 1:1.1) has very recently been incorporated into a commercial mass spectrometer (Finnigan MAT 900), with additional features that allow the ion beam to be scanned across the detector (6) rather than to be recorded in consecutive steps. On the other hand, it has a relatively long "deadtime" (5 μsec); two ions arriving within a shorter time interval would appear as one signal at an intermediate position and have to be discarded.

A 1" array detector based on the EOID principle (Fig. 1) has recently been incorporated into two commercial instruments, KRATOS MS-50 (7) and the KRATOS Concept (8), covering a mass range of 1:1.04. We have constructed a 2" array detector (9,10) for the JEOL HX110/HX110 tandem mass spectrometer, which has a 3" focal plane covering a 1:1.10 range. The reduced range (1:1.066) was dictated by the availability of only a 2" array of 2048 photodiodes. Previously, all EOIDS utilized the fiber optics as a means to transmit the photon beam through the wall of the vacuum envelope of the mass spectrometer, allowing for operation of the photodiode array at atmospheric pressure. In our design, it was kept within the vacuum system to allow changing the position of the detector.

Simultaneous ion detection has the additional advantage of much higher sensitivity, compared to a conventional slit/electron multiplier system. The latter measures only that part of the ion beam which passes through the

narrow collector slit, while the rest of the spectrum ($\geq 99.9\%$ of the ion current) is lost. With the array detector covering a range of 1:1.066 (i.e., 6.6% of the mass range), we have improved the sensitivity by a factor of 50-100 (10); increasing the range would further increase the sensitivity proportionally.

The limited range over which ion beams of different mass are in focus along a linear plane in a Nier-Johnson type mass spectrometer presents a more complex data acquisition and processing problem. To record an entire mass spectrum, it has to be assembled from individual segments and the number of consecutive segments required depends, of course, on the mass range covered by the array detector and that of the spectrum. This problem is illustrated by Fig. 2.

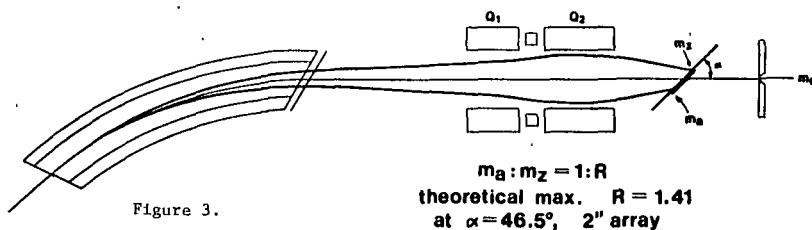
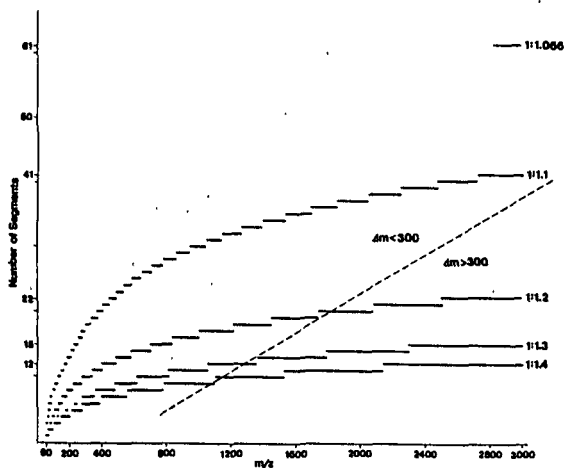
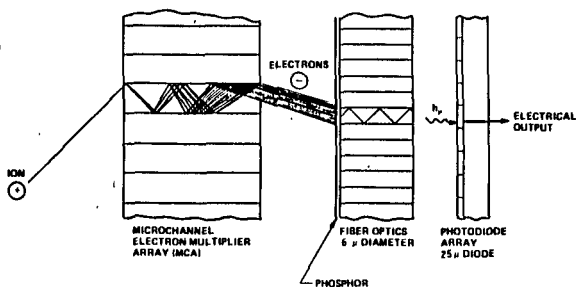
To record a mass spectrum from m/z 60-3000 with a detector covering a range of 1:1.066, the mass spectrometer has to be stepped through 61 segments which then have to be processed to a contiguous spectrum (the 1:1.04 range of the 1" KRATOS detector requires about 100 steps). Increasing the range to 1:1.1 reduces the number of segments to 41. As can be seen from Fig. 2, this is mainly due to the fact that the range (in mass units) covered by each segment becomes progressively smaller at lower mass. To decrease the number of steps required (thus decreasing the time necessary to record the spectrum and further increase the sensitivity), it is desirable to further increase the mass range focused on the MCP. At ranges 1:1.2, 1:1.3 and 1:1.4, the number of segments decreases to 22, 15 and 12, respectively. However, to retain good resolution and mass measurement accuracy, one cannot utilize the full range at higher mass because the fixed number of 2048 photodiodes of a 2" Reticon photodiode array sets a practical limit of 300 mass units per segment in order to provide a sufficient number of data points (>6) per peak. This situation indicates that increasing the mass range focused onto the detector becomes of limited practical utility beyond a certain point. It is therefore desirable to be able to change the range from wide (at low mass) to narrower (at higher mass) to minimize the number of segments required while maximizing the quality of the resulting mass spectrum.

We have constructed such a variable range array detector (11), based on an ion optical principle proposed by Ishihara *et al.* (12), which involves placing a pair of quadrupole lenses between the magnetic field and the collector slit of the JEOL HX110 mass spectrometer. With the proper potentials on the lenses, a focal plane of varying range (theoretical maximum limited to 1:1.41 by the width of the flight tube in the magnet gap) is created in front of the collector slit, providing sufficient space for the variable angle (to vary the mass range) array detector, as illustrated in Fig. 3. Presently, we have achieved a range of 1:1.30. Little would be gained (see Fig. 2) by extending the range even further.

Although we utilize this detector mainly for the recording of tandem mass spectra (11), it is also useful for the measurement of normal FAB spectra, particularly when measuring the molecular weights of compounds of high but unknown mass. In such cases, one can step through the spectrum at the highest angle (mass range) until one encounters a signal. Stepwise reduction of the angle decreases the range covered and thus allows one to "zoom-in" on the signal for measurement of the average mass or, at higher resolution, the monoisotopic mass of the $(M+H)^+$ ion (or others). Detector angle, lens potentials and other parameters are computer controlled (11).

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Capillary Electrophoresis-Mass Spectrometry

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The development of electrophoresis in capillary tubes offers several exciting methods for fast, highly efficient separations of ionic species, separations of macromolecules important in the area of analytical biotechnology, and design of small volume separation based sensors. The history, principles, and applications of capillary electrophoresis have been recently documented by A.G. Ewing et. al.¹ The most common type of capillary electrophoresis in use today is capillary zone electrophoresis or CZE. Other types are capillary gel electrophoresis, micellar electrokinetic capillary chromatography, isoelectric focusing, and isotachopheresis. Of these five methods, all but one, isoelectric focussing, has been coupled with mass spectrometry.

The coupling of mass spectrometry and capillary electrophoresis has a number of constraints. Just as in LC-MS, it must allow the introduction of solutions without severely compromising the vacuum of the ion source. The ionization mode must be universal, allowing highly polar molecules to be ionized or ionic species to be transmitted. There must be a way to complete the electrophoretic circuit. It should not degrade electrophoretic resolution through either band broadening in transfer lines or pressure-induced laminar, or non-plug, flow through the separation capillary. Two approaches have evolved to date, one based on "spray" ionization sources and the other on flow FAB ionization sources.

Richard Smith and coworkers were first to report the direct coupling of CZE and a mass spectrometer using an electrospray interface.² Sensitivities were olympic, low attomole quantities of quaternary ammonium compounds being detected. Jack Henion and coworkers at Cornell used the related ionspray technique for the separation of a peptide mixture.³ Sensitivities were in the femtomole range. Both of these spray methods have the advantage that the electrophoresis capillary exists at essentially atmospheric pressure and therefore, no degradation of the separation due to the pressure-induced flow effects occurs.

A practical method for interfacing CZE with flow FAB would have wide application. It was felt that direct connection was not feasible since the vacuum in the ion source would draw buffer through the CZE capillary and degrade separation efficiency. For this reason, we designed an interface involving an open gap matrix "make-up" coupler.⁴ It had the advantage of allowing the easy completion of the electrophoretic circuit external to the mass spectrometer and ran the CZE capillary at atmospheric pressure. Although we were able to carry out electrophoretic separations, resolution was less than optimal. This system was used to demonstrate the micellar electrokinetic capillary chromatography separation of Leu and Met Enkephalin.⁵ Attempts to improve both resolution and sensitivity using this interface have not yielded very satisfactory results.

Moseley, Deterding, Tomer and Jorgenson have described an improved method of interfacing CZE and flow FAB MS. Based on their very successful capillary LC-flow FAB MS work⁶, they have now reported the direct coupling of CZE and flow FAB MS using the same on-line coaxial continuous flow interface.⁷ By using a small internal diameter capillary, 13 microns, the flow induced by the source vacuum is minimal, on the order of 3 nL per minute. The FAB water/glycerol matrix solution is introduced via a tee through a coaxial outer capillary at the rate of about 1 microliter per minute. The CZE capillary sits back about 1 mm from the end of the matrix sheath capillary and the matrix solution flows over and covers the end of the CZE capillary, thus minimizing vacuum effects. The electrophoretic circuit is completed through the source voltage power supply which was 8KV. Their results, in terms of both sensitivity and separation efficiency, are very impressive. For example, the separation of approximately 400 femtomoles each of

the two basic dipeptides, arginyl phenylalanine and lysyl alanine was reported. The number of theoretical peaks in both cases is almost 300,000. Their data also includes an example of CZE MS/MS on 130 femtomoles of peptide.⁷

For the purposes of our research at Penn State, sampling from single cells⁸, injection volumes in the picoliter range are very desirable and capillaries with less than 10 micrometer ID's are often used in the single cell work using electrochemical detection. For this reason, the Tomer/Jorgenson approach is very attractive to us and we have set out to duplicate their CZE/FFAB/MS coaxial interface design. In our preliminary work, we detected 3 picomoles of Substance P and the number of theoretical plates was about 40,000. Since this was a single component, it may have simply been drawn through the electrophoresis capillary by the source vacuum. However, changing the applied potential changed the migration time in direct proportion to the change in the voltage and, therefore, true electrophoresis was taking place. The mass spectrum showed a signal to noise ratio of over 100 to 1 for the MH⁺ ion at m/z 1348 and it seems reasonable that femtomole range sensitivities will ultimately be obtained.

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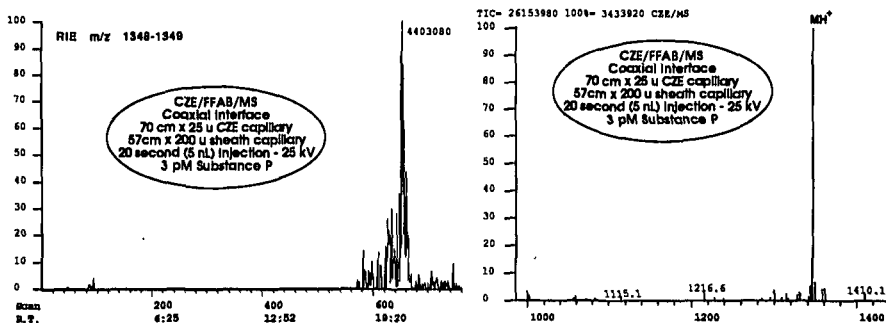
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ANALYSIS OF DATA RATE REQUIREMENTS FOR HIGH PERFORMANCE GC/MS: THE CASE FOR TOF-MS WITH TIME-ARRAY DETECTION

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For accurate reconstruction of chromatographic peak height, area, and position, 10 data points per standard deviation unit (σ) are needed assuming a Gaussian peak. For a 1-second wide (FWHM) peak, now being achieved in high performance gas chromatography, this corresponds to 20-30 samples (data points) per second. For current scanning filter mass spectrometers, this sampling rate is achievable only with selected ion monitoring (SIM) or over a very limited mass scan range. When such rapid full mass scans are possible, the detection limits are reduced at least 1000-fold over SIM. The under sampling of scanned mass spectra also results in varying intensity distortions of the mass spectra. Such "skewed" spectra reduce the reliability of spectral matching programs and interfere with the application of "clean-up" programs to sort mixture spectra from overlapped chromatographic peaks into clean mass spectra for each component. These problems can potentially be avoided with non-scanning or array-detector mass spectrometers such as magnetic dispersion with array detector, FTMS, and time-of-flight (TOF) MS. None of these array detector MS instruments currently meets the scan rate, sensitivity, mass range, and mass resolution required by modern high-performance chromatography. We believe TOF-MS has the best chance to achieve these objectives in the near future.

A data rate of 20-30 useable full mass spectra per second requires that the ion detection rate be quite high. Specifically, at least 400 ions must be detected in the base peak alone for each scan file if its intensity is to have a precision of 5% or better. Assuming the base peak contains 1/20 of all the ion intensity in the spectrum, at least 8,000 ions must be detected per scan file for quantitation at 5% precision. In TOF-MS, an ion pulse is formed every 100 μ s to produce 10,000 transient wave forms per second at the detector, each containing full mass spectral information. With an integrating transient recorder (ITR) (1), 100 to 1000 successive transients are summed to produce 10 to 100 scan files per second. At 20 scan files/s, each of the 500 transients summed must, on average, represent 8,000/500 = 40 detected ions (minimum level for 5% precision in quantitation). For a reasonable dynamic range, the maximum ion detection rate should be at least five times the minimum quantitation level, or ~ 200 ions per transient. This eliminates the time-domain converter (arrival time detector) as a detection device since it is limited to only a few ions per transient. At the minimum detection level (S/N = 3), the ion detection rate at the arrival time of interest must be 10 times the background count rate for that mass. For a background of 10 ions/s in a given mass (time) window, the count rate for minimum detection must be 100 ions/s or one ion every 100 transients. Clearly, each ion event at the detector must advance the ADC in the transient recorder at least one count. The dynamic range of the TOF-ITR system with an 8-bit ADC would be equal to saturation rate/detection rate = $28/0.01 = 10^4$, a very good value.

The sample ionization and mass separation processes must be capable of delivering ions to the detector at the above calculated rates. At the minimum quantitation level ($\pm 5\%$), the detected ion current must be at least (8,000 ions/scan file) $\times$ (20 scan files/s) $(1.6 \times 10^{-19}/\text{ion}) = 2.6 \times 10^{-14}\text{A}$. In TOF-MS, ion pulse formation can make inefficient use of a gaseous (continuous flow) sample. The efficiency of chopping an ion beam, continuously extracted from the source, is only about 50 parts per million. The efficiency of continuous ion formation with pulsed extraction from the source can be 1% or higher. Assuming an ionization efficiency of 10^{-5} , the overall efficiency for conversion of sample molecules into detectable ions is 10^{-7} for pulsed ion storage source. The sample flow rate to the source to produce a $2.6 \times 10^{-14}\text{A}$ detected ion current would be 2.5 pmole/s. For a 1-s peak, the minimum quantitation level would thus be 2.5 pmoles and the minimum detection level, 100 fmole.

An MS chromatographic detection system of this type has been built in this laboratory around a CVC-2000 TOF instrument and an in-house integrating transient recorder. It produces 1-40 scan files per second. For time-lag focusing, unit mass resolution is obtained over a mass range of 2.5:1 for any given time lag setting (2). The detection limit is about 1 pmole at 20 scans/s, comparable to that of scanning filter instruments in SIM mode, but in our case, with near full spectral range and with no spectral skew. Novel approaches will be required in the data systems to process data at the required scan file generation rate and to review chromatographic run files with tens of thousands of scan files.

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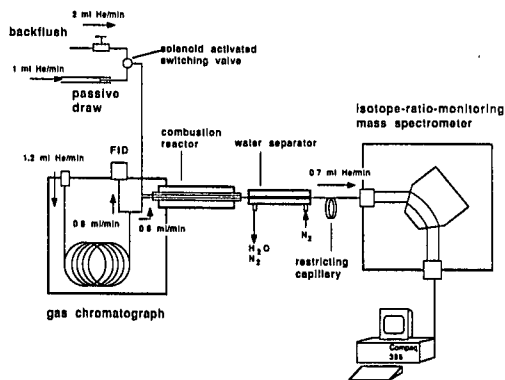
Isotope-Ratio-Monitoring Gas Chromatography Mass Spectrometry

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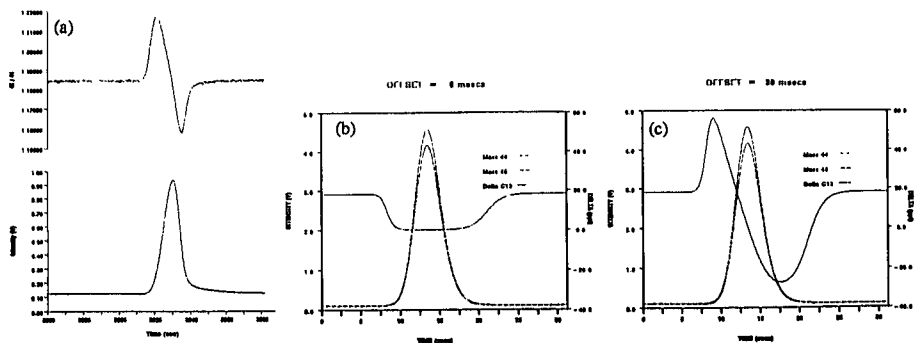
Particularly for applications in the natural sciences, there is great interest in the precise measurement ($\pm 0.001\%$ ^{13}C , 95% confidence interval) of carbon-isotopic compositions of individual organic compounds. To avoid problems associated with conventional, dual-inlet mass spectrometer systems in this application, we have extended the development of isotope-ratio-monitoring gas chromatography mass spectrometry (irmGCMS, ref. 1). A schematic diagram of the instrument is shown in Fig. 1. A vitreous silica capillary leak withdraws CO_2 , traces of O_2 , and He from a post-column combustion reactor with $V < 30\ \mu\text{L}$. Pressure in the reactor is stabilized at 1 atm and O_2 is supplied by dissociation of CuO at 800°C . Manipulation of gas flows allows diversion of solvent peaks. The ion source has a conductance of $\sim 1.6\ \text{L/sec}$ (air), about 4X greater than a conventional isotope-ratio source. The source housing is differentially pumped by a 300 L/sec turbomolecular pump. Rise times of all three collector amplifiers are carefully matched at 60 ms. A specialized data system integrates ion currents for selectable time intervals in the range 60-240 ms.

Figure 1. Isotope-ratio-monitoring gas chromatograph mass spectrometer.



Replicate injections of small peaks of CO_2 yield highly reproducible integrated ion current ratios. Specifically, for peaks containing $0.67\ \text{nmol}\ \text{CO}_2$, the measured relative standard deviation of

Figure 2. Chromatographic separation of isotopic species (see text).



45/44 ion-current ratios is $\sigma/R = 0.013\%$ (2). At the observed ionization efficiency of 6×10^{-5} ions/molecule, the shot noise limit is 0.006%. For 22 nmol peaks, $\sigma/R = 0.002\%$ (limit 0.001%).

When high-resolution capillary columns are employed, combustion of chromatographic peaks reveals significant resolution of isotopic species. Shown below in Fig. 2 are (a) ion currents and 45/44 ratio across a chromatographic peak (C_{21} , *n*-alkane, methyl-silicone column), (b) signals expected in the absence of chromatographic resolution of isotopic species, (c) signals expected for a $^{13}C^{12}C_{20}H_{44} - ^{12}C_{21}H_{44}$ separation of 30 ms. The ^{13}C -containing species elutes more rapidly. This behavior is the result of a vapor-pressure (*i. e.*, equilibrium) isotope effect. To avoid degradation of isotopic accuracy, care must be taken to integrate ion currents across the full width of chromatographic peaks. The phenomenon must also be taken into account during deconvolution of overlapping peaks.

The accuracy obtainable in measurements of isotopic compositions of individual alkanes is shown in Table 1. Isotopic compositions are presented in terms of $\delta^{13}C_{PDB}$ values and expressed in terms of permil units (‰). A standard deviation of one permil is equivalent to $\sigma/R = 0.1\%$. The uncertainties are considerably increased relative to those obtained following injection of peaks of CO_2 . Correction for CO_2 contributed by combustion of column bleed and uncertainties associated with standardization (*i. e.*, placement of the isotopic compositions relative to an isotopic standard) are primarily responsible for this degradation. In order to obtain standard peaks that do not interfere with normal alkanes, samples are spiked with perdeuterated *n*-alkanes. The δ values of the deuterio-alkanes are accurately known, and the δ values of unknown peaks are referred to them.

Acknowledgements. We appreciate financial support from Chevron Oil Field Research Corporation and provision of isotopic standards by Martin Schoell and Robert Carlson of Chevron.

Table 1. Isotopic compositions of individual alkanes.

Carbon Number	$\delta^{13}C$		Std. Dev. (irm-GCMS)	Residual Delta (‰)
	irm-GCMS	Conventional		
11	-26.82	-27.26	0.30	0.44
12	-32.78	-33.26	0.59	0.48
13	-26.77	-26.99	0.53	0.22
14	-28.46	-28.65	0.26	0.19
15	-28.53	-28.68	0.37	0.15
16	-34.14	-34.23	0.38	0.09
17	-25.41	-25.66	0.18	0.25
18	-34.41	-34.63	0.26	0.22
19	-28.98	-29.00	0.36	0.20
20	-28.39	-28.21	0.20	-0.18
21	-27.29	-27.57	0.43	0.28
22	-34.09	-34.00	0.14	-0.09
23	-27.37	-27.21	0.17	-0.08
24	-31.59	-31.68	0.51	0.09
25	-28.53	-28.15	0.64	-0.38
26	-35.02	-34.64	0.30	-0.38
27	-25.71	-25.46	0.41	-0.25
28	-27.69	-27.43	0.09	0.32
29	-31.19	-30.83	0.31	-0.36
30	-27.85	-27.53	0.12	-0.32
31	-28.68	-28.82	0.08	0.14
32	-27.00	-26.60	0.22	-0.40
33	-30.63	-30.62	0.10	-0.01
34	-27.73	-27.58	0.50	-0.15
35	-26.60	-26.23	0.56	-0.37
36	-27.40	-27.29	0.21	-0.11
37	-29.34	-29.57	0.28	0.23
38	-28.69	-28.32	0.15	-0.37
40	-28.32	-27.84	0.36	-0.48

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Coupling Planar Chromatographies with Mass Spectrometry
and Mass Spectrometry with Optical Spectroscopic Imaging

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Mass spectrometry has been coupled to planar separation techniques using a variety of indirect interfaces; the twenty year history of TLC/MS has been recently reviewed [1]. The capabilities of FAB, SIMS, and laser and plasma desorption for the direct sputtering of TLC plates and other chromatographic media have all been explored. At least one commercial vendor offers a probe accessory that allows the direct FAB analysis of small TLC strips. Our own work has focussed on the development of a grand scale two dimensional sample chamber that allows the full xy spatially-resolved images of samples to be recorded. Concurrent with this work has been extensive investigations into the sputtering mechanism and the details of sampling from a variety of matrices (solid and liquid).

The criteria by which the chromatography/SIMS experiment must be judged include selectivity, sensitivity, and spatial resolution, among others. The selectivity of mass spectrometric detection for chromatography/SIMS is the equivalent of any other mass spectral experiment. Secondary ion mass spectra contain even-electron ions indicative of molecular weight as well as structurally-informative fragment ions. A large collection of SIMS spectra is available in the literature for inspection. The information available from the mass spectrum is of particular use in the chromatography/SIMS experiment when allied with the ability to rescan a chromatogram. Just as in GC/MS, when all ions that comprise a mass spectrum of a pure compound must trace the same peak time profile, in TLC/SIMS, for example, all ions must follow the same xy profile. The sensitivity of the TLC/SIMS experiment has been shown to extend to about 100 pg for discrete samples with high secondary ion yields. The limit is the ability to extract the material from the chromatographic matrix efficiently and without loss of spatial resolution. In general, the sensitivity is lower due to the simple fact that the flux of sample into the source is not compressed into a short time window as with GC/MS. In the latter situation, the absolute intensity of the signal is high, but the persistence short. In chromatography/SIMS, the absolute level of the signal is lower, but the persistence longer; signal averaging increases the sensitivity of the method at the cost of increased analysis time.

Finally, the practical limits of spatial resolution in chromatography/SIMS should be discussed. In the determination of the homogeneity of sample spots, or in the deconvolution of two overlapping sample spots, the highest possible spatial resolution is sought. The liquid metal ion gun discussed in the previous section is capable of providing a focussed spot of 1 micron diameter on the surface of the chromatogram. The minimum detectable number of ions, and thus the practical lower limit of spot size for organic analysis by SIMS, can be estimated by considering the total number of molecules present in a chromatographic spot. For a spot size of 100 microns diameter, 20 microns depth, approximately 10^{14} molecules of a 300 dalton molecular weight compound are present. Assuming a 10^{-4} transmission efficiency for the secondary ion mass spectrometer, a 10^{-3} ionization efficiency, and a 25% extraction efficiency, about 2×10^7 ions will reach the detector. A spot size of 10 microns provides 10^5 ions at the detector, and a 1 micron spot provides 10^3 ions. Ten microns is a reasonable estimate of the lower practical limit in spot size that can be obtained with the chromatography/SIMS experiment, based also on the signal-to-background level that must be attained in the mass spectrum. In routine operation, the FAB and cesium ion guns have been modified to provide a spatial resolution of 0.1 mm. Since many developed spot sizes in TLC are larger than this value, this is sufficient spatial resolution for determination of spot homogeneity, and acquisition of data sufficient for the deconvolution of partially-overlapped spots.

In the last few years, we have developed a number of methods for the application of extraction matrices to the surface of a planar chromatogram, and have explored the sputtering of ions from solid matrices, from liquid matrices, and from matrices that are not quite either. We have shown explicitly that these methods do not cause changes in the spatial orientation of the developed spots on the chromatogram. We have recently developed a surface tracking capillary that is used to bring liquid directly into the vacuum and onto the surface of the chromatogram while sputtering is taking place. The polarity of the solvent can be adjusted as the xy coordinates of the plate are changed to provide varying extraction efficiencies. Experiments also show that the transient matrix does not change the dimensions of the developed spots.

For simple mixtures, the time required to bring sample spots to the point of analysis is not excessively long, especially with automated sample positioning with the piezoelectric translation stages. For more complex mixtures, and larger chromatograms, scan lines can be followed that ultimately intersect sample spots of interest, but overall, the measurements require excessively long periods of time for sample spot siting and analysis. Practically, almost all chromatograms are pre-screened visually before placement within the mass spectrometer. If the sample spots can be visually located, the placement of the sample spot within the point of instrument focus can be monitored through the viewports and plexiglass window of the SIMS instrument. A hand-held UV/vis lamp is also used to illuminate thin layer chromatograms with a fluorescent binder. Dark sample spots on the uniformly fluorescing background are noted by pencil marks, visible again through the ports or windows of the SIMS instrument. Finally, reversible iodine staining, with marking of the sample spots, is also used in preliminary siting of sample spots.

Each of these exercises suggest that an optically-based imaging system should be integrated with the SIMS analysis of chromatograms. Separate imaging of a thin layer chromatogram on a commercial scanning densitometer could be used, with optical spectroscopy data generated in the first xy measurements ultimately correlated with the mass spectra of the organic compounds in a separately determined xy plane. A more satisfactory approach is to simply replace the mass spectrometer with the optical imaging detector, and to leave the sample (in this case, a thin layer chromatogram) in a common coordinate system. Both optical image and mass spectrometric data are then correlated directly in the xy planar coordinates. Reduced sample chromatogram handling also reduces sample contamination and sample degradation.

In the second generation chromatography/SIMS instrument, the quadrupole is replaced by a modern charge-coupled device camera. Sample spots on a thin layer chromatogram are located in visual light, in UV/vis irradiation, or with reversible general and specific color developing reagents. Once the chromatogram is placed on the piezoelectric sample stages of the sample cell, both optical data and mass spectral data are measured within the same planar coordinates. Three detection schemes are sufficient to detect all compounds. With TLC plates coated with a fluorescent coating, illuminating the plate with 254 nm UV light yields a bright plate with dark spots corresponding to the component spots. If a particular compound does not occlude the fluorescence of the plate, and hence is not detectable by this method, the compound may iodine stain. Exposing the plate to iodine vapors turns the component spots brown. Visible light can now be used for the detection, and the iodine pumps away rapidly in the vacuum of the mass spectrometer. If iodine staining fails, a specific color-developing reagent can be used. For instance, reacting polycyclic aromatic hydrocarbons with 2,4,7-trinitro-9-fluorenone yields purplish spots.

The CCD camera can provide data complementary to the mass spectrometry data. For instance, compounds such as polysulfonated dyes are difficult to sputter with SIMS or FAB, but provide a strong visual signal. For some highly colored compounds, the high sensitivity of the charge-coupled device lends to very low detection limits. Figure 3 shows a row-plot of approximately 390 pg of oxazine 4 perchlorate, a laser dye, detected using 366 nm UV radiation. This signal to noise ratio, along with the fact that this row-plot only intersects 1/25th of the total sample, corresponds to a detection limit of about 7 pg (24 femtomoles!). The CCD camera is also being used in current experiments to monitor the sample droplet as it is sputtered, using dyes to monitor diffusion rates from within the bulk and across the surface.

A PRIMER ON ELEMENTAL MASS SPECTROMETRY
ICPMS (SOLUTIONS) VS GDMS (SOLIDS)

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By far the largest number of mass spectrometry applications involve organic samples, yet there exist important needs in elemental analysis that are being met more and more by rapidly growing new mass spectrometric methodology. The two major techniques considered in this report are inductively coupled plasma mass spectrometry (ICPMS) and glow discharge mass spectrometry (GDMS). Each is a form of atomic mass spectrometry, in that the ideal ion source would reduce the analytical sample to simple atomic ions. Unlike organic applications, some form of relatively high energy is required to create the atomic reservoir and to effect ionization.

EXPERIMENTAL

Quadrupole based instruments are used with both ICPMS and GDMS, although a magnetic sector unit is the chief commercial unit for the latter. Figure 1 shows one type of GDMS instrument.

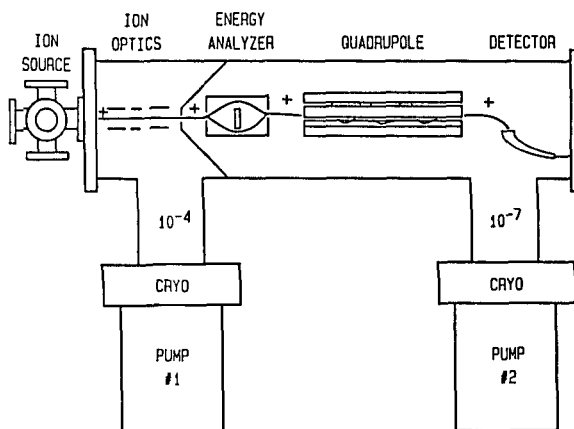


Figure 1. Cross section schematic of a glow discharge mass spectrometer.

The low resolution of quadrupoles can cause problems if polyatomic species occur at the same nominal mass as atomic ions. Even the sector instrument cannot resolve many of the more common problems, although in general this source is not particularly prone to form polyatomics.

RESULTS AND DISCUSSION

Elemental mass spectrometry has many advantages in the competitive trace element analysis field. Spectra are quite simple, compared to optical emission spectra, as exemplified in Figure 2.

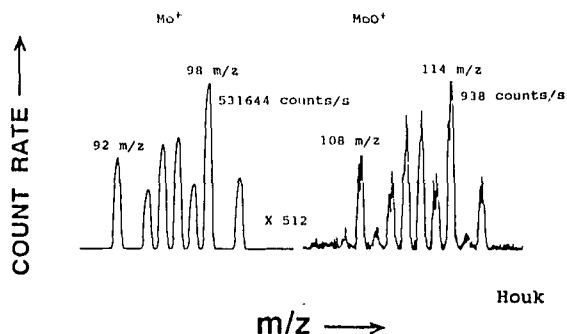


Figure 2. ICPMS spectrum of molybdenum solution.

Elemental mass spectrometry is also very sensitive. Both techniques commonly exhibit ppb detection limits. Solution sensitivity is often given in parts-per trillion. Experimental simplicity is also often cited as an advantage, at least in comparison to many other techniques of comparable sensitivity. Both metals and nonmetals can be determined, as opposed to primarily metals in optical emission and adsorption. Matrix effects, while certainly not absent, are at a minimal level, particularly with GDMS. The isotopic information obtainable with each technique also stands as an advantage relative to other trace element methods.

The applications to which these two techniques have been directed are ever growing. ICPMS is useful for solution samples or any materials that can be easily dissolved. High acid or solids content in the solutions can, however, cause problems in trace analysis. GDMS is particularly valuable for samples that profit from analysis directly in the solid form. For example, the analysis of high purity metals is a major application, since no contamination-mixing is required. Table I shows results of an analysis of high purity indium.

Table I. Impurities detected in high purity indium by GDMS.
Values in ppm. (VG Isotopes)

	Sample I (99.9999%)	Sample II (99.999%)
Mg 24	0.017	0.013
Al 27	0.056	1.40
Si 28	0.020	0.15
P 31	0.006	0.003
S 32,34	0.70	0.5
K 39	0.76	0.26
Ca 44	0.23	0.26
Fe 56,54	1.46	1.80
Cu 63,65	0.12	0.15
Zn 64,66	0.018	0.006
Cd 111,112	0.015	0.027
Sb 121,123	0.17	0.018
Te 125,126	0.018	0.035
Tl 203,205	0.33	0.81
Pb 206,208	0.45	3.76
Bi 209	0.004	0.033

Nonmetal samples, such as geological materials, can be analyzed by either technique, although with additional steps. For ICPMS, often difficult dissolutions are required with attendant problems. GDMS needs a conducting sample, so some metal matrix must be mixed with the sample before it is compacted into a suitable electrode, with the possibility of introducing impurities. Still, excellent results have been obtained with each technique for such difficult sample types, given the proper analytical preparation.

RIMS and TIMS and APPLES and ORANGES - A COMPARATIVE SUMMARY Larry J. Moore, Washington Regional Center, Atom Sciences, Inc., College Park, MD 20742

Thermal ionization mass spectrometry (TIMS) has a long and distinguished record in the performance of precise and accurate isotope ratio measurements for those elements amenable to the variant forms of the thermal ionization mechanism. Within the last several years laser resonance ionization mass spectrometry (RIMS) has been conceived and reduced to practice in trace metal and isotope applications peculiar to its sensitivity and selectivity advantages. The purposes of this paper are to discuss the principles and instrumentation used for TIMS and RIMS, summarize the current status of their development, and illustrate similarities and contrast differences between the techniques.

The basic thermal ionization phenomenon results in the spontaneous emission of ions from a hot surface onto which has been deposited the element, or a salt containing the element of interest. The Saha-Langmuir equation describes the ionization process:

$$\frac{n^+}{n^0} = A \exp \frac{(W-IP)}{kT}$$

Where n^0 = neutral atoms, n^+ = positive ions, $A = g^+/g^0$ = statistical weight ratio of ionic to atomic species leaving the surface, IP = ionization potential of the element being ionized, W = work function of the surface, k = Boltzmann constant and T = temperature in Kelvin.

In general the ionization efficiency is greatest for those elements with low ionization potentials, and for refractory metal substrate surfaces with high work functions. In the early history of the development of this technique, the alkali and alkaline earth elements were among the relatively few of the periodic table that were deemed amenable to this ionization process. Subsequently it became apparent that if the ionization potential of the element were sufficiently high, its electron affinity would permit the formation of negative ions. This observation led to the ability to determine the halides using negative ions. In the interim between the conception of TIMS and the 1960's, the precision and accuracy of isotope ratio measurements was improved from a few percent to a few tenths of a percent. The ensuing decade into the 1970's witnessed the improvement of isotope ratio precisions to a part in ten thousand, and accuracies approaching this level. Current commercially available TIMS instrumentation is capable of isotope ratio precisions in the parts per million range.

In parallel with these improvements, and driven principally by application needs in the geological community and nuclear industry, ingenious improvements have occurred that extend thermal ionization to many other elements of the periodic table that were previously considered intractable. The advent of ionization enhancement agents, such as the silica gel/phosphoric acid process (1) allowed the analysis of submicrogram quantities of lead (and other high ionization potential elements) and provided a powerful tool for environmental, biological and geological investigations through the

measurement of lead isotope ratios. The negative ion analog of thermal ionization advanced analogously, using lanthanum hexaboride coated surfaces (2) to reduce surface work functions and permit extremely efficient ionization of halides and other non-metals previously immune to ionization. A collective result of these advances has been to extend the ionization capability, at varying sensitivities, to more than two-thirds of the elements in the periodic table. More recent developments in the analysis of small actinide samples suggest that time efficiencies may be greatly improved through total integration of the isotopic data (3).

Although chronologically somewhat later in conception and practice, energetic primary ion bombardment beams were used to sputter materials and form secondary ions directly. During the thirty or so years since its inception, secondary ion mass spectrometry (SIMS) has been refined analogously to its thermal ionization predecessor, and is widely used today for the spatial characterization of materials. The fast atom beam (FAB) primary ion source, although developed initially for the direct analysis of liquids, is also being used to determine stable isotope ratios in biomedicine (4).

Thermal ionization and ion sputtering utilize the ions formed directly in the ionization phenomenon. For most elements and/or their attendant matrices, the analyte ions formed directly represent the minority of species produced in the phenomena, whereas the neutral species (including a large proportion of atoms) predominate, often by as much as several orders of magnitude. If it were possible to access this neutral atom population, it might be possible to exploit these species for more sensitive analyses, and/or to extract new information regarding the origin and character of the species. Laser resonance ionization spectroscopy (RIS) (5), combined with mass spectrometry (RIMS), provides a unique opportunity to access the neutral species formed in conventional ionization processes.

Basic advantages of the RIMS technology are: sensitivity, in that each atom that crosses the active volume of the laser can be ionized with unit efficiency; selectivity, such that the tunable laser can ionize specific elements and isotopes in the presence of billions of disparate atoms; generality, in the ability to ionize every element in the periodic table, except helium and neon (6). Since the (RIS) ionization process utilizes neutral atoms and is independent of the directly formed ions in the atomization phenomenon, the atomization and laser ionization steps may be temporally and spatially segregated. This segregation permits the optimization of the parameters associated with the ionization efficiency, such as geometric expansion of the atom plume with time; overall system efficiencies of eight to twenty percent (i.e., of 100 atoms sputtered from a surface, eight to twenty are registered at the detector of the mass spectrometer) have been achieved. Another benefit of the temporal segregation is that the ions formed directly in the atomization phenomenon may be suppressed or extracted to prevent their interference in the mass spectrum.

Biomedical applications are currently underway using RIMS, in

medically diverse areas such as neonatology, neurology, diabetes, and hematology. A method to determine copper in microliter serum samples has been developed (7) and methods are underway for ultra-trace iron, zinc, chromium, aluminum, molybdenum and vanadium in extremely small samples. Femtogram sensitivities have been demonstrated for stable chromium isotope tracers, and picogram levels of chromium have been resolved in microliter blood samples for the purpose of determining red blood cell volume, mass and survival. Extremely simple sample preparations are required.

A detailed comparison of relative advantages among RIMS, TIMS and SIMS/FAB is beyond the scope here; however, selected comparison may be made among a few key factors. Sensitivity: Clearly TIMS and SIMS can achieve overall ionization and system efficiencies of up to a few percent for selected elements. However, a substantial fraction of the elements, including some noble and transition metals, such as rhenium, osmium and chromium, may be analyzed more efficiently with RIMS, even with the limited duty cycle of the pulsed laser system. Selectivity: In principle, and in incipient practice, this is a major advantage of RIMS derived from the selectivity of the tunable laser. Whereas TIMS and SIMS generally require complex sample preparation and purification procedures for elemental/isotopic analysis, a one or two-step process has been sufficient for most RIMS applications. Some TIMS selectivity can be achieved through the use of negative (molecular) ions and thermally selective atomization, but this is not generally applicable. In conventional mass spectrometry, greater general selectivity has been achievable through increased engineering of the ion optics to provide higher resolution, but with attendant loss in sensitivity. In comparison, the 'engineering' with RIMS occurs with the laser selectivity, permitting simpler ion optics and no loss in sensitivity. Accuracy and Precision: TIMS is clearly the leader, providing isotope ratio precisions in the ppm range, and accuracies approaching the parts per hundred thousand range. Analogous RIMS precisions less than one percent and accuracies within two percent have been achieved, and further improvements may be expected. In the current state of development, RIMS precisions and accuracies are analytically useful and competitive for those elements for which there is superior sensitivity.

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PRODUCTION OF IONS FROM CHARGED LIQUID DROPLETS IN ELECTROSPRAY, THERMOSPRAY, AND RELATED TECHNIQUES

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In recent years spray techniques have become increasingly popular as a means of ionizing high molecular weight, nonvolatile ionic compounds. The spectacular results obtained by electrospray during the past two years (1-3) on large peptides, small proteins, and oligonucleotides have further whetted the interest of the biological community. While there is great interest in these techniques, there is also considerable confusion about the details of the apparatus, the mechanisms involved, the distinctions between various approaches, and the relative merits and capabilities of each. The ambitious goal of this presentation is to at least partially clarify some of these issues.

For purposes of this discussion, and will apologies to techniques which don't quite fit in one of these categories, we may classify the techniques according to the method used for producing the spray. These then become electrospray, thermospray, and aerospray (as suggested at Sanibel Island by John Fenn) to include those techniques which employ gas flow nebulization. In all of these techniques we can separate out five more or less distinct steps in the overall process evolving from a liquid flow to ions in the low pressure gas required by the mass spectrometer. These are the following:

1. Nebulization
2. Droplet Charging
3. Vaporization
4. Ionization
5. Ion transport

1. Nebulization

In electrospray the liquid emerging from a sharpened, small bore hypodermic needle is nebulized by direct application of a high electric field to the surface of the liquid. This produces very fine droplets apparently charged to their Rayleigh limit, and works best at very low flow rates on the order of a few microliters per minute. In aerospray devices, such as that pioneered by Iribarne and Thomson (4) and further developed and applied by Covey and Henion the liquid is nebulized by a high velocity jet of gas directed either transverse to or coaxial with the liquid nozzle. In thermospray the high velocity gas jet is generated in situ by vaporization of a portion of the liquid, which provides very efficient nebulization into relatively small droplets and provides a convenient source of heat for vaporizing large liquid flows. Thermospray generally works best at the higher end of the flow rate regime extending to several mL/min, while aerospray seems to perform best at intermediate flows.

2. Droplet Charging

In electrospray the droplets are charged directly in the nebulization process. This results in the smallest and most highly charged droplets of any of the spray techniques. In aerospray charging is by application of a high electric field to the aerosol. The detailed mechanism of this "induction" charging is not entirely clear (at least to me) but probably results from removal of ions of opposite charge produced in the spray and from polarization and disruption of initially neutral droplets. In thermospray the major charging mechanism is the statistical charging resulting from violent disruption of the liquid containing ions in solution. The latter technique produces essentially equal populations of positive and negatively charge droplets, while the former two produce droplets of the same sign as the applied voltage.

3. Vaporization

In all of these techniques production of molecular ions from the charged droplets requires nearly complete vaporization of the solvent. In the first two techniques the heat of vaporization is supplied by a relatively high flow (ca. several L/min) of heated gas at atmospheric pressure, while in thermospray the heat is supplied to the liquid in the capillary while producing the spray and downstream by heating the vapor at reduced pressure. As has been noted by Henion (6) thermospray can be done at atmospheric pressure, but the performance is poor, presumably because loss of ions by recombination is more important at higher pressures.

4. Ionization

Two basic mechanisms have been proposed for the production of molecular ions from highly charged liquid droplets. These are basically the ion evaporation model proposed by Iribarne and Thomson (7) and the Rayleigh disintegration model discussed by Roelgen and coworkers. (8) Considerable controversy remains concerning the relative importance of these two models, partly because it has proven rather difficult to design experiments that will unambiguously discriminate between them. In the ion evaporation model partially solvated molecular ions are evaporated in competition with evaporation of neutrals as a result of lowering the barrier to ion evaporation resulting from the high electrical field at the surface of a highly charged droplets. For large droplets their stability is clearly set by the ability of surface tension to resist the repulsion of the excess charge. This Rayleigh disruption may either lead to subdivision into two more or less equal droplets or because of local perturbations may expel its excess charge as a jet of small charged droplets. When the Rayleigh disruption has proceeded sufficiently, as it must as vaporization continues, eventually we may end up with only one sample molecule in a charged micro droplet. Further evaporation of solvent gently releases a molecular ion into the gas phase. It may not be possible, or necessary, to distinguish between these mechanisms, since both work best if we produce the maximum number of the smallest size droplets charged to their Rayleigh limits, and then cause the droplets to completely evaporate their solvent molecules.

5. Ion Transport

The most difficult part of the process appears to be getting the molecular ions removed from their birthplace and transported to the vacuum of the mass spectrometer. For compounds significantly more polar than the solvent, all of these spray ionization techniques appear capable of very high ionization efficiencies, probably approaching 100%, but efficient extraction from a high pressure environment in the presence of large quantities of condensable vapor is nontrivial. In both electrospray and aerospray, this is accomplished by sweeping dry gas in the opposite direction to ion transport to sweep away the vapor while an electrical field is applied to cause the ions to drift in the desired direction. In thermospray heat is applied to prevent condensation, and the sampling is done from a lower pressure. In all cases the major loss of sensitivity is due to the very low efficiency with which ion sampling is done.

Summary

All of the techniques discussed have provided useful results, and all have their limitations. To date thermospray is the most widely applied primarily because of its ability to handle normal analytical LC flows and to provide sensitive detection of a wide range of compounds under a variety of LC conditions. Electrospray has provided the most spectacular results on high molecular weight compounds, presumably because it provides the most efficient method for producing the highest specific charge, but applications have been limited by the low flow rates required. Variations of the aerospray approach show considerable promise because of their ability to bridge the gap between electrospray and thermospray. The recent development of capillary electrophoresis techniques should provide a boost for electrospray because of the obvious compatibility of the two techniques.

DESORPTION TECHNIQUES: HOW THEY ARE DIFFERENT

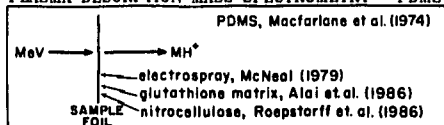
Robert J. Cotter

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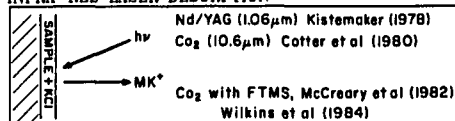
The so-called "desorption" techniques are many and varied. The different methods employ primary particle energies from the 5 KeV to 100 MeV range or photon densities of greater than 1 MW/cm^2 , and a complex array of matrices including glycerol/thioglycerol, salts, nitrocellulose surfaces, glutathione solutions, finely divided platinum powder and the UV-absorber: nicotinic acid (Figure 1). What they have in common is that they are all effective in producing "soft" ionization of large and complex molecules directly from the condensed phase, and have thus been invaluable for the elucidation of the structures of biomolecules. They are different from electron impact (EI) which uses 70 eV electrons to ionize molecules already in the gas phase and results in extensive fragmentation, or chemical ionization (CI) which is soft, but requires that the samples be volatile.

These high energy projectiles accomplish this remarkable feat of soft ionization because the energy is absorbed and isomerized by the substrate holding the sample before it reaches the molecules of interest (Figure 2). It is this part of the process that has received the

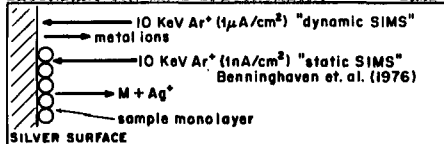
PLASMA DESORPTION MASS SPECTROMETRY PDMS



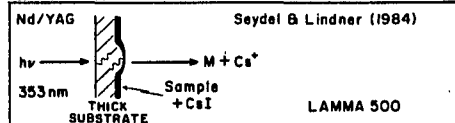
INFRA-RED LASER DESORPTION



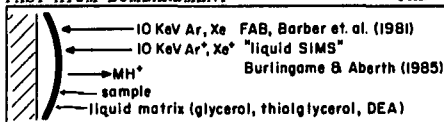
SECONDARY ION MASS SPECTROMETRY SIMS



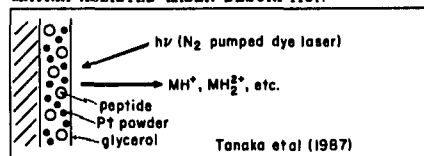
SHOCK WAVE DESORPTION



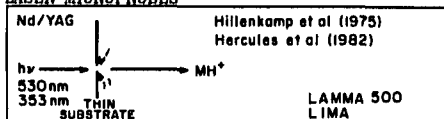
FAST ATOM BOMBARDMENT FAB



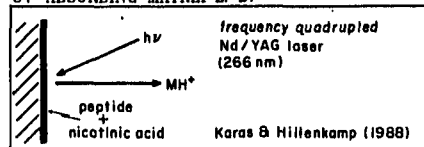
MATRIX ASSISTED LASER DESORPTION



LASER MICROPROBES

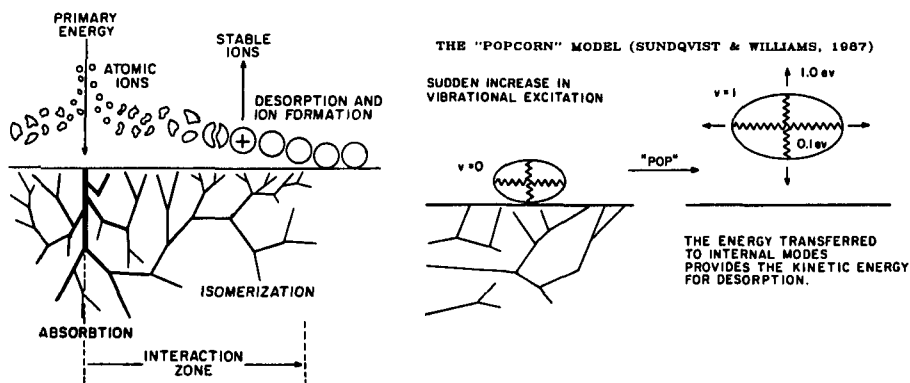


UV-ABSORBING MATRIX L. D.



most attention from physicists and theoreticians attempting to shed some light on the "mechanisms." "Thermal spikes," "collision cascades" and "popcorn" (Figure 3) models have all been proposed. As the molecular particles leave the surface of the substrate, it is not clear whether they leave with a charge ("preformed" ions) or acquire protons by collision with "reagent" ions present in the dense plasma. What is clear, however, is that the spectra from all of the techniques resemble CI spectra, i.e. they consist of stable even-electron ions which are internally quite "cool." This low internal energy may result from the fact that internal modes are not highly excited by the desorption process ("popcorn" model) or from a cooling process after desorption due to "solvent shedding."

ENERGY ABSORPTION, ISOMERIZATION AND THE FORMATION OF SECONDARY IONS: THE MECHANISMS

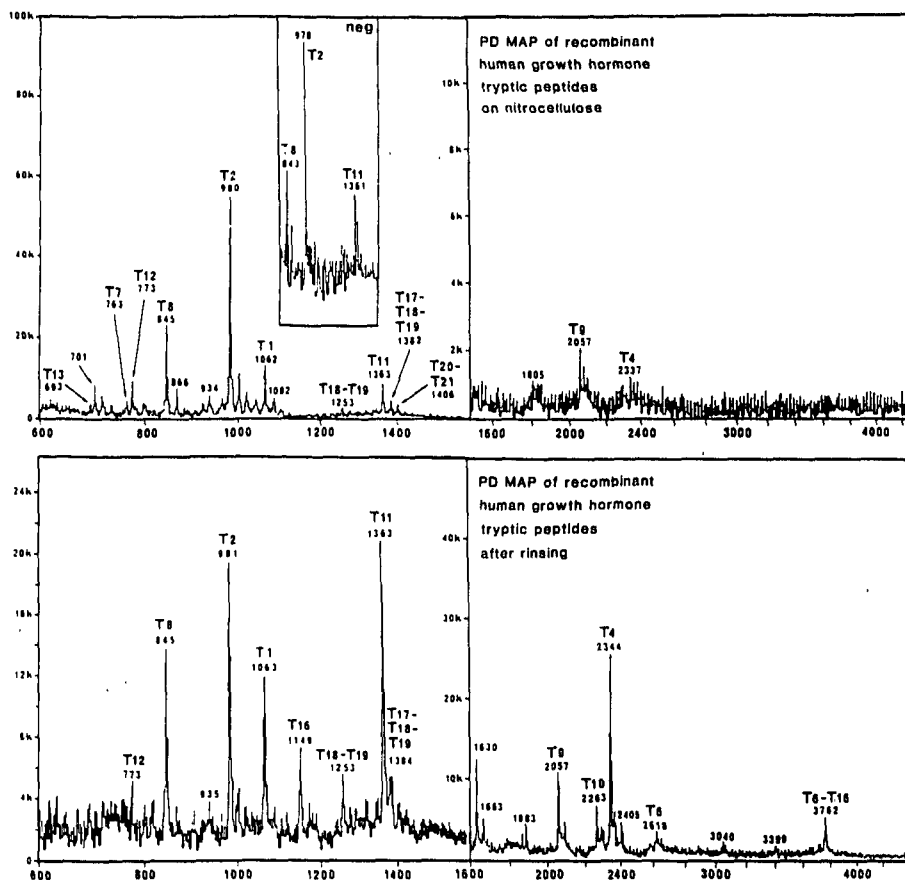


The different desorption techniques give remarkable similar spectra, which can be interpreted with almost the same rigor that EI spectra can be interpreted. While it is not clear (in the complex multi-body collisional process of ion formation) where the proton is attached, heteroatoms (those which contain non-bonding electrons) are as good a place to start as in the interpretation of EI spectra. Thus, a proton attached to the N-atoms in each of the amide bonds of a peptide or the O-atoms in each glycosidic bond of a polysaccharide can be used to explain fragmentation of the adjacent bonds. The interpretation differs from EI, however, in that H-transfers are assumed where appropriate to the formation of an even-electron ion. Thus, the Y' N-terminal sequence ions from a peptide contain two additional protons: one resulting from proton attachment to the amide nitrogen and the other a H-transfer accompanying fragmentation. This close attention to protons is crucial when one considers that glutamic acid and glutamine, aspartic acid and asparagine, and glucose and glucosamine all differ by one mass unit.

But, most interesting is how desorption spectra differ. The attachment of alkali ions to form molecular ion species (and some of the fragments) is more prevalent in IR laser desorption (see Figure 1) than in fast atom bombardment, although spectra from both methods follow the same even-electron rules. The protonated oligosaccharides produced by fast atom bombardment tend to produce little fragmentation, but cleavage of the glycosidic bonds can be

improved by permethylation or peracetylation of the sample. On the other hand, the fragmentation of MK^+ ions produced by IR laser desorption is extensive, but results almost exclusively in two-bond ring cleavages (Figure 4). Derivatization, in this case, actually reduces fragmentation.

Hydrophobicity plays an important role in desorption techniques. In a mixture of peptides, the more hydrophobic residues reside on the glycerol surface in fast atom bombardment, and "suppress" the ion signal from the hydrophilic residues. Hydrophobicity plays an equally important role in plasma desorption mass spectrometry, but for different reasons. Figure 5 shows the PDMS spectrum (PD-MAP) of the tryptic digest of recombinant human growth hormone (rHGH) obtained from Genentech. The sample had been deposited on a nitrocellulose surface, and when washed produced the spectrum shown in Figure 6. In this case, the hydrophilic peptides were removed by washing and allowed observation of the hydrophobic residues (many of these at higher mass).



INTERACTIONS OF SOLIDS, LIQUIDS, AND VAPORS IN ICP-MS: POTENTIAL FOR ADVANCES

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The advent of inductively coupled plasma mass spectrometry (ICP-MS) has provided a great advance in the capability to determine elements at levels typically two orders of magnitude below what is possible by inductively coupled plasma optical spectrometry (ICPAES). This has consequently opened up many avenues of opportunity for ICP-MS to explore approaches that were effectively closed to ICPAES. Examples include the use of coupled techniques, such as liquid chromatography ICP-MS (LC/ICP-MS) and flow injection ICP-MS (FI/ICP-MS), where the absolute amount of analyte passing to the detector in a transient mode is extremely low. However, even in these situations, there is a further need to improve system performance.

Some important areas in which ICP-MS might be usefully improved are: (1) lower detection limits (2) reduced isobaric interferences (3) improved short and long term precision. There are clearly many ways in which these goals might be pursued, but we have attempted to determine the manner in which the sample introduction process may influence these parameters. Specifically, we have carried out a systematic series of studies in which the primary aerosol is heated in one chamber, then the resultant partially evaporated aerosol is cooled in a subsequent chamber. The manner in which the evaporation processes take place is shown in Figure 1.

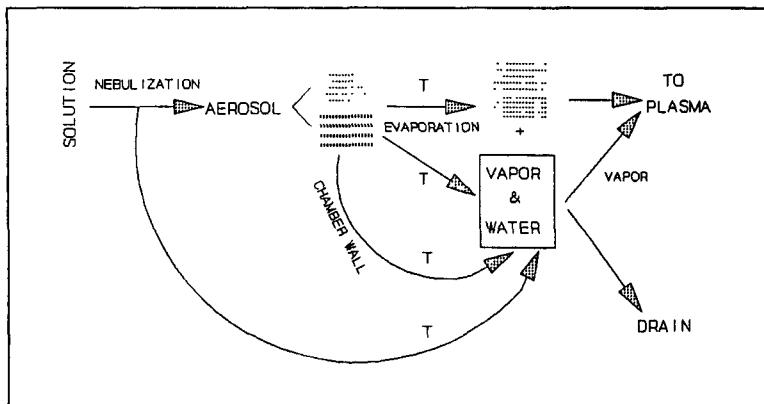


Figure 1. Model for aerosol evaporation: aerosol and vapor transport.

While the concept of the heater/condenser experiment is certainly not new, the manner in which it has been carried out has led to a number of significant advances both in our understanding of basic processes taking place in the interface, and in important aspects of performance, such as improved detection limits. The basic system is shown in Figure 2.

A number of basic studies with aerosol evaporation processes have been carried out, paying particular attention to: (1) the influence of the evaporation process on the aerosol drop size (2) the analyte mass transport to the plasma (3) the solvent aerosol mass transport to the plasma and (4) the solvent vapor mass transport to the plasma. It has been demonstrated that as the heated evaporation chamber temperature is raised, the mean drop size decreases non-uniformly as the temperature is raised. At higher chamber temperatures, the solvent is largely evaporated, which allows a much higher percentage of the analyte, now in the form of smaller, more concentrated drops, to reach the plasma. The mass transport of solvent aerosol remains constant at all desolvation chamber and condenser temperatures, and the solvent vapor transport is determined only by the condenser temperature.

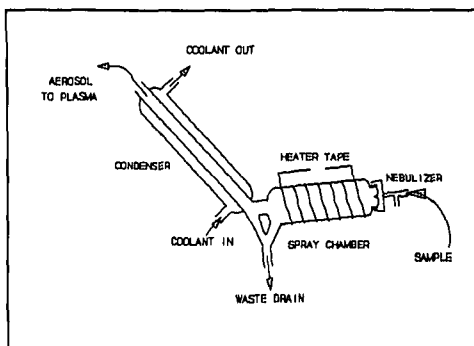


Figure 2. Schematic of desolvation system for ICP/MS.

The net result of controlling the evaporation chamber and the condenser at optimum temperatures is to cause an increase in analyte mass transport passing to the plasma of approximately one order of magnitude. With a certain amount of element-to-element variation, this increase in analyte mass transport is reflected in a corresponding increase in signal, and decrease in detection limits for the elements tested. This behavior is contrasted with that experienced in ICPAES, where increases in background level in the plasma largely counteracted the influence of analyte mass transport increase. In the case of ICPAES, therefore, much smaller signal-to-noise ratio enhancements were observed for the same sample introduction setup as that used for ICP-MS.

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PLASMA MASS SPECTROMETRY FOR CHROMATOGRAPHIC DETECTION

by

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Inductively coupled plasma source mass spectrometry (ICP-MS) affords high sensitivity and low levels of detection for elemental analysis. When coupled with chromatographic methods for separation, it provides unparalleled low level detection and speciation for elemental species of toxicological or environmental concern. It is well known, that various elemental forms have markedly different toxicities as demonstrated, for example by elemental mercury, when compared with methyl mercury compounds. This discussion illustrates that sub-ng to pg detection levels are attainable for various arsenic and tin species after separation with high performance liquid chromatography (HPLC). Other analytical figures of merit such as linear dynamic range and precision are also discussed.

The advantages of HPLC with ICP-MS detection are several: low detection levels, real time chromatograms, multielement or isotope capability, ability to separate interferences from peaks of interest and the potential for measuring isotope ratios on eluting peaks. The interface requirements of HPLC-ICP-MS are relatively simple requiring flows compatible with ICP nebulization rates and minimization of both dead volume and transport of organic solvent vapor to the plasma.

Since urine is the major pathway for elimination of arsenic from the body, it is an important body fluid to monitor for the presence of arsenic. Braman and Roebuck have reported natural levels of 25 ppb or less for four arsenic species of major concern; arsenite (containing As^{+3}), arsenate (containing As^{+5}), monomethylarsonate (MMA) and dimethylarsinate (DMA)¹. In our studies anion exchange HPLC was utilized for separation by employing an amino-bonded phase column with a phosphate/acetate buffer in 30% methanol². Excellent signal to background and good separation are obtained with as little as 2 ng of the arsenic moieties spiked into urine. Detection levels range from 20 to 96 pg for the various As species cited above. In Cl^- containing samples ArCl^+ is formed in the plasma. This has the potential to interfere with any arsenic determination since it occurs at m/z of 75 along with the monoisotopic arsenic. However, in this study by using peak height as the signal, sufficient separation was available so that the potential interference with the arsenite peak was not serious. Comparisons with Fisher Uricheam analyzed standards and NIST certified SRMs showed a good correlation. Linear dynamic ranges (LDRs) were demonstrated at over 3 orders of magnitude. This low level detection capability allowed minimal sample handling. For actual urine samples, only filtering through a 0.2 micrometer filter was necessary prior to HPLC injection. Further detection enhancements at the low pg levels can be achieved by post-column generation of arsine from the eluting species followed by transport to the plasma.

To further assess the utility of HPLC-ICP-MS, studies of alkyltin compounds are discussed. The initial study involved developing separation and detection schemes for trimethyltin chloride (TMT-Cl), tributyltin chloride, and triphenyltin acetate. Both ion

exchange and ion pair HPLC were utilized. Strong cation exchange chromatography was employed with 0.1 M ammonium acetate in 85% methanol as mobile phase³. The ion pair chromatography was done using a C₁₈ column and sodium pentane sulfonate as the ion pairing reagent dissolved in 80% methanol at pH 3. Because of the high amount of organic solvent, background levels were appreciably elevated yielding as high as 11,000 cps, in contrast to the approximately 100 cps attained with equivalent salt concentrations in aqueous solutions. Nevertheless, detection levels ranging from 0.4 to 1 ng were obtained in these experiments, a factor of 1000 lower than those attainable with the same chromatography utilizing ICP optical emission detection³. Precisions on the order of 8% RSD were obtained with both separation types, while LDRs of 3 orders of magnitude were better for the ion exchange chromatography due to the better separation.

With the hope of minimizing the detrimental effects from the organic solvent, micellar liquid chromatography was employed⁴. Sodium dodecyl sulfate was dissolved in purified water at concentrations up to 0.1 M (~3% salt). Below this concentration the separations were not as efficient and above it resulted in clogging problems in the torch injector and the mass spectrometer sampler interface. Only those alkyltin salts of propyl- molecular weight or lower were suitable because of the capacity factor. However, for TMT-CI a detection level of 27 pg was obtained, a factor of 10 lower than the initial study³. Enhanced signal/background was clearly noted in these experiments because the organic solvent was eliminated. Precisions were improved to less than 2% RSD and the LDRs were taken over 3 orders of magnitude.

These studies show that HPLC-ICP-MS can provide detection at the sub-ng to pg levels for selected elemental species of environmental or toxicological concern.

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ATTEMPTING TO REDUCE MOLECULAR ION INTERFERENCES IN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

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In the six years since the introduction of commercial ICP-MS systems there has been little reduction in the levels of interfering molecular ions (relative to atomic (analyte) ions). A variety of techniques such as changes in the differentially pumped interface, changes in plasma chemistry, desolvation and direct solid sampling have been evaluated and have proved only moderately successful in reducing interferences. The origin of the molecular ions is not always clear but in our view is likely the ICP itself and only to a lesser extent ion molecule chemistry in the differentially pumped interface since the number of potentially reactive collisions in the free-jet expansion is calculated to be small (1). The calculation assumes an ideal skimmed beam, free of shock wave interferences, background gas penetration, and scattering losses which occur to varying degree with any real beam source and which could in principle produce substantially higher levels of molecular ions than estimated by the ideal beam theory. Thus in a series of experiments reported here the levels of some molecular ions relative to analyte ions (of similar mass) were measured for different sampler skimmer separations (x_s). In a second series of experiments the use of triple quadrupole mass spectrometry to remove molecular ion interferences was explored with some preliminary results reported below.

The molecular ions studied and the solution concentrations used are shown in Table 1. The aerosol flowrate (at 1200w power, 15 mm sampling depth) was chosen by measuring the analyte signal vs. aerosol flowrate and then measuring the molecular ion ratio at an aerosol flowrate which gave an analyte signal one fifth of the peak response. This procedure approximates the operating conditions normally used to minimize oxide interferences and matrix effects, and was also found to remove some (small) variations in the level of molecular ions at a fixed flowrate with different sampler skimmer combinations.

The measured levels of interfering ions for four sampler skimmer separations are shown in Table 2. It is seen that (i) for all the sampler-skimmer separations there is no substantial reduction in the molecular ions (ii) at $x_s = 8.5$ and 10 mm there is a significant increase in the ratios ArO^+/Co^+ , ClO^+/Cr^+ , and $NaAr^+/Co^+$. The observations are consistent with the expected gas dynamics of the interface. The calculated Mach disc position (x_m) of the free-jet is 9.0 mm from the sampler. For skimmer positions near or downstream of the Mach disc ($x_s = 8.5, 10$ mm) a skimmed beam is not formed and a shock is formed in front of the skimmer; the gas stagnates and then re-expands through the skimmer with a greater chance for ion molecule chemistry to produce molecular ions. Thus the observations of table 2 are consistent with the hypothesis that ion molecule chemistry does not occur to a major extent in the expansion, particularly if the free-jet is skimmed properly ($x_s < x_m$). More practically, substantial reductions in molecular ions were not achieved from these interface changes.

A consideration of the kinetics of triple quadrupole mass spectrometry shows that if the dissociation cross sections for the molecular ions are large and if the scattering loss cross sections for atomic ions are small then substantial reductions in the ratio of molecular to atomic ions are possible by dissociating the molecular ions in the collision cell of a triple quadrupole MS system (ICP-MS-MS). Thus the total loss cross sections (i.e. the sum of dissociation, scattering, reaction etc.) for some atomic and molecular ions were measured here (at 50 eV LAB energy) with the results shown in Table 3. It is seen that (i) the loss cross sections are similar for atomic and molecular ions and (ii) for the atomic ions the cross sections are significantly larger than those expected purely from scattering losses (ca. $2\text{\AA}^2$) (2). It appears that (i) the dissociation cross sections for the molecular ions studied are small ($<5\text{\AA}^2$) and (ii) all species can be lost from the ion beam due to charge transfer to the argon target used. Support for this second postulate comes from (i) the large loss cross section for N_2^+ (which is near resonant with argon for charge transfer) (ii) the observation of Ar^+ as the major reaction product from ClO^+ , Ar collisions with a threshold at that expected for charge transfer (iii) the observation of Ar^+ from Cd^+ , Ar collisions with a measured cross section similar to the loss cross section of Table III.

Finally the use of ion molecule chemistry to significantly reduce molecular ion interferences was investigated. Cerium and Terbium were run to produce Ce^+ , CeO^+ and Tb^+ shown on a logarithmic scale in the spectrum of fig 1a. Oxygen was then used as a reactive gas in the collision cell and a constant addition linked scan carried out with the third quadrupole 16 amu higher than the first. The rare earth ions react with near gas kinetic cross sections to produce MO^+ in the collision cell which is transmitted by the third quad; the oxide ions react to a much lesser extent and so are effectively removed from the spectrum as shown in fig. 1b. Ion molecule chemistry can thus be used, at least to some extent to substantially remove molecular ions from ICP-MS spectra. We also note that ion molecule chemistry could in principle be used to separate atomic isobaric interferences e.g. $^{58}Fe^+$, $^{58}Ni^+$ since the chemistry of these ions is expected to differ.

References

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- (2) P.H. Dawson and J.E. Fulford, Int. J. Mass Spec. Ion Phys. **42**, 295, 1982.

Table 1 - Solution Concentration

Ratio	Solution Concentration
CeO^+/Ce^+	0.1 ug/ml Ce
ArO^+/Co^+	0.1 ug/ml Co
Ar_2^+/Rb^+	0.1 ug/ml Rb
ClO^+/Cs^+	0.1 ug/ml Cs, 2% HCl V/V
$ArCl^+/Rb^+$	0.1 ug/ml Rb, 2% HCl V/V
$NaAr^+/Co^+$	0.1 ug/ml Co, 1000 ug/ml Na

Table 2
Molecular Ion Ratios at Four Skimmer Positions

Ratio	At $x_8 =$ 6.0 mm	At $x_8 =$ 7.5 mm	At $x_8 =$ 8.5 mm	At $x =$ 10.0 mm
CeO^+/Ce^+	0.035	0.025	0.026	0.033
ArO^+/Co^+	1.9	2.9	1.9	5.7
Ar_2^+/Rb^+	6.3	6.8	4.3	4.1
ClO^+/Cr^+	0.65	0.53	0.73	1.9
$ArCl^+/Rb^+$	0.10	0.17	0.10	.20
$NaAr^+/Co$	0.20	0.13	1.0	1.0

Table 3 - Total Loss Cross Sections
50 eV LAB Energy, Argon Target

Ion	σA^2
Cr^+	12
Cd^+	7.5
Se^+	14
Ar_2^+	27
ClO^+	9.5
$ArCl^+$	28
UO^+	17
N_2^+	41

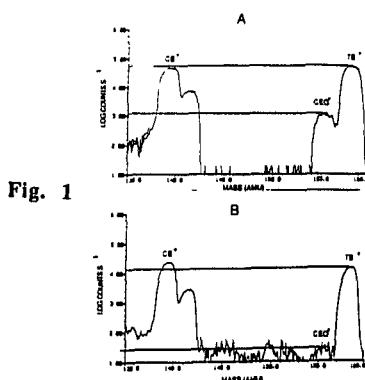


Fig. 1

TIME RESOLVED STUDIES IN GLOW DISCHARGE MASS SPECTROMETRY

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Glow discharge mass spectrometry (GDMS) normally employs a continuous dc argon discharge for sputter atomization and ionization. Pulsed gas discharges have been shown to exhibit certain advantages for enhanced atomic emission, suggesting the possible concurrent improvement for atomic mass spectrometry. For a common average discharge current, decreasing the duty cycle causes higher pulse currents and voltages. This produces greater sputter yield because of the higher energy of the incoming argon ions. Our studies revealed also that advantageous plasma processes occur, encouraging time resolved data collection.

EXPERIMENTAL

A quadrupole-based glow discharge mass spectrometer was used to collect all data. The power supply for the discharge operated in the dc or pulsed mode, when driven by an external pulse generator. A data gate was moved along the pulse envelope by appropriate delays and the resulting spectra compared at selected intervals. All data were collected by pulse counting and displayed on a multichannel analyzer before an eventual hard copy product.

RESULTS AND DISCUSSION

Initial experiments showed that enhanced sputter ablation was observed in the pulse mode compared to dc, as expected. Surprising, however, was the ion pulse shapes that resulted, as shown in Figure 1. The ion profile for the background gasspecies (e.g., OH, CO) was significantly different than that for the sputtered species. A large "afterpeak" resulted in an increase in the analyte signal as shown. By placing the data gate over the afterpeak, a mass spectrum was obtained that showed a high predominance of the analytical sample ions, as indicated in Figure 2. In this way, the often interfering background gas ions are discriminated against.

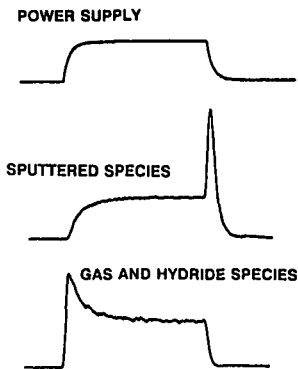


Figure 1. Typical ion profiles from the pulsed discharge.

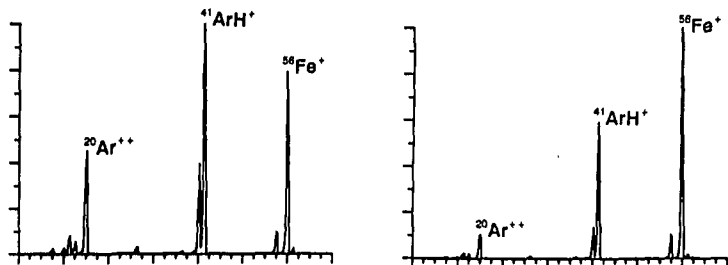


Figure 2. Comparison of mass spectra taken from the plateau vs. the afterpeak region.

Time resolution studies taken throughout the pulse envelope showed the difference in ion signals. Particular differences were observed at the induction period and afterpulse regions. The spectra taken on the plateau were similar to dc spectra, as the discharge reached an equilibrium within 1-2 msec. Comparison of afterpeak vs. plateau also showed that dimers form less readily in the former. The cause of the afterpeak is suspected to be the creation of additional argon metastables as the discharge relaxes. This possibility is supported by results from the introduction of methane, a metastable quencher, which causes a sharp reduction of the afterpeak.

**NEW DEVELOPMENTS IN SOURCES FOR
PLASMA-SOURCE MASS SPECTROMETRY**
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In plasma-source mass spectrometry, appropriate choice of the ion source affects not only sensitivity, but also precision, stability, and accuracy. Currently, the most widely used source for this technique is the inductively coupled plasma (ICP). However, the source commonly employed in ICP-MS has been adopted virtually unchanged from its counterpart long employed in emission spectrometry. There is no reason to believe that changes in operating frequency, plasma-torch configuration, discharge dimensions, or gas composition would not result in a further improvement in ICP-MS characteristics. Indeed, many workers have already noted substantial interelement interferences in ICP-MS and have attributed them alternatively to events that occur in the plasma, to those that arise in the mass spectrometer or ion optic assembly, or to those which arise during the expansion process between the atmospheric-pressure plasma and the lower-pressure ion-optic zones.

An even greater range of alternatives arises if completely different plasma sources are considered for use with mass spectrometry. In fact, one of the most useful sources to date has proven to be the glow-discharge lamp, a device substantially different from the atmospheric-pressure ICP. Another interesting source, which combines many of the attributes of the GDL and ICP, is the microwave-induced plasma (MIP). An MIP is ordinarily sustained at microwave diathermy frequencies (2.45 GHz) and can be operated at either atmospheric or reduced pressure. At atmospheric pressure, a moderate-power MIP exhibits many of the characteristics of the ICP and, indeed, would be expected to yield analytical characteristics that are similar. MIP discharges have the added advantage of operating at lower gas flows and powers than are customarily employed with an ICP. Furthermore, commonly available microwave support structures such as resonant cavities and wave-launching devices are capable of sustaining viable discharges in helium. This capability is especially important when coupled to mass-spectrometric detection because of the high ionization and metastable energies of helium. For example, it has already been shown that a helium plasma is capable of ionizing fluorine efficiently, something which is difficult in an argon-operated plasma. Fluorine is not efficiently ionized in an argon discharge since its ionization energy lies above that of argon.

At reduced pressures (typically 1-10 Torr), an MIP offers some even stronger attributes for coupling with a mass spectrometer. In the first place, the interfacing of a reduced-pressure discharge to a mass spectrometer is inherently more straightforward. Also, at reduced pressure the MIP should generate an even simpler background spectrum. Finally, a reduced-pressure MIP becomes an even more efficient ionization source; electron temperatures in such a plasma have been found to reach as high as 37,000K.

In this presentation, these various alternatives will be explored and compared. It will be shown that there is little operational or performance distinction between inductively coupled plasmas operated at 27.12 or 40.68 MHz. Although the higher-frequency discharge produces somewhat lower ratios of oxide ions to atomic ions and slightly higher ratios of doubly charged to singly charged ions, it yields detection limits that are comparable to or just slightly worse than those found with the 27-MHz plasma. Also, unless appropriate care is taken, a sampling-orifice-linked discharge often distorts results obtained with the higher-frequency plasma.

Conventional ICPs are sustained in a torch having an inside diameter of approximately 18 mm. We have explored the use of smaller torches having inner diameters of 13 mm and 9 mm, respectively. When operating conditions (input power, gas-flow rates, sampling position within the plasma, and sample-uptake rate) are optimized, the smaller plasmas are found to produce acceptable

performance, even though they can be operated at noticeably lower radio-frequency power and gas-flow rates. The 13-mm torch appears to be superior, however, because its larger dimensions makes positioning of the plasma with respect to the mass-spectrometry sampling orifice less critical. With both smaller plasmas, doubly charged to singly charged ion ratios and oxide-ion to atomic-ion ratios are lower than are found with a conventional ICP. However, detection limits range from being slightly better to being a factor of eight worse than are found with the usual torch.

Our mass-spectrometric examination of a helium-supported MIP has produced some surprising results. Although analytical performance levels of the MIP/MS combination were initially encouraging, especially for elements having high ionization energies, we were intrigued by unusually large interferences caused by easily ionized elements and by an extremely large mass-bias effect. This latter effect, which reflects differing sensitivities for atomic ions of differing mass, could be explained quite readily. Helium, being a far lighter gas than argon, produces a supersonic expansion of much greater velocity. As a result, analyte (sample) ions which travel with the expanding helium bath gas achieve a far greater velocity than they would in an expanding argon stream. Because analyte ions were assumed to reach the bath-gas velocity, their kinetic energies and, accordingly, their kinetic-energy differences would be far greater in a helium expansion than in one comprised of argon. The observed mass bias was surmised to be a result of this greater kinetic-energy spread.

To examine our hypothesis more carefully, we elected first to study in detail an argon expansion, since it had been examined also by other workers. In these initial studies, emission, fluorescence, and electrical-probe measurements were performed, with the optical observations occurring in both the atmospheric-pressure plasma and in the reduced-pressure expansion (interface) zone. Surprisingly, we found that even in the relatively high-pressure (nominally one torr) region in the interface, charge separation seemed to be virtually complete. By the time the expanding plasma plume reaches the second (skimmer) orifice, positive-ion concentrations exceed those of electrons by factors of at least 50. Furthermore, concentrations of selected sample ions in the expanding stream were affected noticeably by concomitant ions. In particular, the greater the mass of the concomitant ion, the lower was the observed concentration of the selected analyte ion. We believe these findings suggest one possible source of the mass-dependent interference that many workers have already observed in ICP-MS.

Carrying these studies further, we have measured ion kinetic energies at several locations in the expanding plasma plume by converting our mass spectrometer into a retarding-plate analyzer. Kinetic-energy measurements taken in the second stage of our vacuum system (held at approximately 10^{-3} torr) show little difference in kinetic energies of analyte ions expanding in an argon stream. In fact, the kinetic energy attained by most ions approximates that expected for the argon bath gas at the plasma temperature. We believe this finding results from the dominant influence of the bath-gas ions near the retarding plate. In contrast, when kinetic-energy measurements are made in the lowest-pressure region of our instrument (nominally 10^{-6} torr), mass-proportional kinetic-energy differences are observed among analyte ions. These latter investigations will be described in greater detail in a companion poster presentation from our research group.

ION KINETIC ENERGIES IN THE LOW-PRESSURE ZONES OF AN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER

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Most inductively coupled plasma mass spectrometers (ICP-MS) consist of two or three differentially pumped stages. In these systems the analyte-containing plasma is sampled at atmospheric pressure and a portion of this sampled plasma expands into subsequent mass-spectrometer stages. In available ICP-MS systems the first stage has been designed to optimize the skimming of the supersonic free-jet expansion described by gas-dynamics theory. However, many of the equations that specify the geometry of the supersonic free-jet expansion assume a perfect, homogeneous, neutral gas. A departure from these ideal conditions that occurs in ICP-MS instruments can be attributed to the presence of ionized species, the addition of an analyte-containing aerosol, the entrainment of air, and/or a non-homogeneity in plasma-source temperature. Such departures complicate trajectory modeling of neutral bath gas and ionic species in the ICP-MS interface.

The trajectories of neutral species, though complicated, can be described by modifications to ideal gas-dynamic theory that take into account deviations from ideal-gas behavior. One such deviation, observed in our experiments, is that ion trajectories are influenced not only by the gas dynamics of collisions with neutrals, but also by electrostatic interactions between ions with large collisional cross-sections. These large cross-sections arise from low gas-kinetic temperatures that species attain in the first expansion stage. One of the approaches chosen to evaluate these forces has been to examine ion movement in the mass-spectrometer interface.

Ion kinetic energies have been measured in both the second and third stages of an ICP-MS that has been converted to a retarding-plate analyzer. These studies have been coupled with Langmuir probe analysis (1), used to characterize the constituents of the expanding plasma, and have led to the identification of an on-axis positive space-charge region (sheath-like in nature) that extends from the skimmer cone into the second stage approximately 10^{-3} torr of the mass-spectrometer interface. Ion kinetic-energy measurements in this second stage reaffirm the existence of this "sheath" formation. Mean ion kinetic energies measured in the second stage for light and heavy ions are similar (see Figure 1). This phenomenon is believed to be due to a large bath-gas-ion population and substantial ion collisional cross-sections. In the second stage, pressures are sufficient to allow ion-ion interaction, thus reducing the mobility of the analyte ions, which are being "swept" along by the bath-gas ions. The focusing of analyte ions is believed to be achieved indirectly through manipulation of the bath-gas ions. In the third stage, ion kinetic energies cover a range of approximately 5 eV from lithium (7 amu) to uranium (238) (Figure 2). This observed ion kinetic-energy spread is attributed to a low third-stage pressure (normally 10^{-6} torr). In the third stage, analyte ions are less influenced by ion-ion interactions, and are thus able to exhibit mass-dependent kinetic energies. In the ICP-MS third stage individual ions are expected to be focused on the basis of their own kinetic energies.

The use of ideal-gas-dynamic theory alone is not sufficient to model the sampling process employed in an ICP-MS system. Such theories do not take into account ion-ion interaction. The

present experiments have allowed us to identify some of the forces that determine ion trajectories in the mass-spectrometer interface and are being used to redesign both the ICP-MS first-stage interface and ion-optic system.

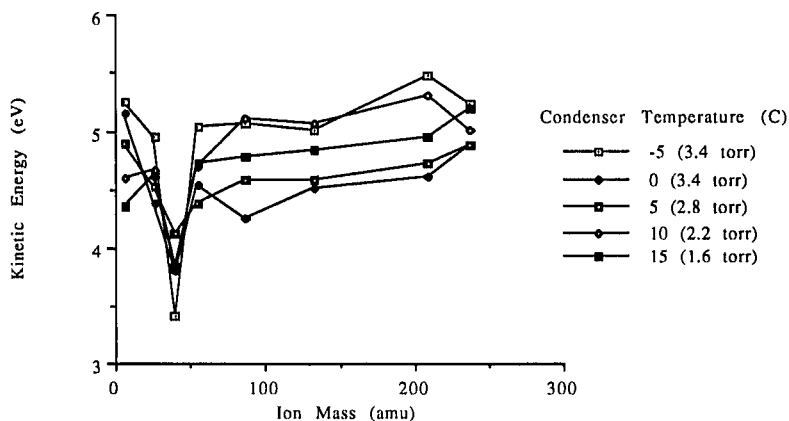


Figure 1. Mean ion kinetic energies for second-stage retarding. Optimum skimming conditions were used (e.g. first-stage pressure and desolvator condenser temperature).

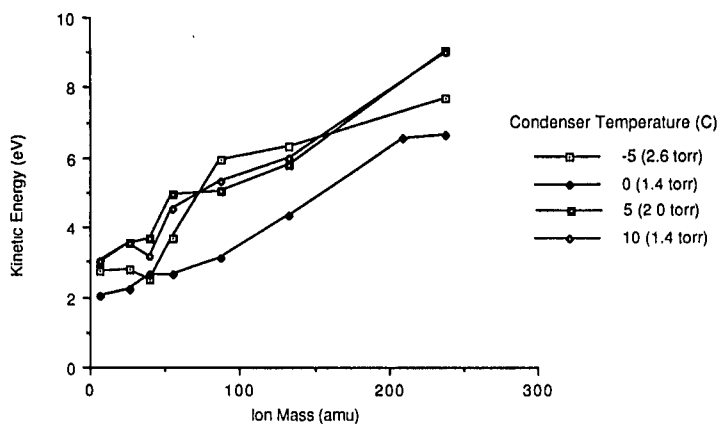


Figure 2. Mean ion kinetic energies for third-stage retarding. Optimum skimming conditions were used (e.g. first-stage pressure and desolvator condenser temperature). A 0.5 mm orifice separated the second and third stage.

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SAMPLE AEROSOL, PLASMA GAS COMPOSITION AND SAMPLER-SKIMMER GEOMETRY EFFECTS
IN INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

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The inductively coupled plasma (ICP) ion source used for elemental analysis by mass spectrometry (MS) was adapted directly from the inductively coupled plasma used for elemental analysis by optical atomic emission spectrometry. This plasma is a high temperature Ar plasma maintained with a kilowatt RF supply running at 25 or 40 MHz and samples are normally introduced as aqueous aerosols. However, it is becoming increasingly clear that Ar alone may not be the best plasma support gas for ICP-MS.

The analytical performance of ICP-MS can be enhanced by the utilization of mixed gas plasmas. A mixed gas plasma is one in which part of the Ar of one or more of the three torch flows (outer, intermediate, central) is replaced by another gas such as N_2 , O_2 or air. With the addition of 5% N_2 to the outer gas flow, signal levels for many analytes (M^+) can be modestly enhanced (factors of 2 to 4). The most significant enhancements occur for those elements that have high first ionization potentials and for those elements that are strong oxide (MO) formers. On the other hand oxide signal levels (MO^+) are significantly reduced (an order of magnitude) by the addition of N_2 to either the outer or the central gas flow and a mixed gas plasma can also reduce the level of some background and matrix related species. These include ArO^+ , $ArOH^+$, Ar_2^+ , ClO^+ , and $ArCl^+$. Finally, some background species are increased by utilization of a N_2 mixed gas plasma. These include N^+ , N_2^+ , NO^+ , and ArN^+ along with a general increase in the overall background level.

Most samples are introduced into the inductively coupled plasma as aqueous aerosols. However, in ICP-MS, the introduction of samples as aqueous aerosols introduces more complexity to mass spectrometry measurements than it does to optical emission measurements. It is now recognized that a general reduction of the solvent load is beneficial in ICP-MS. The effect of various levels of aqueous solvent load reduction on analyte, analyte oxide and background species will be illustrated. These include use of a simple in line condenser, desolvation with a heating chamber and a condenser, and the combination of an in line condenser and a N_2 mixed gas plasma. Finally the background spectral characteristics of a completely dry plasma will be illustrated. In general, as the level of solvent (H_2O) removal is increased oxygen and hydrogen containing species are removed from the plasma. This can be a significant aid to a number of analyses and in fact, the introduction of samples as dry aerosols may become the preferred mode of sample introduction in ICP-MS.

The separation and the orifice sizes of the sampler and the skimmer cones also have a major effect on analyte species distribution and background signal levels in ICP-MS. Sampler-skimmer geometry experiments can be informative particularly for species that may have different formation mechanisms and zones in ICP-MS. For example, species can be formed in the central channel of the plasma, in the outer edges of the plasma (i.e., involving air entrainment), in a boundary layer around the sampler orifice, in the expansion jet or at the Mack disk. The effect of sampler-skimmer spacing on the signals of ArN^+ , $ArOH^+$, Ar_2^+ , ArO^+ , Ar_2H^+ , and several analytes (M^+) and analyte oxides (MO^+) will be illustrated. By increasing the sampler-skimmer distance 0.23 cm over that set by the instrument manufacturer (SCIEX ELAN Model 250), the relative amount of analyte oxide can be reduced but at the expense of analyte signal sensitivity. However, studies with a N_2 mixed gas plasma indicates that the increased sampler-skimmer separation (0.23 cm) may be more optimal. Some signal levels are improved and in particular the background "continuum" level introduced by the N_2 plasma is almost reduced to a normal level with the increased sampler-skimmer separation.

Finally the effect of sampler and skimmer orifice diameter on analyte (M^+) and analyte oxide (MO^+) signals will be shown. In particular, the sampler orifice diameter has a major effect on relative oxide levels. This will be illustrated for orifice sizes ranging from 0.51 mm to 0.94 mm and for analytes including La, Ho, Lu, and Yb. A curious observation will be presented that indicates that the summation of the M^+ and MO^+ signals of an oxide forming element resembles in overall response characteristics (measured as a function of nebulizer gas flow rate) the signal of a non-oxide forming element. This tends to indicate that most, if not all analyte oxides in ICP-MS are formed "downstream" of the ICP.

In conclusion, while at first glance plasma gas composition, sample aerosol processing, solvent load reduction, sampler-skimmer separation, and sampler orifice diameter may seem like disparate system parameters, they are all inextricably related to and impact on analyte and analyte oxide levels in ICP-MS. In a synergic manner, increased knowledge of the effects of these system parameters on signal characteristics should lead to improved performance in ICP-MS.

MASS RANGE EXTENSION IN QUADRUPOLE ION TRAPS

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Quadrupole ion trap methodologies have recently been extended to biomolecules and inorganic materials by coupling desorption ionization techniques to the quadrupole ion trap.¹ However, to fully exploit the capabilities of the ion trap for the analysis of biomolecules, the mass range of the ion trap must be extended. This work describes mass range extension from 650 Da (the mass limit of the commercial ion trap sold by Finnigan MAT) to 45,000 Da.

In the usual ion trap configuration, the maximum detectable mass-to-charge ratio, $(m/z)_{\max}$, is given by:

$$(m/z)_{\max} = 8V_{\max} / [q_{\text{eject}}(r_0^2 + 2z_0^2) \Omega^2] \quad (1)$$

where V is the RF peak voltage; r_0 and $2z_0$ describe the radius of the ring electrode and distance between end-cap electrodes, respectively; and Ω is the angular frequency of the RF drive voltage. The parameter q_{eject} is the point on the Mathieu stability diagram at which ion ejection occurs ($q_{\text{eject}}=0.908$ in conventional operation of the Finnigan ion trap). Hence, the mass range can be extended in four ways: (i) increasing the voltage of the applied RF, (ii) changing the trap dimensions, (iii) decreasing the frequency of the applied RF, or (iv) forcing instability at a different value of q_{eject} on the Mathieu diagram. Todd et al. have reported mass range extension to m/z 1466 through the use of a "reverse" scan, which uses both RF and DC fields and ion ejection at $q_{\text{eject}}=0.27$ along the $\beta_z=0$ line.²

We demonstrate mass range extension through (1) reduction of electrode size (16x extension)³, (2) reduction of the applied RF frequency (1.7x extension), and (3) resonance ejection at lower q_{eject} (45x extension)³. In this work, an Antek cesium ion gun was coupled to a Finnigan ion trap mass spectrometer (Figure 1). To document and evaluate mass range extension, cesium iodide clusters were injected into the ion trap for mass analysis. Figure 2 is a mass spectrum of cesium iodide clusters to 10,000 Da obtained with an electrode assembly of $r_0=0.25$ cm (1/4 radius of the commercial ion trap). Figure 3 is a mass spectrum of cesium iodide clusters to 45,000 Da obtained by reducing the frequency of the applied RF (from 1.1 MHz to 0.92 MHz) and by resonance ejection of stored ions at the $\beta_z=0.01$ line (corresponding to a value of $q_{\text{eject}}=0.014$). A supplementary ac voltage of 10 V pk-pk was applied to the end-cap electrodes during the mass scan. At a main drive frequency of 920 kHz and $\beta_z=0.01$, the frequency of the supplementary voltage is 4.6 kHz. The mass spectrum of $(\text{CsI})_n\text{Cs}^+$ to 16,000 Da obtained by resonance ejection at $\beta=0.04$ and the mass spectrum obtained using a 2.5 mm radius electrode assembly are in excellent agreement (Figure 4). Cs^+ desorption can also be used to inject biomolecules into a 'mass-extended' ion trap. Figure 5 shows the mass spectra of an injected peptide, Angiotensin I. The mass spectrum was acquired by ejection of ions at $q_z=0.42$ ($\beta_z=0.308$, axial modulation frequency=169.9 kHz).

1. R.E. Kaiser, J.N. Louris, J.W. Amy, and R.G. Cooks, *RCM*, submitted.
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Figure 1.

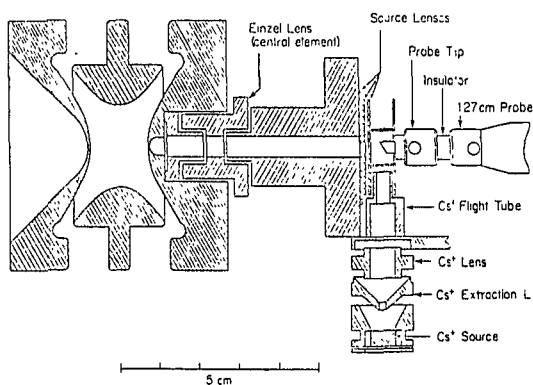


Figure 2.

$(CsI)_nCs^+$

$r_0 = 0.25 \text{ cm}$

$f = 1.1 \text{ MHz}$

$\beta_z = 1.0$

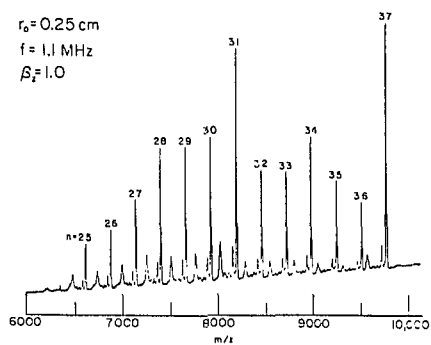


Figure 3.

$(CsI)_nCs^+$

$r_0 = 1 \text{ cm}$

$f = 0.92 \text{ MHz}$

$\beta_z = 0.01$

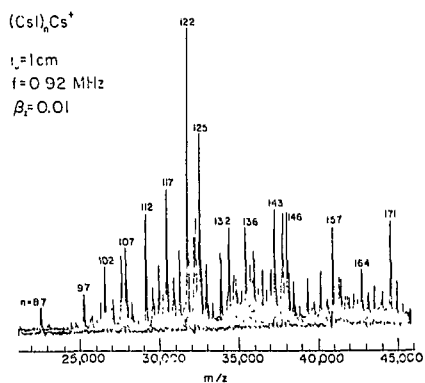
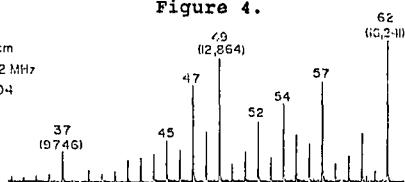


Figure 4.

$r_0 = 1.0 \text{ cm}$

$f = 0.92 \text{ MHz}$

$\beta_z = 0.04$



$r_0 = 0.25 \text{ cm}$

$f = 0.92 \text{ MHz}$

$\beta_z = 1.0$

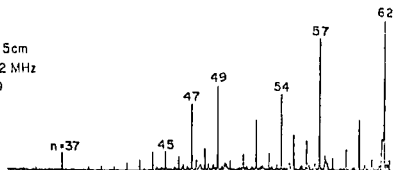


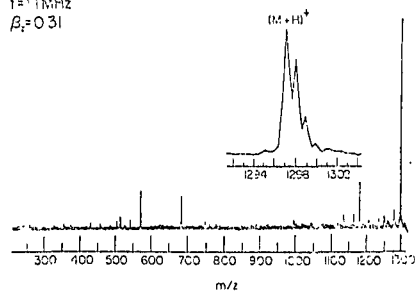
Figure 5.

A-girolensin I

$r_0 = 1 \text{ cm}$

$f = 1 \text{ MHz}$

$\beta_z = 0.31$

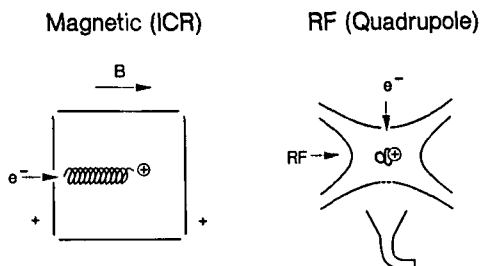


Ion Injection into Ion Traps

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The last few years have seen a dramatic increase in interest in ion trap mass spectrometers, including RF (quadrupole) and magnetic (ion cyclotron resonance) traps (see figure below), for both fundamental and analytical studies. This remarkable growth in interest has stemmed in part from the unique features provided by ion traps, in which sample ionization, mass analysis, and even detection can be performed sequentially in a single region in space. These features include the ability to perform a variety of gas-phase ionization techniques (e.g., EI and CI) and two or more stages of mass spectrometry (i.e., MS/MS/...) without physical modification of the ion trap.

Ion Traps – 2 Major Approaches



Unfortunately, performing sample introduction and ionization in the same spatial region as mass analysis seriously limits the types of samples and ionization techniques that are practical with ion traps. The obvious solution to this dilemma is to perform sample introduction and ionization in a separate ion source, external to the ion trap, and then to inject these ions into the trap for mass analysis. This approach offers a number of advantages, as summarized below:

Why Ion Injection into Ion Traps ?

-
- Remove restriction of low-pressure gas-phase ionization
 - Isolate sample matrix from ion trapping region (reduce problems with ion-molecule reactions)
 - Control number and mass range of ions in trap (minimize problems with space charging)
 - Select polarity of ions in trap (prevent losses due to ion-ion recombination)
 - Improve ion source accessibility (magnetic trap)
-

Ion Injection into Ion Traps (cont'd) - Richard A. Yost and Randall E. Pedder

The range of sample introduction and ionization methods which are available with internal and external ionization are summarized in the table below. Clearly, the major impetus behind the development of ion injection is the desire to employ modern desorption ionization methods (for condensed-phase samples) with ion traps. In addition, the high sensitivity of electron-capture negative ion CI cannot be achieved with ionization within the ion trap, since electrons will not be thermalized for efficient resonant electron capture.

Sample Introduction and Ionization with Ion Traps

	<u>Internal</u>	<u>External</u>
<u>Gas-phase:</u>		
EI	<u>X</u>	<u>X</u>
PCI/NCI (low-pressure)	<u>X</u>	—
PCI/NCI (high-pressure)	—	<u>X</u>
ECNCI	—	<u>X</u>
<u>Condensed-phase:</u>		
LD	<u>X</u>	<u>X</u>
FAB/LSIMS	—	<u>X</u>
Thermospray LC/MS	—	<u>X</u>

It is important to realize that there are two major challenges to meet in developing a practical ion injection system for an ion trap mass spectrometer, as summarized in the table below. The next two tables review the progress in achieving these goals for both RF and magnetic ion traps. Elsewhere in this volume are extended abstracts which describe a number of further advances in ion injection into ion traps, as listed in the final table.

Challenges - Ion Injection into Ion Traps

1. Injecting (getting ions into the trap)
 - Magnetic mirror effect (ICR)
 - RF phase-dependence (quadrupole ion trap)
2. Trapping (keeping them there)
 - Remove ion kinetic energy by collisions or electrodynamic cooling

Ion Injection into Ion Traps (cont'd) - Richard A. Yost and Randall E. Pedder

Ion Injection into RF (Quadrupole) Ion Traps

Concept

1959	U. Bonn	"Es ist unmöglich, ein Ion von außen in das Feld einlaufen zu lassen..."
1968	General Electric	Suggested possibility in patent

Theory

1977-82	India Tech	Injection w/ delayed RF or continuous RF
1980-81	U. Kent	Injection w/ delayed RF
1981	Texas A & M	Injection w/ delayed RF or continuous RF; effect of ion energy, angle, and RF phase

Experimental

1962	Cal Tech	20 μ m diameter Al ions (m/z 2 x 10 ¹⁰)
1984-87	Trent U.	Trapping of CID products of 4 keV ions
1987-88	Purdue U./ Finnigan	EI, LD
1988	McGill U.	Phased injection of 1 m/z with RF cooling
1988	Oak Ridge	CI, atmospheric sampling source
1989	U. Florida / Finnigan	CI, DC quadrupole deflector
1989	Purdue U./ Dow	Thermospray

Ion Injection into Magnetic (ICR) Ion Traps

Experimental

1983	U.C. Irvine / Finnigan	EI, quadrupole beam guide
1984	Nicolet	Dual-cell
1985	U. Virginia / Finnigan	FAB, quadrupole beam guide
1985	Spectrospin	EI, electrostatic lenses
1986	Rice U.	Metal cluster ions, pulsed deceleration
1987	Texas A & M	DC quad deflector, pulsed deceleration
1988	Purdue U.	LD, electrostatic lenses
1988	American Cyanamid	Thermospray, electrostatic lenses

Ion Injection into Ion Traps - ASMS '89

MOD 2:30	<u>FAB up to m/z 50,000</u> - RF ion trap
	Purdue U./Los Alamos
TOA 10:00	<u>Thermospray</u> - RF ion trap
	Purdue U./ Dow
TOF 3:30	External ion source/ <u>trap</u> - Magnetic ion trap
	U. Bremen
TOF 4:30	<u>NCI</u> w/ injection of reagent ions - RF ion trap
	Oak Ridge
TOF 5:15	PCI/NCI <u>off-axis</u> injection - RF ion trap
	U. Florida / Finnigan
RPA 18-23	(External ion source in Magnetic ion traps)
	Nicolet, Spectrospin. U. New South Wales, U.C. Riverside

Two-dimensional FT/ICR

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FT/ICR, compared to classical mass spectrometry, is a method where the different sectors of the latter are replaced by pulse sequences. The "normal" pulse sequence is shown in Fig 1: The minimum time between ionization and detection is a few milliseconds, long enough for all metastable decays to have died away. This minimum time between ionization and excitation can be extended to several seconds and allows different experiments to be performed. This time can be used to introduce additional pulses or pulse sequences in order to get supplementary information that can be represented in additional dimensions. They can be tentatively classified into three categories:

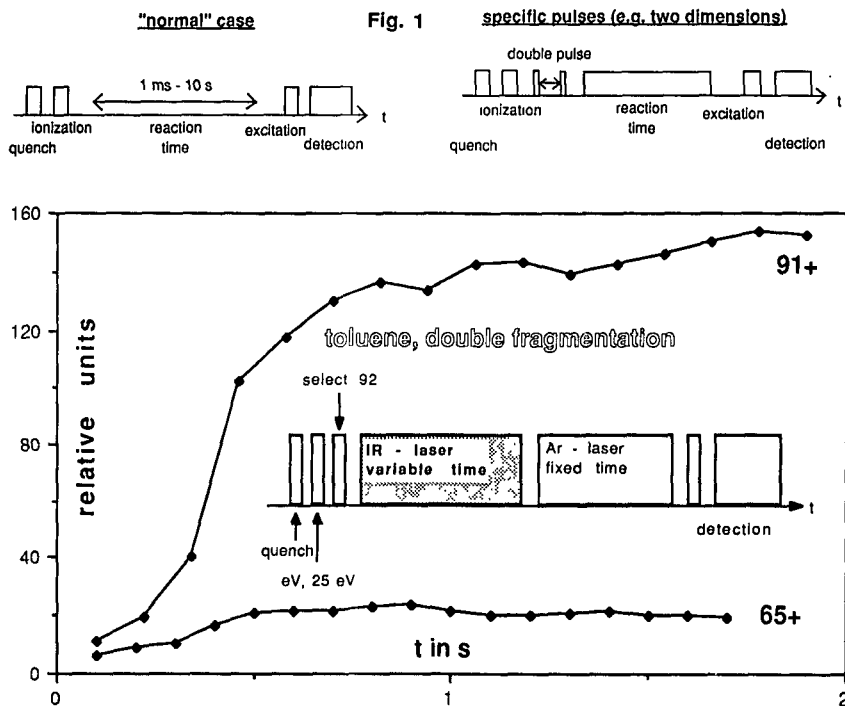
- a.) **nonspecific pulses** such as a pressure increase, a photofragmentation etc. The reaction time is in this case used as an additional dimension to the mass spectrum.
- b.) **semispecific pulses.** The fact that ions of different m/z -values have different resonance frequencies is used e.g. to perform a programmed ejection.
- c.) **specific pulses.** Ions of a given m/z -value do not only have the same resonance frequency, but they are after an excitation in a coherent motion, i.e. they are also characterized by having the same phase. Examples will demonstrate the different possibilities.

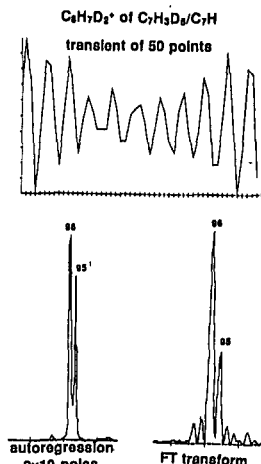
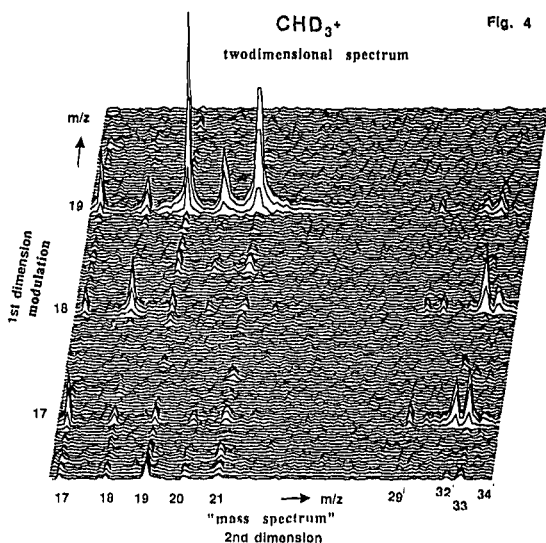
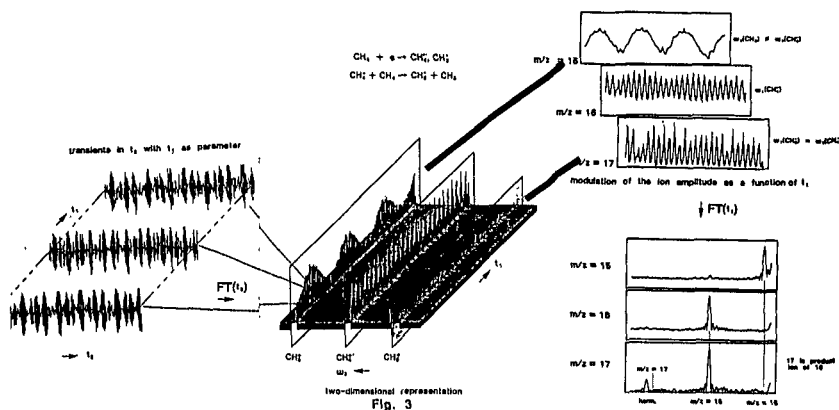
ad a. In FT/ICR it is easy to get the very high resolution that is essential for separating isotope doublets such as $CD_3^{13}CH_5$. However it is necessary to have long transients; this corresponds to pressures in the 10^{-9} Torr range. For the study of ion-molecule reactions the low collision frequency in this pressure range requires very long reaction times. This is particularly cumbersome when working with labeled compounds, because these measurements necessitate a dynamic range that can only be obtained by averaging several thousand spectra. Thus pressure pulsing is a big time saver. The time between ionization and detection can also be used to photofragment an ion. This is demonstrated for toluene cation. Its reactions were subject of many publications. The problem is that the cation has activation barriers for isomerization to other structures (e.g. cycloheptatriene etc.) that are below the appearance energy of the first fragmentation, $C_7H_7^+$. This ion can also have different structures (e.g. benzyl, tropylium). The further fragmentation to $C_5H_5^+$ by a loss of acetylene seems to be a random process that asks for an equilibration of all C and H atoms before fragmentation. The problem is the determination of the ratio of the different isomers $C_7H_7^+$. The pulse sequence is shown in Fig. 3. All fragment ions formed by electron ionization are ejected by a specific ejection pulse. An infrared laser pulse of $10\ \mu m$ wavelength is used to fragment $C_7H_7^+$ with the lowest possible fragmentation energy. The time dependence of the irradiation time allows to estimate the minimum energy needed for fragmentation and its rate constant. The resulting $C_5H_5^+$ is probed by the pulse of an Ar-laser: only one structure (presumably benzyl ion) is decomposed to the $C_5H_5^+$. The results as a function of the irradiation time of the IR-laser are shown in Fig. 2

ad b. The type of measurements described above have the disadvantage that a single ion must be isolated before the start of the reaction sequence. The use of frequency pulses of large amplitudes allow the ejection of selected ions. The problem is to obtain a good separation of two ions having a small mass separation. The notch ejection is a convenient method to eject all ions except one mass [1]. It is also easy to program. By using techniques such as the S-matrix of the Hadamard transform one is able to lower the number of measurements considerably by measuring the reactions of several selected ions at the same time. However the existing software does not tolerate a complex ejection program, as it is needed by such a method in order to improve the signal-to-noise-ratio. The application of this technique in mass spectrometry has first been proposed by McLafferty et al. [2]. A new sweep technique (SWIFT), where the ejection is programmed in the frequency domain, some amplitude smoothing phase shift is applied and the resulting complex spectrum transformed in the frequency domain facilitates considerably the programming [3]. The resulting signal is used to obtain the tailored ejection.

ad c. Beside the evident advantage of improving the signal-to-noise ratio, the Hadamard technique suffers from the disadvantage that a complex ejection program is necessary and that only a prescribed number of predefined m/z values can be used. Whereas the former disadvantage could be avoided by a suitable software, the number of precursor ions that has to be known in advance is inherent to the method. Phase-sensitive

pulsing as it is used in the 2D-method can circumvent this problem [4]. The principle of the method is the following: In a first pulse the ions in the mass range of interest are excited as usual, bringing the ions in a coherent motion. After a delay that varies between 0.1 μ s and 1 ms the pulse is repeated. If the excited ions are in phase with their resonant frequency in the second pulse, they will be further excited and thus ejected. On the other hand, if the ions are in antiphase, they are deexcited back to thermal rotational energy. By increasing the selected increments Δ and taking additional scans, the concentration of the ions with thermal rotational energy varies as a function of Δ . This concentration will be modulated on successive scans with a frequency that depends on their m/z value. During a reaction or a photofragmentation this modulation frequency is transferred to the product. At the end of the experiment the scans are subjected to a two-dimensional Fourier transform: the first dimension is characteristic for the m/z value of the precursor ion, the second dimension gives the resonance frequency of the product ion. The procedure is shown in Fig. 3 at the left are shown three transients with different Δ values. The results after a first transform in t_2 is shown in the waterfall presentation of the middle: from left to right is the normal mass spectrum, from the front to the behind (still as a function of t_1). The difference in the modulation frequencies between $m/z = 15$ and 16 is clearly visible. An improved picture is shown at right, where also the second transform (in t_2) is executed. $m/z = 17$ has no frequency of its own, but only the frequency of its precursor ($m/z = 16$) is seen (the higher frequency is a harmonic). In Fig 4 a waterfall representation of the ion-molecule reactions in CHD_3 is shown. There are evidently a few more reactions present beside the classical proton- and hydride-transfer reactions. The FT transform is a method that works better with many data points. This is of no problem in a unidimensional case. However in the two-dimensional transform, the number of points to be stored might exceed the available memory. Parametric methods such as autoregression or maximum entropy methods [5] need only a few data points, but a high signal-to-noise ratio. An example of a modulation transient is presented in Fig. 5. The signal-to-noise ratio is such that both methods should give comparable resolution. Note that in the autoregression method the intensity is proportional to the peak surface, not the height. In both cases unit resolution is achieved with only 50 data points.





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NEW METHODS OF ION EXCITATION AND ISOLATION IN FTMS

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To evaluate methods of ion isolation and ion excitation for Fourier transform mass spectrometry (FTMS), the effect of the excitation method must be determined for the ions that are to be excited and also for ions that are to remain unexcited. The reproducibility of the method must also be considered. Here we evaluate the stored-waveform inverse Fourier transform (SWIFT) excitation method [1] with respect to ease of use, selectivity (resolution), the effect on ions undergoing excitation (or ejection), and the effect on ions undergoing isolation.

In the SWIFT method, the operator defines the excitation in terms of the ion kinetic energies (ion orbital radii) and resolving power to be achieved over the specified mass ranges. The result of this spectral definition process is referred to as the "requested excitation spectrum". Inverse Fourier transform methods [2] are used to calculate the SWIFT excitation waveform that will produce result defined by the requested excitation spectrum. The principal advantage of SWIFT excitation over other excitation methods is that the operator needs only to specify the desired results -- no guesswork or tuning is involved. In theory, a SWIFT waveform can be calculated for any requested excitation spectrum.

We have found that at high magnetic field strengths the ejection of ions over a wide mass range occurs by z-axis (trapping) ejection rather than by ejection in the x,y-plane (the plane perpendicular to the direction of the magnetic field). The extent of z-axis ejection is proportional to the cube root of the mass of the ions to be ejected and increases with the frequency (mass) range over which ions are to be ejected. These factors can be used to design highly efficient SWIFT waveforms. By using SWIFT, we have isolated trace components in a complex mixture in the presence of interferences that have the same nominal mass [3], and we have obtained daughter ion spectra for parent ions that require a resolving power of 10,000 to separate [4].

The SWIFT waveform can be designed to leave an isolated ionic species with any desired kinetic energy. This was verified experimentally by measuring the signal intensity for an ionic species immediately following isolation of that species with a SWIFT waveform. The SWIFT waveform was designed to leave the isolated ionic species with a kinetic energy of 0.18 eV. The signal intensity for the isolated ionic species was 4.1% relative to the signal intensity for the same ionic species excited to a 0.5 cm orbital radius. This corresponds to a kinetic energy of 0.18 eV, which is in excellent agreement with the predicted value.

Kerley and Russell have described an ion isolation method (referred to as "ion partitioning") that uses a Fourier transform mass spectrometer with a dual trapped-ion cell [5]. Ions that are trapped in one of the two trapped-ion cells are excited to an orbital radius that is larger than the radius of a hole in the common electrode (the conductance limit) between the two cells. The desired ionic species is then selectively de-excited to a small orbital radius, and it is transferred to the other cell by grounding the conductance limit. This experiment can also be performed by using a SWIFT excitation waveform to selectively excite all ions except the desired species to a larger orbital radius. The SWIFT approach is simpler than the "excitation/de-excitation" approach because only one SWIFT waveform is required to isolate the desired species, and no guesswork or "tuning" is required.

Applications of the SWIFT technique include single-ion or multiple-ion monitoring [3], high-resolution parent ion selection [6] for tandem mass spectrometry (MS/MS), and complex experiments such as Hadamard transform FTMS[3]. We have found that the results of SWIFT ex-

citation are more reproducible than the results of swept-frequency excitation for the detection of high-mass ions. A tentative explanation for this observation is that swept-frequency excitation waveforms may contain a net dc component that can cause the ions to drift off-center and be ejected [7]; SWIFT waveforms are designed with no net dc component.

Selected-reaction monitoring is a highly specific identification method that can be performed with SWIFT techniques. In this MS/MS experiment, a SWIFT waveform is used to simultaneously eject daughter ions and accelerate parent ions to a chosen kinetic energy. If a target gas is introduced into the trapped-ion cell, the accelerated parent ions can undergo collision-induced dissociation (CID) and the resulting fragment ions are detected by SWIFT multiple-ion monitoring. Figure 1 shows an example of SWIFT selected-reaction monitoring for GC/MS/MS detection of n-butylbenzene. Figure 1a shows the total ion current chromatogram for a mixture of ten compounds. Figure 2a shows the total ion current chromatogram for selected reaction monitoring of the CID of the molecular ion of n-butylbenzene (m/z 134) to produce the m/z 91 and m/z 92 fragment ions.

In conclusion, SWIFT excitation is an efficient and selective method for ion excitation and ion isolation in FTMS. The effects of SWIFT excitation are well-defined for ions undergoing excitation as well as for ions undergoing isolation.

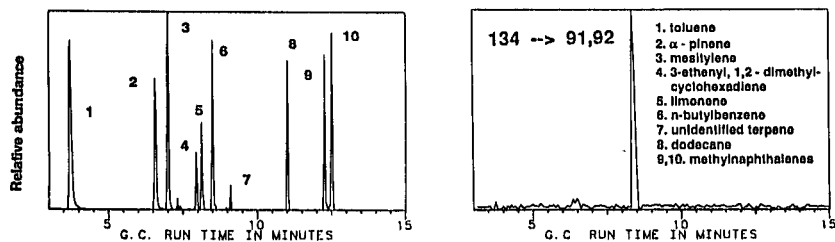


Figure 1.

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A TRIPLE STAGE QUADRUPOLE GLOW DISCHARGE MASS SPECTROMETER:
THE DISSOCIATIVE REMOVAL OF POLYATOMIC INTERFERENCES

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Elemental mass spectrometry seeks to reduce all ionic species to the atomic form. Unfortunately, real-world atomic ion sources are imperfect, creating numerous polyatomic ions that potentially (and actually) interfere with atomic analytical ions of the same nominal mass. High resolution instrumentation solves only some of these problems and then at a high cost. We have taken a different approach by drawing on collision induced dissociation effects to break apart undesired polyatomic ions.

INSTRUMENTATION

A commercial triple stage quadrupole mass spectrometer (Figure 1) was used for all experiments. The conventional ion source was replaced by a glow discharge source with a direct insertion probe.

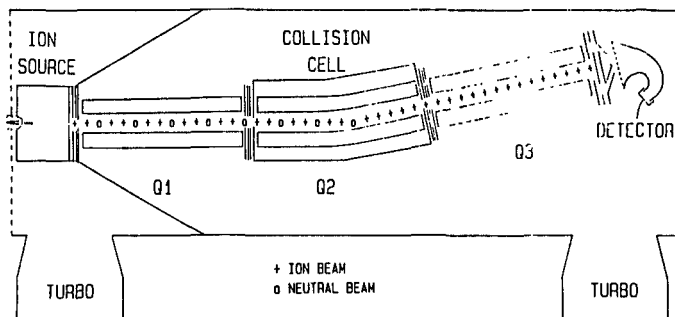


Figure 1. Triple stage quadrupole with glow discharge source.

An important aspect of the spectrometer was the nonlinear ion flight path, as fast neutrals from the glow discharge ion source can cause noise problems in line-of-sight quadrupole systems. The second quadrupole (Q3) was used as the collision cell by introducing millitorr levels of argon to interact with the ion beam.

RESULTS AND DISCUSSION

The original aim of this study was to determine if polyatomic ions could be dissociated to a great degree, thus reducing interference problems. An interesting aspect that arose during the experiments was the ability to perform certain diagnostics on the ion source processes. An example of the CID effect on GDMS spectra is shown in Figure 2, in which the ions from species like Ar^+ , OH^+ , and H_2O^+ are repressed by collisions with argon atoms, leaving a spectrum enriched in analyte ions. Aside from this directly understandable analytical advantage, the triple stage quadrupole permits the acquisition of parent and daughter spectra.

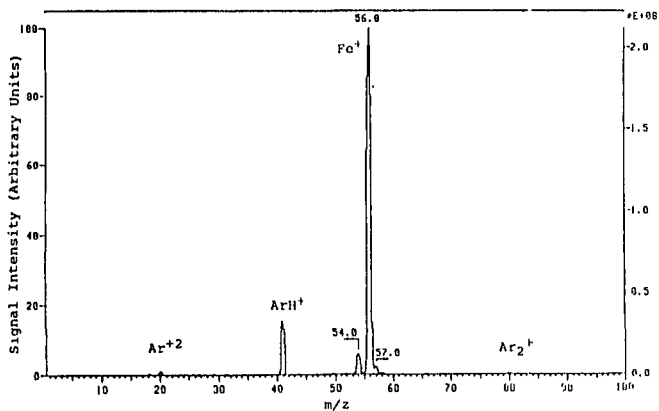


Figure 2. Effect of CID on removal of background peaks in GDMS.

Figure 3 illustrates the effect of scanning Q1, using Q2 for collision, and setting Q3 to pass on m/z 56.

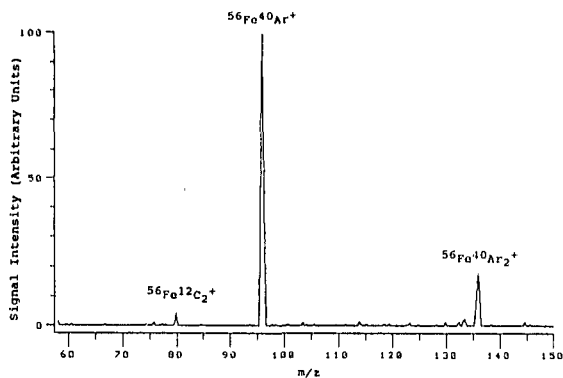


Figure 3. Parent spectrum for 56 amu ions.

Apparent are the species which dissociate to produce Fe^+ , indicating some of the polyatomic ions that form in the discharge. Daughter spectra may also be obtained to show what species result from certain collisions. The TSQ thus offers both practical and fundamental opportunities for analytical studies.

EXPECTED REGULATORY AND GUIDANCE REQUIREMENTS FOR CONTROL OF AIR TOXICS

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The U.S. Environmental Protection Agency (EPA) and many State and local air pollution control agencies are currently developing regulations and/or guidance for control of hundreds of air toxics. Gas chromatography/mass spectrometry will be required to measure emissions for hundreds to thousands of chemicals in the immediate future (now to the next few years). The sampling and analysis protocols will have to consider how to measure relatively low concentrations of numerous chemicals in the same emission stream. The protocols will be necessary for both testing to obtain data to develop emission limits and for compliance testing. Sound regulations depend on sound data and sound data depend on sound methods. Thus, the greatly accelerated standards development schedule will necessitate a greatly accelerated schedule for methods development.

Some people may doubt that hundreds of air toxics regulations will really be promulgated in the next few years, considering the small number of national emission standards for hazardous air pollutants that have been promulgated under Section 112 of the Clean Air Act in the last 19 years. However, this situation will change for two reasons. First, this low output of Federal standards has caused a number of States to forge ahead with their own regulations rather than waiting longer for EPA. Some of these States are developing test methods to support their regulations but many are forging ahead without developing test methods first. They hope that the methods will be developed by the time that enforcement decisions are necessary.

The second reason is that Congress has also noticed that EPA has not promulgated many hazardous air pollutant standards and has indicated via bills such as the House of Representatives Bill Number 4 - also called the Dingell Bill - that EPA must greatly accelerate the development of standards for air toxics. EPA expects that some bill, not necessarily HR4, but some bill, will be passed within a year that will require a large number of Federal standards in a short time.

The States and Congress are acting because they and EPA believe that control of air toxics is important to the safety and the health of all citizens. Air toxics include over 3,000 compounds detected in ambient air. Many of these are known or suspected carcinogens or have serious non-cancer effects such as respiratory disease, birth defects, or neurotoxicity. Also, many contribute to ozone formation, stratospheric ozone depletion, or indirect exposures. Air toxics are emitted directly from both stationary industrial sources and mobile sources and are also indirectly emitted via atmospheric transformation.

Agreement on the control of air toxics is complicated by disagreement on the perception of the nature of the problem versus reality. For example, some people say that no one has the right to pollute and the quantitative estimates of risk are crude and err on the side of underestimation and thus stringent control, including shutdowns, may be warranted. Other people say that pollution is the price we pay for an industrialized society, that society implicitly considers the risks and benefits of industrialization, and quantitative estimates of risk are crude and err on the side of gross overestimation and thus controls should be reasonable and generally not warrant shutdowns. The reality is that the limited available data can be used to argue either perception and reality probably lies somewhere in between. In most cases, control technology or pollution prevention, or other mitigation measures are available that can substantially reduce potential risk but not reduce the risk to zero. At current levels of control, air toxics are estimated to account for 1,600 - 3,000 cancer deaths per year. Estimates of the average urban individual lifetime cancer risk range from 1 in 10,000 to 1 in 1,000. Pollution is responsible for less than 5 percent of the 400,000 annual cancer deaths for all causes. Thus, air toxics represent a "high risk/high priority" problem area.

Regrettably, the portion of the air toxics problem that is currently quantifiable is limited to those chemicals for which there are both emission data and health data. However, the advent of industry self-reporting via the SARA 313 Toxic Release Inventory as part of the Superfund Community Right to Know Legislation should help. The partial report for 1987 identified over 300 toxic chemicals that totalled over 2.4 million pounds of emissions. Also, EPA's "Unfinished Business" Comparative Risk Project of 1987 reaffirmed the air toxics program area as high risk/high priority for both cancer and non-cancer risk. A number of studies of urban risk have estimated excess cancer of 1 to 30 deaths per year per million population and individual lifetime risks of 1 in 10,000 to greater than 1 in 1,000. A non-cancer study showed approximately half of the 197 chemicals studied exceeded the Acceptable Daily Intake (ADI) and 7 percent exceeded the Lowest Observed Effect Level (LOEL). For example, for chloroform, of 171 facilities for which exposure data were obtained, 162 exceeded the ADI, 50 exceeded the ADI x 10, and 4 exceeded the LOEL.

Information like this has elevated EPA's and Congress' concern about the status of the Section 112 program. Too often the current Section 112 program has taken a very long time to decide and redetermine actions within EPA and in court. Now the court is requiring final action on the remand for five benzene source categories by August 31, 1989, and a decision on whether or not to regulate nine additional benzene source categories by August 5, 1989. EPA is recontinguing development of hazardous air pollutant standards for 11 pollutants and over 20 source categories for which development had been suspended pending the benzene decisions. The pollutants include hexavalent chromium, ethylene oxide, asbestos, trichloroethane, perchloroethane, coke oven emissions, methylene chloride, carbon tetrachloride, chloroform, ethylene dichloride, and butadiene. Also, seven other air toxics regulations under other authorities are currently being developed. These pollutants include some of the previous pollutants plus dioxin/furan, hydrogen chloride, pathogens, cytotoxicants, various metals (arsenic, beryllium, cadmium, nickel, mercury, lead), chlorinated benzenes, chlorophenols, formaldehyde, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls. Some of these will be proposed this year and most of these regulations should be proposed within 2 years.

To ensure that EPA does actually follow through on these standards and many more, Congress plans to revise Section 112 of the Clean Air Act. The Dingell Bill includes a list of 185 chemicals which EPA must assess and a schedule for assessments and regulations. After enactment of the revisions, EPA must publish a list of pollutants within 3 months, consider additions and deletions to the list, and publish a source category list within 12 months. Standards for ten source categories would have to be promulgated within 2 years, 25 percent of the source categories within 4 years, 50 percent within 7 years and all categories within 10 years. The standards would be based on Maximum Available Control Technology (MACT) and would require evaluation of the residual risk within 7 years of promulgation. Where the residual risk is significant, additional regulations would require up to 90 percent additional risk reduction. The main thrust of the Dingell Bill is to increase the number of standards promulgated by including mandatory schedules and lessening the difficulty of the initial decisionmaking by delaying the residual risk evaluation until 7 years after promulgation of MACT. Although the residual risk assessment is delayed, the test methods cannot be delayed because selection of MACT will depend on expected emissions and because the regulations will need to include enforceable emission limits.

The development of these methods is a huge task. The number of pollutants and interfering combinations and emission streams is almost overwhelming. Draft budget estimates for developing methods in the normal ways are far above the available resources. EPA doesn't have the answer yet. Innovative approaches will be necessary. Cooperative projects with EPA, States and local agencies, and industry are being considered. Clean Air Act Amendments to require industry to develop test methods are being considered. Surrogate pollutants will be used in some cases to represent broader categories. EPA will consider all reasonable suggestions to achieve the goal. Your suggestions would be greatly appreciated.

ROLE OF MASS SPECTROMETRY IN EXPOSURE ASSESSMENT PROGRAMS

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After nearly two decades, the role of mass spectrometry in air pollution research remains as strong as ever in use with problems that have as their central thrust, chemical characterization and quantification. For example, every facet of the risk assessment model (Fig. 1) from source to health effects employs mass spectrometry with an assortment of ancillary attachments (GC, HPLC, FAB, etc.). A major research area encompassing mass spectrometry has been human exposure assessment studies where people's exposure to air pollutants have been determined. Exposure data as gleaned from population studies are important input into dose, metabolism and pharmacokinetic/toxicokinetic studies. With this information toxicological impact profiles and thus health effects of pollutants are established (1).

Mass spectrometry based technology has been used to identify sources of volatile organic chemicals (VOCs), e.g., those derived from building materials, environmental tobacco smoke (ETS), consumer products, attached garages, etc., which have an impact on indoor air quality and thus human exposure. Methodology has been developed which permits the collection and transfer of VOCs from personal, indoor, and outdoor air, and breath samples to GC-MS for qualitative and quantitative analysis (2-4). Examples of this successful application are demonstrated in the Total Exposure and Assessment Methodology (TEAM) studies (4-6).

The original assumptions concerning air pollution were: (a) outdoor air was the major route of human exposure, and (b) automobiles and industrial emissions were the major sources. With the advent of personal monitoring technology and results from direct human exposure monitoring studies, these assumptions have been significantly tempered. The TEAM studies have provided a wealth of actual human exposure data to VOCs and to semivolatile chemicals (e.g. pesticides, polynuclear aromatic hydrocarbons).

The TEAM program is a probability-base study where the participants selected for monitoring represent the entire population of a defined geographical area. This methodology allows inferences to be made to the general population from monitoring data on a smaller subset of individuals concerning exposure to as many as 30 VOCs. TEAM studies have been conducted with more than 700 people over the past nine years in Elizabeth and Bayonne, NJ, Devils Lake, ND, Greensboro, NC, Los Angeles, CA, Pittsburg, CA, and Baltimore, MD (4-7). In many areas follow-up studies have been conducted on the same participants in another or same season of the year. Several important discoveries have been made from these studies:

- (a) indoor air levels for many VOCs are higher than outdoor ambient levels;
 - (b) personal air levels are generally less than indoor air levels but greater than outdoors;
 - (c) significant source of human exposure to hazardous substances occurs from indoor air pollution, since most individuals spend 70 to 90% of their time indoors;
 - (d) energy conservation measures tend to decrease ventilation, which increases pollutant build-up indoors;
 - (e) within a home, room-to-room variations in concentrations are remarkably small, indicating good air mixing;
 - (f) similar exposure levels are observed in later years for the same season for the same individuals; however, different exposure levels may occur in different seasons of the year;
 - (g) breath levels, an indicator of actual exposure, best correlated with personal air levels;
 - (h) in-home and public building source emission rates were highest for those VOCs in consumer products used as room air deodorizers (e.g. p-dichlorobenzene, limonene, α -pinene) and for VOCs associated with ETS (e.g. benzene, styrene), dry-cleaned clothes (e.g. tetrachloroethylene), mobile sources/attached garages (e.g. benzene, xylenes, octane), and
 - (i) VOC exposure levels correlate with activity patterns of people.
- A recently conducted TEAM study examined hypotheses regarding major sources of exposure to several VOCs (5,8). Among these hypotheses were the following:
- (a) exposures to tetrachloroethylene are mainly due to dry-cleaned clothes in the home or wearing them;

- (b) exposures to aromatic VOCs may be partially due to storage of cars, lawn mowers, gasoline, kerosene, etc., in attached garages or other areas in the home; and
(c) hardware stores may be important sources of exposure to many toxic VOCs.

This study was designed to allow testing these hypotheses in 11 homes over a period of 3 or 5 days. Participants performed activities according to a set schedule--for example, all participants picked up freshly dry-cleaned garments on the second day of the monitoring period, and wore a freshly-cleaned garment on a later day. Personal, indoor and outdoor air samples and breath samples were collected for each participant over the 3-day or 5-day monitoring period. Air exchange rates were also measured to allow the calculation of source emission rates. Table 1 gives the peak 12-hr average tetrachloroethylene levels in nine homes before and after introducing the garments into the house. Five-fold to over an order of magnitude increase was observed in tetrachloroethylene levels, depending on the number of garments cleaned and the air-exchange rate in the home. The participant's actual exposure or dose is reflected by the levels measured in breath.

In addition to homes and public buildings, indoor air quality is also of concern in highly confined areas such as in submarines and in space flight. Air quality in nuclear submarines has been evaluated, using similar mass spectrometry based techniques as used in the TEAM studies. Selected VOC levels in one submarine that has been briefly studied is given in Table 2. VOC levels were an order of magnitude higher than those found in homes. The total organic levels (TOL) in each of the samples were also determined and found to be over two hundred times higher than the levels observed in the TEAM studies. Molhave's (9) research suggests TOL levels above 5 mg/m^3 may impair judgement and response/motor coordinating functions in some individuals. In the TEAM studies, ten percent of all air samples had TOL levels above 5 mg/m^3 (10,11).

In summary, our understanding of human exposure to chemicals has been greatly facilitated by the use of mass spectrometry based measurement techniques. Significant research remains to be performed, however, in all facets of the risk model (Fig. 1). For example little information exists concerning complex mixtures containing polar volatile and semi-volatile chemicals along the pathway of the risk model. The application of supercritical fluid and high performance liquid chromatography in combination with MS will receive considerable attention in future exposure assessment programs.

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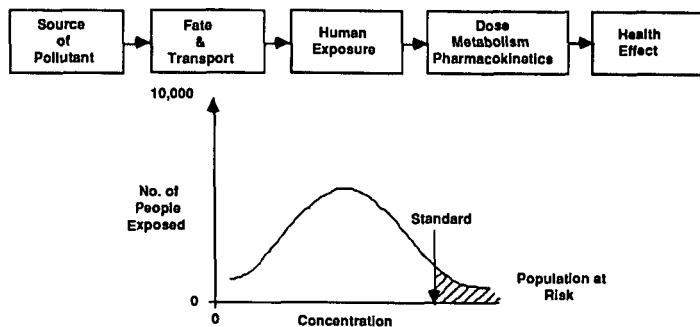


Figure 1. Components of Risk Assessment

TABLE 1. PEAK 12-HR AVERAGE TETRACHLOROETHYLENE LEVELS ($\mu\text{g}/\text{m}^3$) IN NINE HOMES BEFORE AND AFTER THE INTRODUCTION OF DRY-CLEANED CLOTHES.

	HOME									MEAN	S.D.
	A	B	C	D	E	F	G	H	I		
BEFORE											
LIVING ROOM	6	27	9	35	5	16	10	7	12	14	10
BEDROOM	7	64	7	37	7	NM	11	7	10	19	21
PERSONAL AIR	NM ^a	NM	11	34	22	35	8	10	12	19	12
BREATH	6	28	9	30	7	14	10	3	9	13	10
AFTER											
LIVING ROOM	181	142	45	297	97	20	56	37	11	98	94
BEDROOM	116	198	50	262	96	16	40	50	8	93	86
PERSONAL AIR	185	303	53	244	198	17	42	90	12	127	108
BREATH	35	58	14	61	29	10	17	9	12	27	20
NO. GARMENTS CLEANED											
	5	4	4	10	9	3	4	4	2		
MEAN AIR EXCHANGE RATE (ACH)											
	1.3	1.0	0.5	0.3	1.0	1.2	0.8	0.8	2.1		

^aNM = NOT MEASURED.

TABLE 2. SELECTED VOLATILE ORGANIC CHEMICAL LEVELS ($\mu\text{g}/\text{m}^3$) IN SUBMARINE AIR.

CHEMICAL	GALLEY	CREW BERTHING AREA	ENGINE ROOM	AUXILIARY MACHINE ROOM	MISSILE COMPARTMENT
1,1,1-TRICHLOROETHANE	168	77	139	8.3	75
TRICHLOROETHYLENE	93	82	36	69	87
TETRACHLOROETHYLENE	53	44	24	37	23
CHLOROBENZENE	19	19	16	19	18
p-DICHLOROBENZENE	0.5	0.4	0.3	0.3	0.4
n-OCTANE	164	161	142	151	147
n-NONANE	328	346	368	379	342
n-DECANE	402	424	469	481	421
n-DODECANE	333	150	197	222	331
BENZENE	67	332	41	50	25
ETHYLBENZENE	81	86	110	93	86
STYRENE	21	27	13	21	23
o-p-XYLENE	371	388	444	411	387
α-PINENE	16	15	6.6	12	16
LIMONENE	142	104	38	73	138
TOL (mg/m^3)	248	214	232	252	238

DIRECT AIR MONITORING USING AN ION TRAP MASS SPECTROMETER

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Air monitoring by mass spectrometry has typically been performed by doing atmospheric pressure ionization (API). API has been demonstrated to be very sensitive for certain types of compounds but very insensitive to other compound types. Other drawbacks to the use of API that are characteristic of one or the other of the typical API sources, i.e. beta emitter sources and corona discharge sources, are: clustering; the need for large volume pumping; ionization saturation; the need for bottled gases; and frequent source maintenance. An alternative approach that has been used sometimes is to leak air directly into an electron ionization or chemical ionization source. The major drawback to this approach is that the sample pressure is typically reduced by seven orders of magnitude or more with a corresponding reduction in the concentration of the analyte. Thus an analyte present at a part per million in ambient air has a number density of 2×10^{13} molecules/cm³, but this is reduced to 3×10^5 molecules/cm³ if the sample is leaked into a conventional ionization source at a pressure of 1×10^{-4} torr (an analyte pressure of 1×10^{-11} torr).

We have developed a source that is intermediate to these two pressure regimes and that overcomes many of the operational disadvantages of traditional API sources. The source is called an atmospheric sampling glow discharge ionization (ASGDI) source and it has been described in detail previously.¹ This source operates in a pressure range of 0.2-1.0 torr. An important feature of this source is that the ionization is controlled predominantly by kinetics, not thermodynamics as is generally the case with API sources. Thus, the ASGDI source is especially useful for negative ion analysis for compounds which readily undergo electron capture.

The electron capture rate can be up to two orders of magnitude faster than the rate for ion/molecule reactions. Therefore in the ASGDI source, in which kinetic control is dominant, efficient formation of negative ions that may not be the most thermodynamically stable ions can occur. This also means that trace impurities that can change the ion chemistry in thermodynamically controlled ion sources will have little effect on the ASGDI source.

Once ions are formed, a suitable mass spectrometer is needed for the analysis step. Some of the characteristics of the ideal mass spectrometer are: high sensitivity, simplicity, small size, and MS/MS capabilities. The three-dimensional quadrupole ion trap, known as an Ion Trap Mass Spectrometer (ITMS) has these characteristics. We have interfaced the ASGDI to an ITMS for direct air monitoring. A schematic of the system is shown in Figure 1. The system is housed in a six inch per side cube with six inch conflat flanges. Ions are pulsed into the ITMS from the ASGDI source via an einzel lens which has a split middle element which serves to deflect the beam during periods when it is not desired to inject ions into the ITMS. The normal electron gate pulse from the standard ITMS electronics is used for this deflection. For negative ions the normal electron gate voltages are used while for positive ion injection this voltage is floated up by use of a 300 volt battery. Details of the system can be found elsewhere.²

It has been found that several parameters affect the efficiency of ion injection from the ASGDI source into the ITMS and the appearance of the mass spectrum that is obtained. The main parameters are: the nature of the bath gas; the pressure of the bath gas; and the rf level of the ring electrode of the ion trap. Collision-induced dissociation (CID) that occurs during injection, along with the injection efficiency, affect the appearance of the observed mass spectrum. The amount of CID is dependant upon the same parameters as the injection efficiency.

For bath gases, the lighter the bath gas, the more efficient the ion injection. This can probably be attributed to less scattering upon collision of the ion with the bath gas. Injection efficiency shows an increase with bath gas pressure with a maximum that is dependant upon the bath gas. For H₂ and He this maximum is in the range of 1.5 to 3 millitorr. It is observed that CID decreases with increasing bath

gas pressure. A likely explanation for this is that at higher pressures the ion energy is damped more and thus while there are more collisions at higher pressure, the ions have less kinetic energy and thus the energy of each collision is reduced.

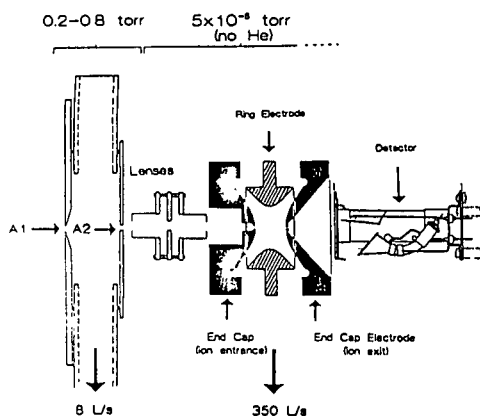
The injection efficiency shows a mass dependent maximum versus the rf level of the ring electrode. Thus, while a wide range of masses can be injected at a given rf level, the relative intensities observed in the mass spectrum will depend upon the rf level. As for the amount of CID, this increases with increasing rf level. This can be attributed to the increased kinetic energy that the ions have once they are trapped in the ITMS. Overall, the data suggest that the CID that is observed in the ion injection process occurs mainly after the ions are in the ITMS, not in the ion injection einzel lens.

An example of the performance capabilities of the system is shown in Figure 2. For this experiment the head space over a small amount of trinitrotoluene (TNT) was being sampled. Ions were injected for 0.3 milliseconds which calculates as a maximum sample input of 50×10^{-15} grams. This signal increases linearly with injection time up to the point where the ion trap is filled up with ions (i.e. ca. 10^6 ions). Further improvements in sensitivity can be achieved by filling the ion trap with ions and then ejecting out all the ions except the analyte ion of interest and then repeating the process.

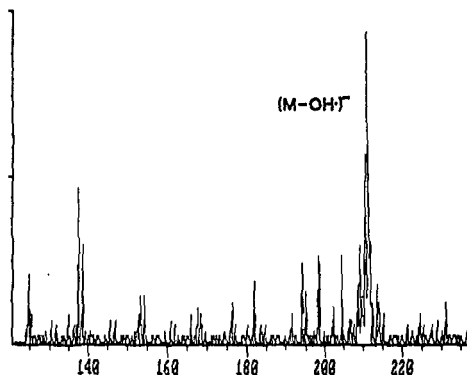
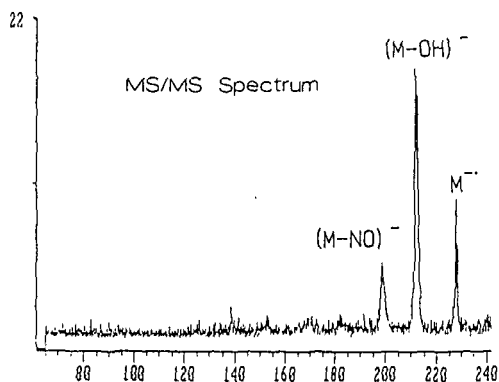
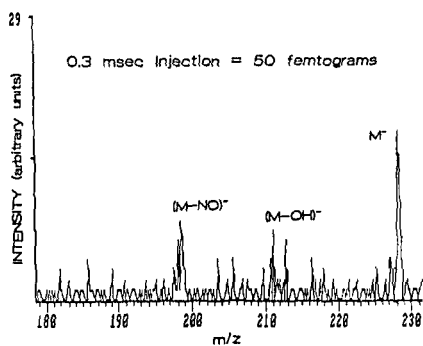
An important feature of the ITMS is its efficiency in doing MS/MS experiments.³ For positive ions an overall MS/MS efficiency of 75-80% is often observed. There is slightly more variation with negative ions since some negative ions will undergo electron detachment more readily than CID. However, for negative ions with electron affinities of a couple of eV the overall MS/MS efficiency is usually similar to that observed for positive ions. Figure 3 shows the MS/MS spectrum of TNT. The top spectrum is the average of 20 scans, taking a total of seven seconds to acquire. The bottom spectrum is a single scan acquired in 0.35 seconds.

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ION INJECTION Mass Spectrum of TNT



ATMOSPHERE ANALYSIS ON BOARD NUCLEAR SUBMARINES

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A mass spectrometer that is capable of real-time air monitoring also must be highly reliable since most situations that require real-time monitoring dictate continuous operation. The implication is that the atmosphere being monitored can change at anytime from a benign situation to a dangerous one. It is therefore of utmost importance that instrumental downtime be reduced to a minimum even at the expense of ultimate analytical performance.

The monitoring of closed environments requires determining the concentration of both life gases and trace species, the former taking precedence. In the early 1970's the Central Atmosphere Monitoring System, Mark I (CAMS-I), was developed and deployed on board all American nuclear submarines. This application represents the greatest number of mass spectrometers used for real-time monitoring. The CAMS-I is a fixed collector, permanent magnet mass spectrograph; an analogous commercial instrument is sold by Perkin Elmer Applied Sciences as a medical gas analyzer and a chemical process control instrument. CAMS-I has proven to be a highly reliable and accurate instrument. During the 1980's a new generation submarine atmosphere analyzer has been designed, built, and extensively tested.

In light of the proven performance of the CAMS-I, why was it necessary to develop the CAMS-II? At the time the CAMS-I was designed it was not feasible to use computers to control the instrument due to their size, cost, and reliability. Due to the extraordinary progress in microelectronics, computer control of an atmosphere monitor became feasible. Thus the CAMS-II was designed as a computer-controlled magnetic scanning mass spectrometer of Nier-Johnson geometry, Figure 1. The CAMS-II incorporates both a Faraday cup collector for the intense species requiring accurate measurement and an electron multiplier operated in the pulse counting mode for trace species. A comparison of the two CAMS systems is shown in Table I.

Table I. CAMS Design Requirements

Requirement	CAMS-I	CAMS-II
Mass Range	2-135	1-300
Resolution	1:28	1:300
Detection limit	6 ppm	0.1 ppm
Electron Energy	fixed	programmable
Monitored gases	8	unlimited
Programmable	no	yes
Data Recording	no	yes
Video Display	no	yes

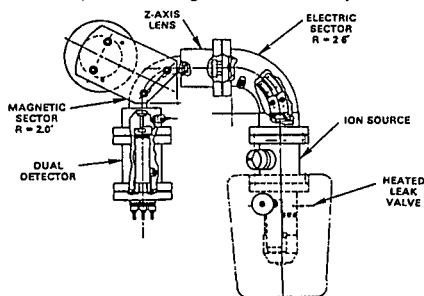


Figure 1. Schematic of the CAMS-II Analyzer.

Before being accepted by the Navy the CAMS-II has undergone an extensive testing program. The final stage of the testing consisted of two years of continuous operation on board a submarine. The CAMS-II performed flawlessly during this period. It did not require any adjustments or calibration maintaining its resolution and mass accuracy within specifications. The CAMS-II is factory calibrated and the calibration factors are burned into its read-only memory. Periodically a verification gas is sampled to assure correct operation.

During normal operation the CAMS-II monitors selected ions in order to analyze the following species: hydrogen, water, nitrogen, oxygen, carbon dioxide, Freon 12, Freon 114, Freon 113, aliphatic hydrocarbons, aromatic hydrocarbons, and benzene. The first five species are analyzed using the Faraday cup detector; the last six are analyzed using the multiplier. This analysis requires about 20 seconds. Every seven hours a complete mass scan between mass 2 and 300 is recorded for future analysis if needed. Figure 2 shows this data from both detectors for mass 44; the slit in front of the Faraday cup is 6 times wider than the slit in front of the multiplier and offset from the true focus of the mass spectrometer.

The ability of the CAMS-II to detect trace species depends upon several factors: inherent sensitivity, interfering ions, and sample transport. Currently the sensitivity of the CAMS-II is about 500 ions/sec/ppm for oxygen. Thus

detection of species at concentrations of a few ppb is feasible. In fact ^{134}Xe has been detected by averaging 50 spectra recorded on a submarine. This isotope is present in air at 8 ppb and the dwell time of the instrument as it is scanned is 20 msec/point. Thus each point represents a one second dwell time. Since the background count rate is only a few counts/minute the main noise source is counting statistics.

Interfering ions are always a problem in direct mass spectrometric analysis. One of the reasons a magnetic analyzer mass spectrometer was selected was its high abundance sensitivity. Figure 3 shows a log plot of the mass spectrum in the mass range of 30 to 50 amu. The background intensity in this region is equivalent to the intensity of a peak due to about 2 ppm of a contaminant. Figure 4 shows the background in the region of 75 to 95 amu. The peaks due to krypton are clearly observable; the natural abundance of ^{83}Kr is 130 ppb. Figure 5 shows the same region of the mass spectrum when a test gas containing 2.5 ppm of benzene, 6.5 ppm of toluene, and 200 ppm of Freon-12 is sampled; note the scale change for the ordinate. Both benzene and toluene are readily detectable.

Sample transport is always a problem when analyzing trace species. The CAMS system samples through stainless steel sample lines at ambient temperature. Materials such as toluene and higher aromatics require a long time to reach equilibrium in stainless-steel lines at ambient temperature; since the CAMS samples continuously, this is only a limitation for situations in which the sample composition is changing rapidly.

In conclusion, the CAMS-II has demonstrated that a mass spectrometer can accurately detect and measure atmospheric species from concentrations of a few ppb to one hundred percent in real time. It is highly reliable having operated continuously without recalibration or other adjustments for more than two years.

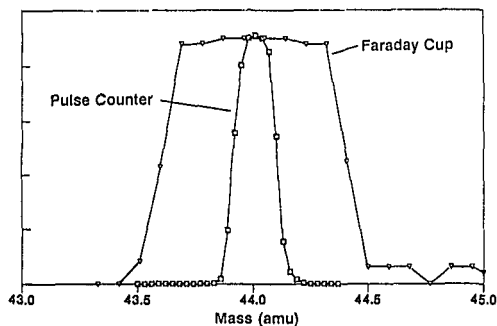


Figure 2. Scan of mass 44 with both detectors.

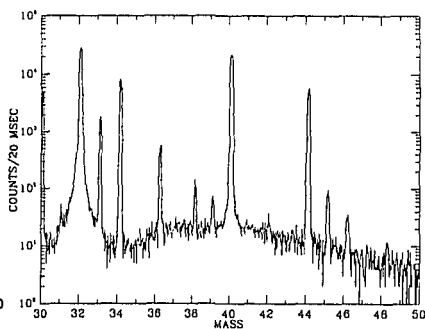


Figure 3. Background scan.

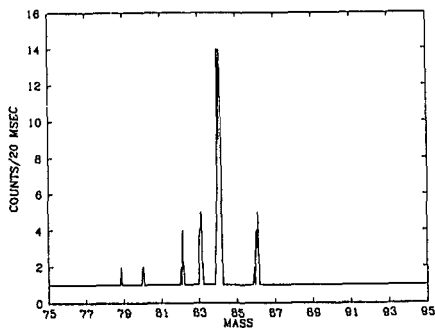


Figure 4. Background scan, only Krypton peaks are evident.

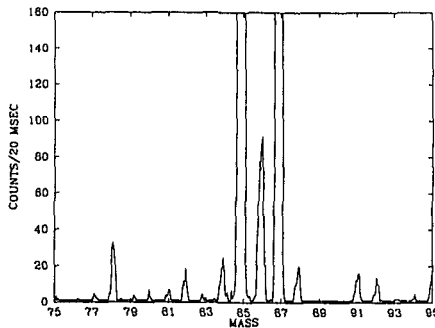


Figure 5. Same scan as Fig. 4, but sampling test gas.

**A Rapid Immunoaffinity Cleanup GC/MS Method for
Determination of 3-Methyladenine in Urine**

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INTRODUCTION 3-Methyldeoxyadenosine, a major adduct formed by reaction of DNA with alkylating agents such as N-methyl nitroso compounds (1), is rapidly repaired by base excision to release 3-methyladenine (3-MeAde). As 3-MeAde has been shown to be excreted in a dose dependant manner via urine, without catabolic breakdown, its measurement in urine may provide a useful marker for prior exposure to methylating agents (2).

The previously available Gas Chromatography/Mass Spectrometry (GC/MS) method for urinary 3-MeAde suffered from the disadvantage that it required a lengthy clean-up involving XAD-column chromatography and HPLC (3). The recent development of a polyclonal antiserum to 3-MeAde offered the possibility of ELISA-based quantitation without the need for GC/MS, but the presence of cross-reactive purines in human urine rendered this approach difficult (4). However, a method which has proved successful is the combination of easily used immunoaffinity columns and GC/low resolution MS carried out on a relatively inexpensive benchtop mass spectrometer.

EXPERIMENTAL Immunoaffinity columns were prepared with a rabbit antiserum specific for 3-MeAde in batches of from 5 to 20 columns at a time. Each column, containing 1 ml of antibody-modified Sepharose gel, was shown to retain quantitatively up to 100 ng of [³H]-3-MeAde. Columns are reusable and no detectable carry over was found from one sample to another even after a column had been recycled up to 30 times.

Two ml of urine, spiked with 50 ng d₃-3-MeAde internal standard, applied to the column is sufficient for reliable analysis. Evaporation of a 1M acetic acid column eluate provides a sufficiently clean sample for direct analysis of the t-BDMS derivative by selective ion monitoring of the (M-57)⁺ ions of 3-MeAde (m/z 206) and a d₃-3-MeAde internal standard (m/z 209).

GC/MS was carried out on a Hewlett Packard 5890 GC equipped with a cold on-column injector, coupled to a Hewlett Packard 5970A Mass Selective Detector operated at 70 eV in the electron impact mode. The GC column (Hewlett Packard Ultra-2, 0.2 mm i.d. X 25 m, film thickness: 0.33 µm) was heated from 55°C at injection to 270°C at 40°C/min and then at 10°C/min to 300°C where it was held for 3.5 min. The mass chromatogram (Fig. 1) for a typical human urine sample (~ 7 µg 3-MeAde/24 h) shows the excellent cleanup achieved by the immunoaffinity cleanup step. The limit of detection is less than 0.5 ng/2ml urine.

RESULTS AND DISCUSSION In collaboration with Dr S.H. Lu of the Chinese Academy of Medical Science in Beijing, People's Republic of China, the method has been applied to the analysis of 3-MeAde in the urine of subjects in two geographical regions having differences in the incidence of oesophageal cancer.

Only slight differences were observed between the two groups (Table 1). Work is in progress to determine to what extent 3-MeAde present in the diet would affect such measurements as it is known that, in animals, any 3-MeAde ingested is excreted unchanged in the urine (2).

ACKNOWLEDGEMENTS This work is supported by the U.S. National Cancer Institute (Grant No. 1-U01-CA 48473-01)

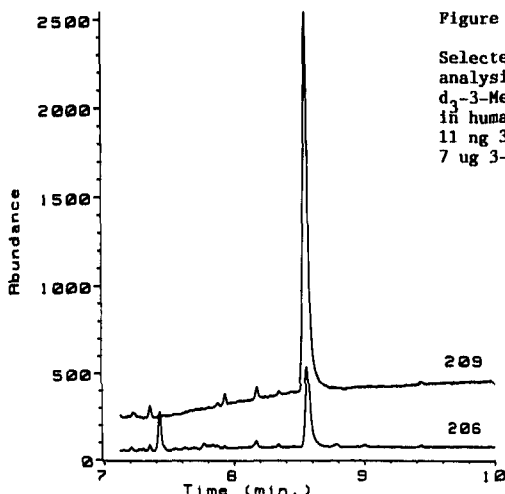


Figure 1

Selected ion monitoring traces for the analysis of 3-MeAde (m/z 206) and d₃-3-MeAde internal standard (m/z 209) in human urine. This sample contains 11 ng 3-MeAde/2 ml urine implying about 7 ug 3-MeAde per 24 h.

Table 1

Urinary 3-MeAde levels in 43 subjects from High (Lin-Xian) and Low (Yu-Xian) risk areas for oesophageal cancer in Northern China

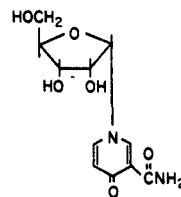
	Mean (ng/mg Creatinine)	Standard Deviation (ng/mg Creatinine)	N
Lin-Xian County	12.3	14.0	21
Yu-Xian County	13.0	" 14.0	22

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ISOLATION AND CHARACTERIZATION OF AN UNUSUAL NUCLEOSIDE FROM HUMAN URINE.
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For a number of years, our laboratory has been engaged in the isolation and characterization of novel nucleosides excreted in the urines of cancer patients and normal subjects. In continuation of our studies, we wish to report the finding of a first α -nucleoside, 1- α -D-ribofuranosylpyridin-4-one-3-carboxamide (I) (α -4-PCNR) from human urine (Fig. 1). The unknown (I) was isolated from a 24 hr urine collection of a normal subject (190 μ g) by a previously described procedure¹. It was isolated in elevated amounts in the urines of two chronic myelogenous leukemia (CML) patients (400 μ g, 460 μ g).



(I)

The UV spectra of unknown urinary nucleoside (I) exhibited a λ_{max} at 257 nm at pH 1.3, 258 nm at pH 6.0 and 259 nm at pH 11.0 and were similar to the UV spectra of β -4-PCNR, previously isolated and characterized in our laboratory². The CD spectra of (I) exhibited a negative band in aqueous solution at 254 nm. The differences in retention times between unknown I and β -4-PCNR on RP-HPLC and in the chemical shifts attributed to the anomeric protons in the D_2O NMR spectra (6.05 ppm for (I) and 5.71 ppm for β -4-PCNR) further suggested that the unknown may be an α -anomer of the β -4-PCNR.

1- α -D-ribofuranosylpyridin-4-one-3-carboxamide (α -4-PCNR)

Fig. 1

The GC-MS spectra of a trimethylsilyl derivative of (I) (Fig. 2) showed the highest mass ion at M/Z 558 with characteristic fragment ions identified in the tetra (TMS)₄ derivative of β -4-PCNR². These included a $M-15$ ion at 543 from the loss of CH_3 , a $M-28$ ion at 530 attributed to the loss of CO , the unusual $M-45$ ion at 513 indicating a loss of CH_3NO and the ion at M/Z 349 resulting from the sugar portion of I. The ions at M/Z 349, 259, 245, 243, 230, 217 and 169 characteristic of unmodified ribose preclude a substitution on the sugar. These data suggest that the underivatized urinary unknown I has a molecular weight of 270. The EI mass spectra of underivatized (I) has the highest mass ion at M/Z 270 confirming a mol. wt. of 270 and fragment ions consistent with a ribose nucleoside. These include the base ions at M/Z 138 ($b+H$), and 167 ($b+30$). Ions at M/Z 150 and 121 formed by the elimination of 17 amu, most likely represent a loss of NH_3 while a loss of 44 amu from the elimination of $CONH_2$ gives M/Z 93 ($b-COHN$). The ion at M/Z 133 indicates an unmodified ribose. These data suggest that the unknown nucleoside I may be an isomer of β -PCNR. FAB spectra of the natural unknown I in glycerol gave a $M+H$ ion at 271.0931 ($C_{11}H_{15}N_2O_6$) further supporting the molecular weight of 270 ($C_{11}H_{14}N_2O_6$).

The NMR spectrum of (I) in D_2O exhibited the following chemical shifts (δ) and J values (Hz): 8.72 (d, 1, $J_{2,6} = 2.1$ Hz, 2-H), a pair of doublets at 8.02 and 7.98 (d, 1, $J_{6,2} = 2.3$ Hz and $J_{6,5} = 2.3$ Hz, 6-H), 6.75 (d, 1, $J_{5,6} = 7.6$ Hz, 5-H), 6.05 (d, 1, $J_{1,2} = 4.7$ Hz, 1'-H). The synthesis of the α -nucleoside I was achieved by condensation of pyridine-4-one-3-carboxamide with 2,3-isopropylene-1,5-di-O-acetylribofuranose in the presence of TMS triflate and hexamethyldisilazane in refluxing dichloroethane, followed by deblocking³.

By comparison of UV, NMR, mass spectra and CD data and HPLC retention times of the urinary material with those of the authentic α and β anomers, the nucleoside (I) was assigned the structure of 1- α -D-ribofuranosylpyridin-4-one-3-carboxamide.

MASS SPECTRA

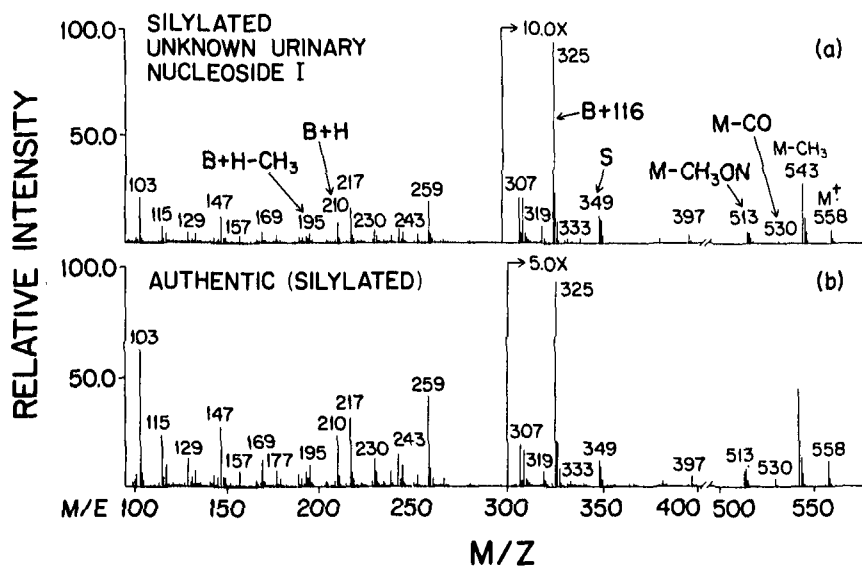


Fig. 2

As to the origin, α -4-PCNR is structurally related to nicotinamide adenine dinucleotide (NAD). Isolation of α -NAD⁺ from numerous sources, including animal tissue, have been reported. These reports have led to speculations concerning its *in vivo* origin and its role in metabolic functions⁴. Although the biological occurrence and significance of the α -NAD⁺ is unclear, the possibility of α -NAD⁺ as a source α -4-PCNR remains attractive.

The *in vitro* anomerization of natural β -4-PCNR as a source of α -4-PCNR was excluded by carrying out proper control experiments on β -4-PCNR under the conditions used to isolate and purify natural (I). Detection of both α and β anomers in a riboside fraction of urine, processed by an alternate milder one-step affinity chromatography procedure³, further supported the existence of natural α -4-PCNR in urine. The significance and possible role of this naturally occurring α -4-PCNR as an agonist or antagonist in some metabolic pathways remains to be investigated.

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**LASER IONIZATION FOURIER TRANSFORM MASS SPECTROMETRY
FOR THE ISOMERIC DIFFERENTIATION OF ALKYLATED NUCLEOSIDES ***

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Alkylation of cellular deoxyribonucleic acid (DNA) is one form of DNA modification induced by carcinogenic agents. For example, alkylating agents such as N-methyl-N-nitrosourea react with DNA to generate methyl adducts of all four nucleic bases (adenine, guanine, cytosine, and thymine), including several isomers of each base. Since isomeric compounds often exhibit remarkably different biological activity, the structural characterization of these DNA adducts requires sensitive and unambiguous identification at the isomeric level. O⁶-methyl guanine and O⁴-methyl thymine are two compounds of specific biological interest which must be identified and differentiated from the other methyl isomers. Laser ionization Fourier transform mass spectrometry (FTMS) is quite useful for the examination of modified nucleic acid constituents, since this technique can provide detailed structural information for picomoles of non-volatile, thermally labile compounds such as modified nucleosides.

Laser ionization of methyl nucleosides generates negative ions which include an (M-H)⁻ ion for the nucleoside as well as an ion corresponding to the free nucleic base (formed by elimination of the sugar ring). The collisional dissociation of these negative ions from the various methylated adducts of thymine, cytosine, and adenine gave fragment ions which could be used in most cases to determine the position of methyl substitution, as summarized in Table 1. For example, O⁴-methyl thymine fragmented by loss of methanol, whereas O²-methyl thymine lost CH₃. Accurate mass measurements of the parent and daughter ions as well as sequential CID experiments (MS³) were used to examine the fragmentation of these ions.

Deuterium labelling has also been used to help elucidate the positions of alkyl substitution for these compounds. The negative ions mentioned above do not undergo deuterium exchange in the gas phase, since these ions are very weak bases due to resonance stabilization. However, these nucleosides will undergo selective deuterium exchange with D₂O in solution. For example, the amino hydrogens of the nucleosides will undergo rapid deuterium exchange whereas the C8 hydrogen of the nucleoside will exchange more slowly. As a result, deuterium exchange can be used to identify the position of methyl substitution in some cases, since the location of the methyl group will determine the number of exchangeable hydrogens which are present on the nucleoside.

These structural studies have been extended to include large DNA adducts as well. Laser ionization of amino polycyclic aromatic hydrocarbon (PAH) adducts of guanine also generated characteristic negative ions. An intense (M-H)⁻ ion at m/z 317 is observed in the negative ion laser desorption spectrum of C8 para-aminobiphenyl guanine. Collisional dissociation of this ion generates several daughter ions, shown in Figure 1, which provide structural information for the adduct. Loss of NH₃ from this ion implies that the N1 and N² positions of the guanine base are unsubstituted. The daughter ion at m/z 168 corresponds to (aminobiphenyl)⁻, and provides the information necessary to identify the adduct. The ion at m/z 193 corresponds to (aminobiphenyl - H + CN)⁻ and indicates that the aminobiphenyl adduct is attached to the guanine at the C8 position. Several C8 amino-PAH guanine adducts have been examined and the fragmentation pathways observed in Figure 1 appear to be present for all of the adducts.

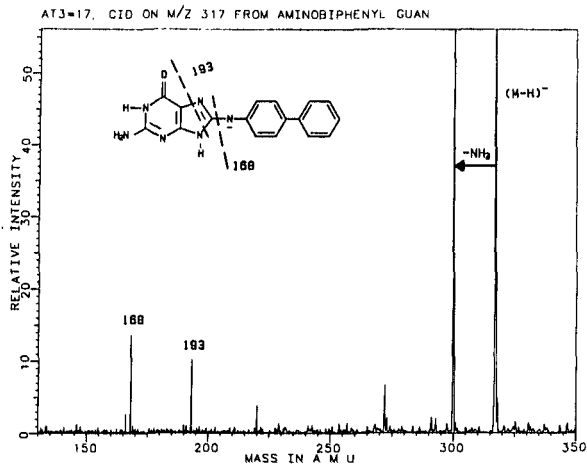
* Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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Table 1. CID of negative ions from methyl nucleosides

Compound	Parent Ion	Daughter ions observed		
O ⁴ -methyl thymine	139	(M-CH ₃ OH) ⁻	CNO ⁻	CN ⁻
O ² -methyl thymine	139	(M-CH ₃) ⁻	CNO ⁻	CN ⁻
3-methyl thymine	139		CNO ⁻	CN ⁻
5-methyl cytosine	124	(M-NH ₃) ⁻	(M-HNCO) ⁻ CNO ⁻	CN ⁻
3-methyl cytosine	124		CNO ⁻	CN ⁻
1-methyl adenine	148	(M-C ₂ H ₃ N) ⁻		CN ⁻
N ⁶ -methyl adenine	148	(M-CH ₃) ⁻	(M-CH ₄) ⁻	CN ⁻
3-methyl adenine	148	(M-CH ₃) ⁻	(M-CH ₄) ⁻	CN ⁻

Figure 1



FAB/MIKES ANALYSIS OF CYCLIC NUCLEOTIDES AND RELATED COMPOUNDS

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Cyclic nucleotides are a group of compounds which contain a heterocyclic base, a ribofuranose ring and a phosphate group. They differ from other free nucleotides in that the phosphate group is present as a cyclic phosphodiester moiety, and one of the naturally occurring cyclic nucleotides, adenosine 3',5'-cyclic monophosphate (cyclic AMP), has been shown to have a key role in mammalian metabolic regulation, acting as the intracellular mediator of the action of the majority of mammalian short term sudden effect hormones, for example adrenalin and glucagon (1). A second cyclic nucleotide, guanosine 3',5'-cyclic monophosphate (cyclic GMP), has more specific regulatory roles, functioning as part of the signal transduction mechanism which responds to light perception in the mammalian eye and as a mediator of atrial natriuretic protein action (2), while a third, more recently established, cyclic nucleotide, cytidine 3',5'-cyclic monophosphate, has a suggested involvement in the regulation of cellular proliferation (3). These biochemical functions of cyclic nucleotides led to their description as second messengers (the hormone or other agonist is termed the primary messenger) and make them potential pharmacological targets of great significance. Analysis of cyclic nucleotides is extremely difficult due in part to their low cellular concentrations and also due to their molecular instability. While traditional methods of mass spectrometry have not been of any wide applicability in this field because of the instability of volatile cyclic nucleotide derivatives, the advent of fast atom bombardment mass spectrometry and mass-analysed ion kinetic energy spectrum scanning has significantly changed this situation.

Application of FAB/MIKES analysis to cyclic nucleotides has provided a very effective means of distinguishing 3',5'-cyclic nucleotides, the metabolic regulators, from their 2',3'-isomers, breakdown products of nucleic acids, by the presence of a major fragmentation in the 3',5'-isomers producing a prominent peak at an m/z value corresponding to protonated base plus 42 mass units ($[BH_4 + 42]^+$), and the absence of any such prominent peak in the MIKE spectra of the 2',3'-cyclic nucleotides (4). FAB/MIKES analysis of partially purified extracts, from both mammalian and plant tissues, subjected to sequential chromatographic procedures, have provided the first unambiguous evidence of the presence of cyclic CMP in mammals (5) and of cyclic GMP in higher plants (6). Refinement of the purification procedure has now enabled the first demonstration of the natural occurrence of uridine-, inosine- and deoxythymidine-3',5'-cyclic monophosphates (cyclic UMP, cyclic IMP and cyclic dTMP) in mammalian tissues (7) and in higher plants (8), in the latter case including the exciting observation that cyclic CMP was found in higher relative concentrations in the rapidly proliferating tissues, that cyclic UMP was found only in these regions, and that cyclic IMP and cyclic dTMP were found only in tissues dividing slowly (8).

Analysis of cyclic nucleotide-related enzymes by FAB/MIKES has also proved successful. Cyclic CMP has been identified as one of the products resulting from the incubation of dialysed cell-free preparations from rat brain, liver and kidney with cytidine 5'-triphosphate (CTP). In addition to cyclic CMP and unchanged CTP, the products of the reaction were found to include cytidine monophosphate (CMP) and cytidine diphosphate (CDP) together with four novel cytidine compounds identified as cytidine 3',5'-cyclic

pyrophosphate, cytidine 2'-monophosphate-3',5'-cyclic monophosphate, cytidine 2'-O-aspartyl-3',5'-cyclic monophosphate and cytidine 2'-O-glutamyl-3',5'-cyclic monophosphate (9). This evidence provided the first unequivocal proof of the natural occurrence of cytidylate cyclase activity; the four novel cytidine cyclic phosphates identified provide an explanation of the discrepancies in previous reports which led to controversy concerning cytidylate cyclase.

Analysis of the cyclic nucleotide-related enzymes has now been taken a stage further with the quantitation of cyclic nucleotide phosphodiesterase activity (10). Characteristic peaks of the substrate, cyclic AMP, and product, AMP, were identified in positive FAB mass spectra and MIKE scans of the protonated molecular ions. By spiking enzyme incubates with known quantities of cyclic AMP and AMP and measuring peak heights in the MIKE spectra of both spiked and unspiked samples, the concentrations of cyclic AMP and AMP in solution at the end of a series of enzyme incubations have been estimated. From the data obtained the kinetic parameters, K_m and V_{max} , showed very close agreement with those obtained by radioactive assay.

Further application of FAB/MIKES has been made in the examination of derivatives used in studies of the biochemical function of cyclic nucleotides. The technique has provided the only rapid means of confirming the retention of the cyclic phosphodiester group during the synthesis of the 2'-O-succinyl, 2'-O-succinyl-proteinyl- and 2'-O-succinyl-tyrosinyl-methyl-ester-derivatives of cyclic nucleotides essential for the development of radioimmunoassay, the major method of cyclic nucleotide estimation. Similarly the identity of lipophilic cyclic nucleotides, synthesized to enable the cyclic nucleotides to permeate through cellular membranes, has been confirmed by FAB/MIKES analysis, which has been used to determine the position of butyrylation in N⁶,2'-O-dibutyryl adenosine 3',5'-cyclic monophosphate, N⁶,2'-O-dibutyryl guanosine 3',5'-cyclic monophosphate and N⁶,2'-O-dibutyrylcytidine 3',5'-cyclic monophosphate (11).

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An Investigation of Ionization Mechanisms During
Liquid Secondary Ion Mass Spectrometry (LSIMS): Evidence for
One-Electron Reduction of Oligonucleotides and Modified Oligomeric Analogs.

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The base sequencing of oligodeoxynucleotides, DNA/adducts, and modified oligonucleoside analogs is the goal of this work. Oligodeoxynucleotides and modified oligonucleoside structures show intense molecular anions in negative ion LSIMS spectra. In addition, cleavages along the backbone allow for bidirectional (3' and 5' ion series) base sequencing of the DNA chain (Grotjahn *et al.*, 1985). This phenomena has been used to sequence oligonucleoside analogs containing non-phosphodiester backbones (Griffin *et al.*, 1988) as well as analogs containing unnatural base or modified sugar residues neither of which can be enzymatically digested via conventional sequencing methods. Negative ion LSIMS uniquely provides the needed data (Figure 1).

A comparison of experimental (Figure 2a) and calculated (Figure 2b) isotopic cluster patterns for the (M-H)⁻ anion of d(pA)₈ shows poor agreement; Bovine insulin oxidized B-chain and β -cyclodextrin give excellent agreement between experiment and theory. This indicated the molecular anion region of d(pA)₈ was recorded without discrimination and that ionic species other than (M-H)⁻ are present - one electron reduction (Williams *et al.*, 1987) is suggested. The molecular anion cluster pattern can be greatly influenced by the electron affinity of the matrix (Figure 3). Spectral deconvolution reveals the presence of several ionic species in the molecular anion cluster including a radical anion and an MH⁻ species. A theoretical molecular anion isotopic cluster can be synthesized which closely matches the experimental results (histogram in Figure 2a).

Oligodeoxynucleotides up through 18-mers have been ionized and their spectra deconvoluted by solution of a set of simultaneous equations. A critical look at LSIMS data is timely. Since one-electron reduction appears to be mass dependent, the average mass of the molecular anion envelope will shift to higher mass leading to a potential mass measurement error. Moreover, careful use of isotope ratios to track biological pathways or determine isotopic enrichment is necessary and consideration must be made of the ions present. Deconvolution of the molecular anion cluster is used to estimate the relative concentrations of ionic species present.

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Figure 1

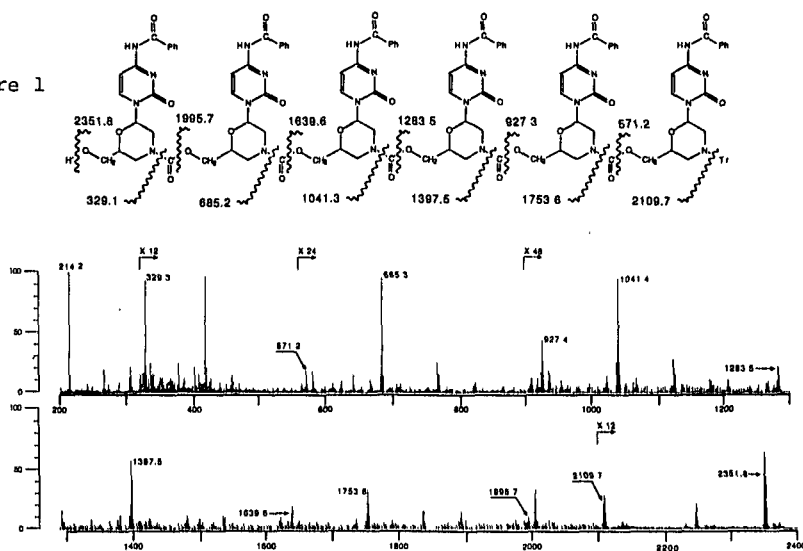


Figure 2a

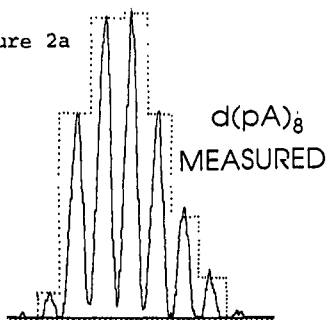


Figure 2b

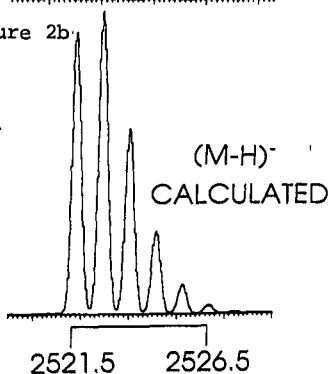
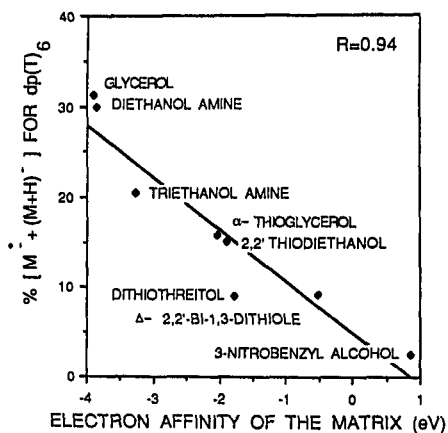


Figure 3



Research supported by National
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QUANTIFICATION OF EPOXYEICOSATRIENOIC ACIDS BY CAPILLARY
COLUMN CHROMATOGRAPHY/NEGATIVE ION CHEMICAL
IONIZATION MASS SPECTROMETRY

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The epoxyeicosatrienoic acids (EETs) are cytochrome P-450 metabolites of arachidonic acid (1). The EETs are endogenous constituents of rat liver, kidney and lung and have been detected in urine (2). They possess a diversity of biological activities including the ability to stimulate peptide hormone release from endocrine cells, mobilize microsomal Ca^{2+} from aortic smooth muscle and anterior pituitary cells, inhibit arachidonic acid-stimulated platelet aggregation and inhibit vasopressin stimulated water transport (2). In order to help determine the role of EETs in endogenous biochemical processes it was necessary to develop highly sensitive and specific method for their analysis. It was of particular interest to develop methodology that would allow their quantitative determination to be carried out from stimulated human platelets. Capillary gas chromatography/electron capture negative ion chemical ionization mass spectrometry (GC/NICIMS) assays have been developed for a number of eicosanoids previously (3). This methodology has now been applied to the analysis of the EETs.

Analysis of the pentafluorobenzyl (PFB) ester derivative of 14,15-EET was carried out by GC/NICIMS. The mass spectrum showed an intense ion at m/z 319 (M-PFB), together with fragment ions at m/z 303 (M-PFB-O) and m/z 301 (M-PFB- H_2O). The 5,6-, 8,9- and 11,12-EET-PFB ester derivatives showed identical spectra. The PFB ester derivatives of the four regioisomers could not be separated by capillary column chromatography. During the course of these studies it was noted that artifactual formation of EETs from arachidonic acid could occur by autoxidation. It was found that this undesirable reaction could be minimized by removal of arachidonic acid prior to derivatization and by adding butylated hydroxytoluene (BHT) as an antioxidant.

Previous studies had shown that EETs are esterified into phospholipids (3) and so we required a method that could be used for the quantification of esterified EETs. Methodology has been developed that involves phospholipase A_2 (PLA_2) hydrolysis of extracted phospholipids. Addition of BHT during hydrolysis minimized autoxidation of released arachidonic acid. Hydrolysis was carried out with PLA_2 at 22 °C for 5 min in the presence of 0.1 % BHT. The crude product obtained from PLA_2 hydrolysis was submitted to reversed phase HPLC for further purification. Purified EETs were hydrogenated with $\text{Rh}/\text{Al}_2\text{O}_3$ and then esterified with pentafluorobenzyl bromide (PFB). Hydrogenation of double bond in EET molecule facilitated complete separation of the 5,6-, 8,9-, 11,12- and 14,15-EET PFB esters. GC analysis was carried out on an HP-17 fused silica capillary column (25 m x 0.25 mm I.D.) (Figure 1). The NICI mass spectrum of hydrogenated 14,15-EET-PFB showed an intense M-PFB anion at m/z 325 together with ions derived from fragmentation at the epoxide moiety at m/z 225 and m/z 241 (Figure 2). Similar types of fragment ions were observed with the other three EETs. Oxygen-18 labelled EETs were synthesized for the use of internal standards in quantitative analysis. Good linearity was observed between the peak area ratio of m/z 325 (M-PFB for $^{16}\text{O}_2$ -EET) to m/z 329 (M-PFB for $^{18}\text{O}_2$ -EET) and weight ratio of $^{16}\text{O}_2$ -EET to $^{18}\text{O}_2$ -EET in the range of 1 to 10 ng/mL. The present method was applied to quantification of EET released from cyclooxygenase-inhibited thrombin stimulated human platelets. It was shown that 5.2 ± 2.8 ng/ 10^9 platelets ($n = 4$) of EETs were released. The possibility that this trace amount was formed as a consequence of autoxidation of arachidonic acid released during the phospholipase hydrolysis cannot be excluded.

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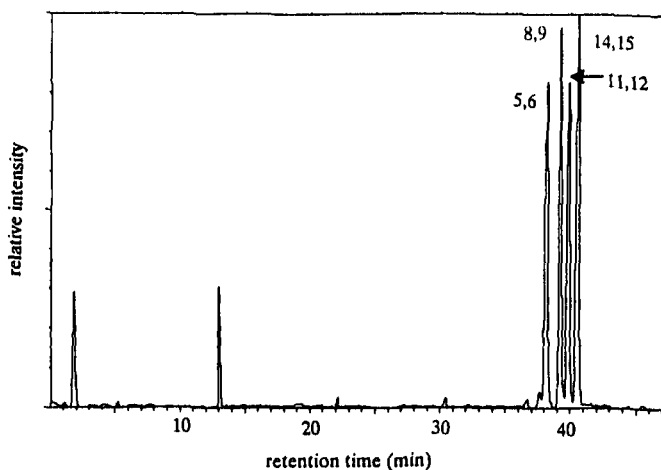


Figure 1: Separation of epoxyeicosatrienoic acid pentafluorobenzyl esters on a 30 m HP-17 column.

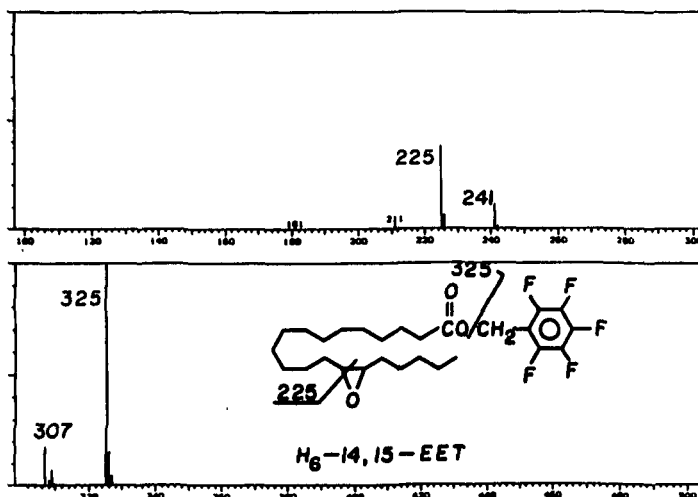


Figure 2: Electron capture negative ion chemical ionization mass spectrum of 14,15-epoxyeicosatrienoic acid pentafluorobenzyl ester.

Acknowledgement

Supported by NIH grants DK 38226 and AM 26657.

Because only partial purification of several phosphoinositides was obtained using TLC and silicic column chromatography, and because triethanolamine matrix ions were present in all FAB mass spectra, B/E linked scanning with collisional activation was carried out in order to eliminate matrix ions and other contaminating ions. Although two LPI species co-eluted during chromatographic purification, for example, B/E linked scanning of the respective deprotonated molecular ions provided essentially pure daughter ion spectra which were free from contaminating ions. (See Figures 2 and 3.) B/E linked scanning also facilitated the identification of structurally significant fragment ions. In all of the B/E scans, abundant daughter ions were typically detected which corresponded to palmitate (m/z 255) or linoleate (m/z 279) (Figures 2 and 3.) There was no evidence of any other fatty acids. Other daughter ions included m/z 433, 409, 315, 241, and 153, corresponding to linoleoyllysophosphatidic acid, palmitoyllysophosphatidic acid, glycerophosphoinositol, dehydrated inositol phosphate, and glycerophosphate, respectively.

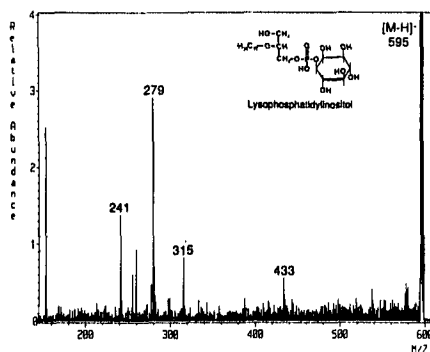


Figure 2. B/E linked scan of $[M-H]^-$ (m/z 595) of linoleoyl LPI from carrot cells.

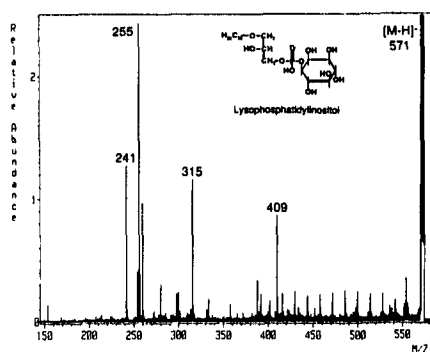


Figure 3. B/E linked scan of $[M-H]^-$ (m/z 571) of palmitoyl LPI from carrot cells.

Palmitoyl,linoleoyl-PI was the only phosphatidylinositol detected in carrot cells. In contrast, arachidonoyl, stearoyl-PI, linoleoyl,palmitoyl-PI, and linoleoyl,linoleoyl-PI were detected in animal PI samples used as chromatography reference standards. Jensen and coworkers (3), using FAB MS/MS, detected small amounts of fatty acids other than palmitic and linoleic acid in soybean PI. As for the other phosphatidylinositols detected, LPI and PIP were also shown by mass spectrometry to contain acyl groups corresponding exclusively to linoleic (18:2) and palmitic (16:0) acid. These measurements represent the first physico-chemical evidence of these phosphatidylinositols in plant cells. Investigations are in progress to determine whether PIP₂ and LPIP are present in carrot cells.

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ENHANCED DETECTION OF DIAGNOSTIC ACYLCARNITINES
IN PLASMA AND TISSUE UTILIZING FAB COUPLED
WITH TANDEM MASS SPECTROMETRY

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It is possible to characterize a range of inborn errors in the metabolism of branched-chain amino acids and fats by the identification of diagnostic acylcarnitines. These metabolites are usually analyzed in biological matrices (e.g. urine, blood and tissue) by fast atom bombardment mass spectrometry (FAB-MS). The high level of chemical interference in such matrices, however, limits the applicability of the method. This paper describes a new method utilizing partial chemical clean-up coupled with FAB- tandem mass spectrometry (FAB-MS/MS) for the analysis of diagnostic acylcarnitines in plasma and tissue samples.

METHODS Acylcarnitines were recovered from plasma and tissue samples according to the procedure outlined in Figure 1. Recoveries of spiked standards were shown to be greater than 86% for both biological matrices. FAB-MS/MS analyses were performed on a VG Tric-3 triple quadrupole mass spectrometer as previously described (1,2). A "precursors of m/z 99" scan was utilized to produce a spectrum of acylcarnitine molecular cations, referred to as an "acylcarnitine profile". A beam of xenon atoms (8Kev) from a saddle-field ion gun was used to achieve sample ionization. The collision gas for CID was argon (0.5 pbar) and the collision energy was 15 eV.

RESULTS The FAB-MS/MS daughter ion spectra of acylcarnitine methyl ester M⁺ ions show a prominent common fragment at m/z 99 (1). This ion is derived from the loss of both the acyl moiety as the corresponding acid, and the quaternary ammonium group as trimethylamine (Figure 2) and represents the "backbone" of the acylcarnitine molecule. The "precursors of m/z 99" scan therefore generates a spectrum of M⁺ ions from acylcarnitines. Figure 3 depicts three FAB-MS/MS acylcarnitine profiles from blood of a normal child (A); a patient with propionic acidemia (B); and a patient with medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (C). In the normal profile, acetyl carnitine (m/z 218) is dominant with lesser amounts of C₃, C₄ and certain other acylcarnitines also present. The abnormal profiles show the expected diagnostic metabolites: propionyl carnitine (m/z 232) for propionic acidemia; hexanoyl carnitine (m/z 274), octanoyl carnitine (m/z 302), and decenoyl carnitine (m/z 328) for MCAD deficiency. The identification of these acylcarnitines was confirmed by infusion of stable isotope-labelled carnitine to an affected patient (Figure 4).

Diagnostic acylcarnitine profiles have also been obtained from tissue samples (2). Detection limits for this method have been estimated to be 0.5 nmol/ml in plasma and 1 nmol/g wet weight in tissue. By coupling with a continuous-flow FAB interface, samples can be processed very rapidly, making the method feasible for large scale neonatal screening.

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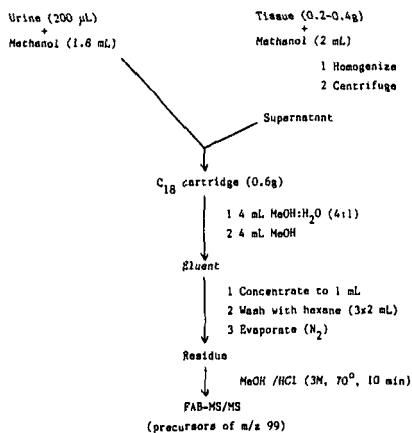


Figure 1. Procedure for the recovery of acylcarnitines from plasma and tissue

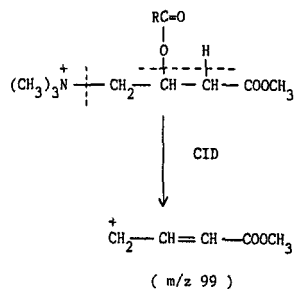


Figure 2. Origin of the m/z 99 ion in the CID spectra of acylcarnitines

Figure 3. FAB-MS/MS acylcarnitine profiles from plasma: (a) normal; (b) patient with propionic acidemia; (c) patient with medium-chain acyl-coA dehydrogenase (MCAD) deficiency

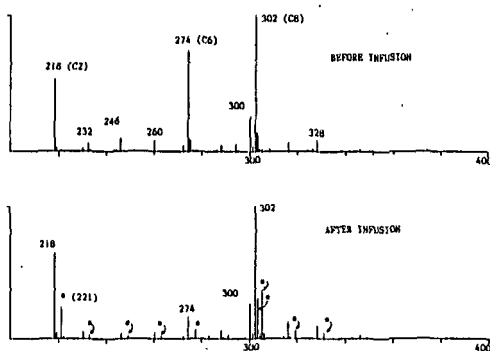
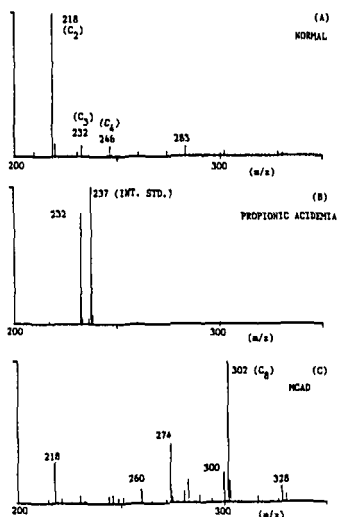


Figure 4. Identification of acylcarnitines in plasma from MCAD deficient child after infusion of $^3\text{H}_3$ -L-carnitine

OBSERVATION OF SEQUENCE SPECIFIC PEPTIDE FRAGMENTATION USING MS/MS/MS SCAN MODES

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Several MS/MS/MS scan modes¹ have been applied to provide sequence specific fragmentation of penta- and octapeptides from pure samples and from mixtures. These experiments have been carried out on hybrid BEQQ and pentaquadrupole instruments with ionization by either fast atom bombardment with Xe atoms or liquid SIMS with Cs⁺ ions.

The reaction intermediate scan², symbolized $\bullet \rightarrow \circ \rightarrow \bullet$, where $\bullet$ represents a fixed mass analyzer and $\circ$ represents a scanning mass analyzer, is the most useful scan for obtaining sequence information because it provides MS/MS/MS spectra of any peptide in a mixture with little or no interference. In the reaction intermediate scan peptide (M+H)⁺ ions are mass selected from the mixture with the first mass analyzer and an N- or C-terminal sequence ion is selected as the final product ion by the final mass analyzer. Intermediate ions between the selected (M+H)⁺ and final product ions are recorded by scanning the middle quadrupole of the pentaquadrupole or by scanning the sector portion of the BEQQ in a B/E linked scan. A, B, and C sequence ions are exclusively observed when an N-terminal containing ion is chosen as the final product ion. A C-terminal containing ion may also be chosen as the final product ion yielding spectra with X, Y, and Z ions exclusively. The N-terminal reaction intermediate scan of peptide I, Tyr-Ala-Gly-Phe-Met-NH₂ (M+H)⁺ m/z 587, obtained on the BEQQ instrument is shown in Figure 1. By selecting the N-terminal tyrosine immonium ion, m/z 136, as the final product ion, only A and B type ions are observed in this spectrum. The C-terminal reaction intermediate spectrum of peptide II, Tyr-Pro-Phe-Pro-Gly (M+H)⁺ m/z 580, obtained on the pentaquadrupole instrument is shown in Figure 2 where the C-terminal containing Y₂⁺ ion was chosen as the final product ion. Only Y type sequence ions are observed and the sequence of the peptide can be readily determined. The detection limit obtained on the newly constructed pentaquadrupole instrument for a leucine enkephalin sample is 4 nmoles. The pentaquadrupole instrument provides more sequence information than the BEQQ instrument due to the ability of the low-energy collisions to generate fragment ions over the entire mass range. In addition, reaction intermediate spectra obtained on the BEQQ instrument contain artifact peaks due to the poor resolution of the linked scans employed.

The selective parent scan, symbolized $\circ ==> \circ \rightarrow \bullet$, where the first part of this scan ($\circ ==> \circ$) represents a neutral loss scan (loss of a CO group in these experiments), produces spectra with exclusively B or X type ions depending on the final product ion chosen. By selecting an N- or C-terminal ion as the final product ion, only B or X ions are observed, respectively. This scan mode provides more specific sequence information than the reaction intermediate scan, but can only be employed on pure peptide samples if interferences are to be avoided. The selective parent spectrum of peptide I obtained on the pentaquadrupole instrument is shown in Figure 3 indicating B type ions only by selecting the N-terminal immonium ion having m/z 136 as the final product ion.

Sequence determination of unknown peptides can be greatly simplified by using the appropriate sequence of MS, MS/MS, and MS/MS/MS experiments.

¹ R.G. Cooks, J.W. Amy, J.C. Schwartz and K.L. Schey, *Adv. Mass Spectrom.*, 11A (1989) 33.

² J.R. O'Lear, L.G. Wright, J.N. Louris and R.G. Cooks, *Org. Mass Spectrom.*, 22 (1987) 348.

Figure 1. Reaction intermediate scan of peptide I in BEQQ instrument.

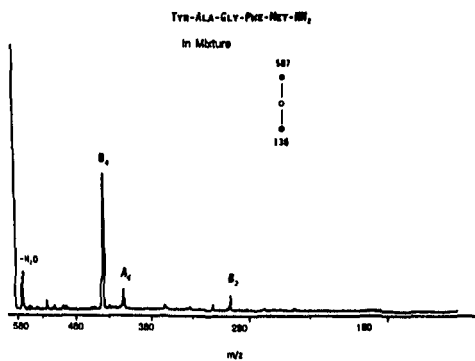


Figure 2. Reaction intermediate scan of peptide II in pentaquadrupole instrument.

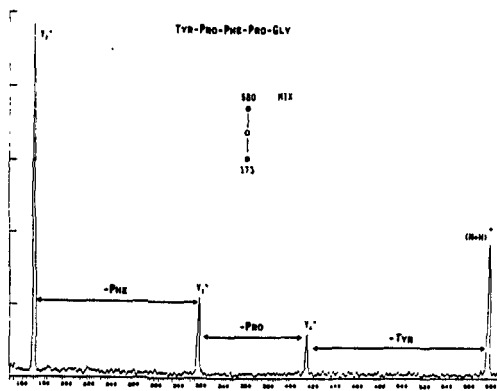
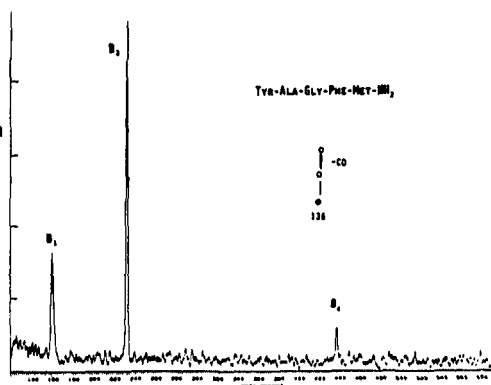


Figure 3. Selective parent scan of peptide I in pentaquadrupole instrument.



TANDEM MASS SPECTROMETRY OF MULTIPLY CHARGED PEPTIDES AND PROTEINS

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In the past two years electrospray ionization (ESI), originally described by Dole and coworkers¹ and reinvigorated by Fenn and coworkers², has received considerable attention as a soft ionization source for mass spectrometry. The ESI method produces intact, multiply charged, gas phase molecular ions of peptides and proteins from aqueous solution with mass to charge ratios (m/z) that are easily within the range of quadrupole mass spectrometers. In fact we have shown that mass spectra of proteins of molecular weight greater than 100,000 daltons can be obtained on an instrument limited to 2000 m/z ³. Because of the possible additional structural information from collisionally induced dissociation (CID) /tandem mass spectrometry, multiply charged ions are likely candidates for examination by this technique, possibly producing all or significant portions of the amino acid sequence.

Although the multiply charged ions give abundant fragmentation, it is still a question whether meaningful fragmentation can be efficiently produced by CID. It could also be anticipated that the charge state of the parent ion might significantly affect the daughter ion spectra both in ions observed and their intensities. Three peptides, mellitin, glucagon, and the synthetic T₁ tryptic fragment of glucagon, and the protein cytochrome-c, have been investigated to examine these issues.

Mellitin is a 26 amino acid peptide of M_r 2846 daltons with six possible positive charge sites. Within the 1400 m/z range of the instrument four charge states, 3+, 4+, 5+, and 6+, are observed. A significant degree of meaningful fragmentation is seen in each of the four daughter ion spectra. The dominant cleavage types are B and Y modes⁴. By combining the daughter ion information from all four ions molecular ions (including daughter ions with m/z greater than that of the parent due to unsymmetrical mass and charge cleavage), the sequence for 20 of the 26 amino acids can be reconstructed⁵. Because some of the same daughter ions appear in more than one spectra and sometimes in more than one charge state, fragment assignments can be made with a good degree of confidence. Interestingly the region not probed is the amidated C-terminus containing four of the six possible charge sites.

Glucagon is a larger 29 amino acid peptide, M_r 3483 daltons, with four possible positive charge sites producing three observable parent ions, 3+, 4+, and 5+. Although daughter ions were observed for each parent ion, only a few amino acid residues (4 at the N-terminus and 9 at the C-terminus) were rationalized with almost no interior fragmentation. However by applying the same techniques to the four synthetic tryptic fragment peptides of glucagon of which T₁ is shown, the entire amino acid sequence could be rationalized.

Horse heart cytochrome-c is a small protein of 104 amino acids, M_r 12,360 daltons, and 23 possible positive charge sites. The MS spectra yields a multiply charged distribution from 9+ to 21+ peaking in intensity around 15+. Although many peaks, clustered around the m/z of the parent, are seen in the low energy CID MS/MS spectra of cytochrome-c, we have not as yet been able to rationalize their identity due to the large number of possibilities. The pattern of these fragments may, however, be useful in a qualitative, peptide mapping fashion. In fact the CID MS/MS spectra of the 15+ molecular ion of cytochrome-c from several different species shown in Fig. 1 demonstrates the dramatic fragment pattern differences for these ions, even between ions that differ by only three and four amino acids, e.g. horse vs. cow and cow vs. rabbit respectively.

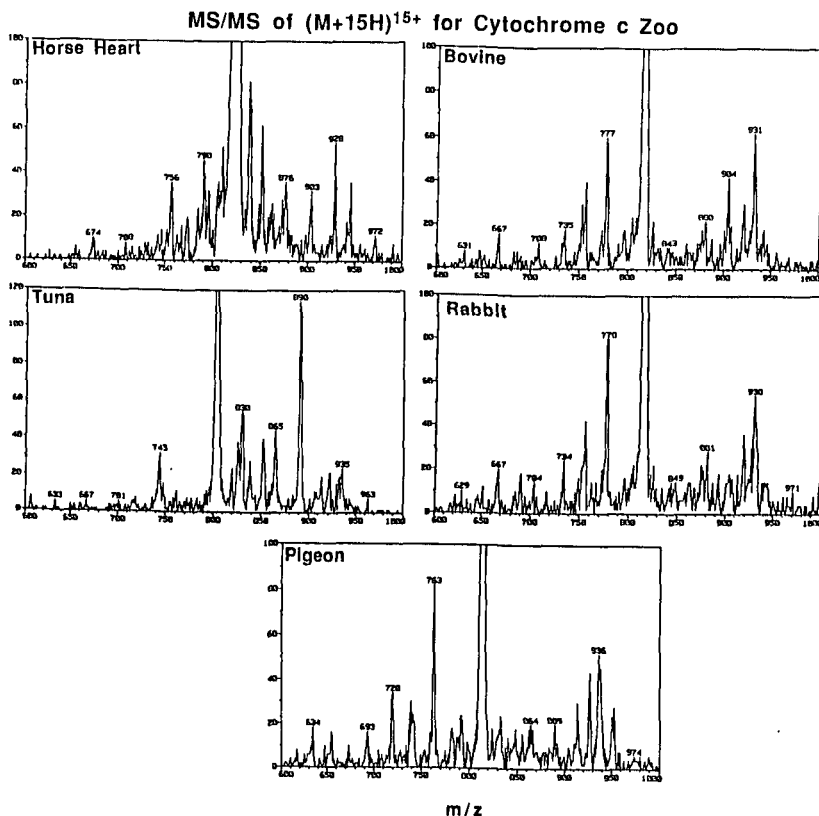
Thus several statements on the CID tandem mass spectrometry of multiply charged molecular ions of peptides and small proteins can be made. They can be efficiently collisionally dissociated into meaningful fragments,

predominantly Y and B type cleavages, providing an information rich spectra which can lead to significant degrees of sequence determination. The redundancy of ions originating from different charge state parent ions enhances confidence in assignment. CID of enzymatic digestion fragments may be required for those molecules that resist CID. Higher charge states yield greater fragmentation, at least using low energy CID, i.e., quadrupole instrumentation. For proteins, although extensive sequence information may not be readily obtainable, CID results appear useful for rapid peptide mapping or "fingerprinting".

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STRUCTURE DETERMINATION OF NOVEL CYCLIC PEPTIDES BY USING TANDEM MASS SPECTROMETRY

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An increasing number of possible anti-cancer agents are being isolated from marine animals and plant life. Many of these compounds are cyclic peptides that contain unusual amino acids, some of which may result from chemical modification by the marine organism of more normal amino acids.

We have developed a methodology which is effective in determining the amino acid sequences of these peptides. The steps are as follows:

- 1) Obtain FAB mass spectrum and verify molecular species
- 2) Determine exact mass of molecular species
- 3) Obtain MS/MS spectrum of $(M + H)^+$ ions
- 4) Compare FAB spectrum and MS/MS spectrum
- 5) Obtain MS/MS spectra of source-produced fragment ions corresponding to significant fragments observed in the MS/MS spectrum of $(M + H)^+$
- 6) Determine exact masses of fragment ions as necessary to verify empirical formulae.

Some of these points appear rather obvious. The significant information available from MS/MS spectra of source-produced fragment ions, however, has often been overlooked. The utility of this approach can be demonstrated with two examples.

A partial FAB spectrum and the MS/MS spectrum of $(M + H)^+$ of the cyclic peptide hymenistatin 1 (isolated from a South Pacific Ocean sponge) are shown in Fig. 1 and 2. Protonation at a basic amide nitrogen results in ring-opening to form a linear acylium ion. Several abundant fragment ions are observed in the MS/MS spectrum; the ions of m/z 780, for example, results from loss of isoleucine from the acylium terminus. From amino acid analysis, the peptide is known to contain three proline residues. Protonation is likely to occur at any one of these three basic sites, resulting in the formation of a mixture of three different, unresolvable acylium ions. The MS/MS spectrum of $(M + H)^+$ is, therefore, a composite of the individual spectra of these three species. Ions of m/z 780, 794, and 796 are observed in both the FAB spectrum and the MS/MS spectrum of $(M + H)^+$. These ions result from losses of isoleucine, proline, and valine, respectively, from the termini of the three different acylium ions. MS/MS spectra of the source-produced fragment ions provide resolution of the different species and allow unequivocal determination of the cyclic peptide sequence. Exact mass measurements are used to verify key empirical formulae.

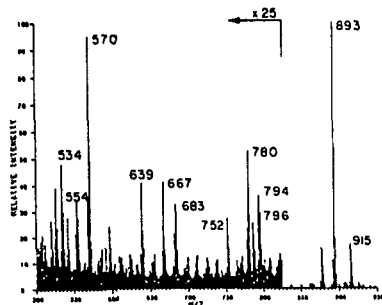
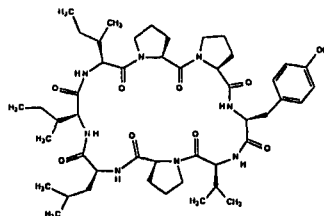
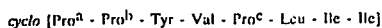


Fig. 1

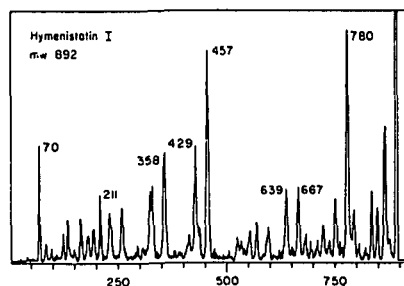


Fig. 2

A second example is provided by the structure determination of dolastatin 13, a cyclic peptide isolated from the Indian Sea Hare *Dolabella auricularia*. The first problem encountered is the determination of the molecular species. The FAB spectrum of the peptide obtained using a protonating matrix (see Fig. 3) produces a apparent $(M + H)^+$ of m/z 888. Addition of a Li^+ salt (see insert), however, results in the formation of $(M + Li)^+$ of m/z 912. The ions of m/z 888 result from loss of H_2O from the protonated molecular species. The MS/MS spectrum of $(M + H)^+$ (see Fig. 4) is also dominated by H_2O elimination. Upon magnification of the data, several other significant ions are observed. There is, however, no clear association between them. MS/MS spectra of the source-produced fragment ions of m/z 487, 646, and 789 clearly show that these ions are not related and result from competitive fragmentation processes. The structure cannot be determined by means of the MS/MS spectrum of $(M + H)^+$ alone. A fragmentation scheme obtained by combining MS/MS data of several fragment ions with exact mass measurements is shown in Fig. 5. Several different competing processes occur; some produce equivalent fragment ions via different routes. The determined structure is shown in Fig. 6. The basic structure of the peptide consists of a six-membered amino acid ring with a two amino acid side chain. A unique cyclic hemiacetal (presumably derived from a Phe-Glu dipeptide precursor) is incorporated in the cyclic portion of the molecule. Cyclization occurs through the theonine oxygen. Another novel aspect of the peptide is that the terminus of the side chain is not a normal amino acid but is rather methyl-glyceric acid.

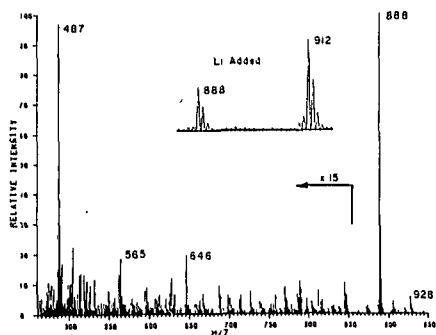


Fig. 3

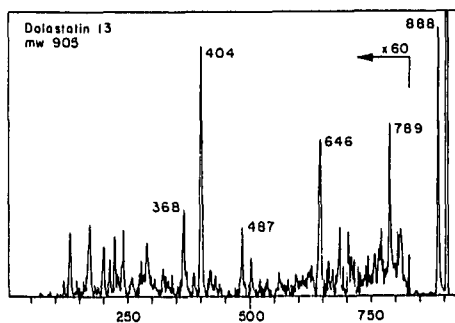


Fig. 4

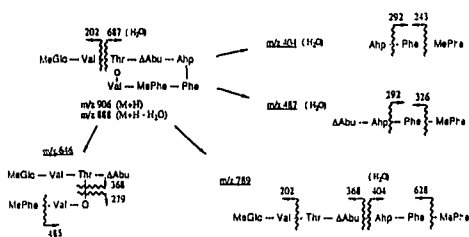


Fig. 5

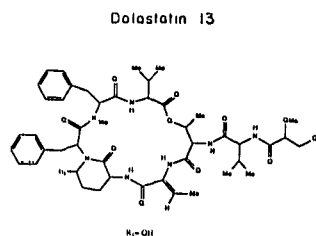


Fig. 6

HIGH vs. LOW ENERGY CID OF PROTONATED PEPTIDES*

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The motivation for this work is best understood from a comparison of Figures 1 and 2, each a CID spectrum of protonated Dynorphin Fragment 1-9 (YGGFLRRIR-OH)⁺, m/z 1137.5. Figure 1 was obtained by using a VG 70-4SE tandem double focussing instrument (EBEB); only a single loading of 0.5 nmol of peptide on the FAB probe was required. Figure 2 resulted from use of a VG ZAB-EQ hybrid tandem instrument (BEQ); multiple sample loadings and scan accumulations were used to obtain this spectrum. The contrast is striking! Test cases used in this work included the Dynorphin Fragments from 1-5 to 1-9 inclusive, and oligomers of phenylalanine up to Phe₈. The latter were studied to study size effects without interference from peptide composition. The EBEB instrument yielded CID spectra of excellent quality under simulated real-world analytical conditions, viz. 0.5-1 nmol of sample consumed; the only exception was Phe₈, for which the precursor beam current of 10⁻¹⁴ A was so low that the quality of the CID spectrum was limited by ion statistics. In comparison the hybrid instrument yielded a good quality spectrum for Phe₈, and a poor spectrum for Phe₇, only after multiple sample loadings with scan accumulation. The EBEB instrument easily produced rich CID spectra for all the Dynorphin Fragment peptides studied here, with only directing effects of composition (basic residues at terminus). The low energy CID spectra were subject to extreme compositional effects, in that highly basic residues (e.g.arginine) stabilised peptides to CID (Fig.2).

The reasons for the low CID efficiency of the hybrid instrument compared with the extremely high efficiencies observed for the same instrument with precursors below 500 Da or so, can be considered in two categories:

- A. Dynamic ion optics of the qQ assembly.
- B. Fundamental aspects of the collisional activation (CA), and subsequent dissociation, of the protonated peptides.

Point A is the subject of current work, but is not a dominant reason here since protonated Gramicidin S (m/z 1141.5) yielded an excellent CID spectrum under conditions identical to those used for Fig.2. It appears that CID of large organic ions at keV energies can access CA mechanisms not available at low energies; this is confirmed by the abundant sidechain cleavages (d,v,w fragments) observed at high energies but not at all below about 300 eV in the hybrid instrument. The most probable candidate is electronic excitation, but this cannot explain the dramatic switch from high to low CID efficiency for low energy CID as the mass is increased above 500-600 Da. It has been proposed that increasing disparity in size between projectile and target demands a switch in the momentum transfer CA mechanism from the "elastic" limit (target interacts with entire projectile) to the "spectator" limit (target atom interacts elastically with a portion of the projectile which subsequently shares the transferred energy/momentum with the spectator portion). (See A.J.Alexander and R.K.Boyd, IJMSIP, in press).

Other qualitative differences include the high abundance of internal cleavage fragments (both N- and C- termini lost) in the low energy case, due probably to the multiple-collision conditions normally employed. Loss of the C-terminus is a low-energy process (appears under unimolecular conditions) but is subject to compositional conditions as yet unknown.



CID OF (YGGFLRRIR-OH)⁺, m/z 1137.6
COLLISION ENERGY 100 eV
ARGON COLLISION GAS, 80% ATTENUATION

* NRCC No. 30277

ANALYSIS OF SYNTHETIC PEPTIDE MIXTURES AND
PROTEIN DIGESTS BY CZE/CF-FABMS

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The application of high-resolution separation techniques coupled with mass spectrometry is a powerful approach to solving problems in the area of protein structure and is especially suited to the characterization of biotechnology products obtained from recombinant DNA methods and automated synthesis (1). We have previously shown that microbore LC/CF-FAB mass spectrometry is a very effective tool in this regard (2,3). Since the CF-FAB carrier solution, 5% glycerol 3% acetonitrile in water, is suitable for the analysis of peptides, we have in this study examined the use of CF-FAB to introduce the effluent derived from capillary zone electrophoresis (CZE) to a mass spectrometer. CZE is a high resolution separation method for charged molecules based on their differential rates of migration in an electric field created by applying a high voltage (20-30 KV) across an open tubular fused silica capillary (10-100 μ m ID) filled with a buffer solution (4). Performing electrophoresis in the "capillary dimension" permits efficient cooling of the process so that Joule heating is minimized, reducing sample zone broadening and thereby increasing separation efficiency. Another consequence of performing electrophoresis in silica capillaries is the creation of a phenomenon known as electroosmotic flow. This flow also contributes to the high resolution capability of CZE and permits a unidirectional non-parabolic bulk flow of capillary solution containing all analytes whether they be cations, anions, or neutrals, toward the cathode.

In order to effect a transfer of CZE effluent to the CF-FAB probe an interface device incorporating a unique combination of features that were previously and separately employed by others (5,6) was developed as shown in Figure 1. CZE was performed using an apparatus constructed at Applied Biosystems Inc. similar to that described by Jorgenson (4). The CZE fused silica capillary was 90 cm long. As indicated in Figure 1, the effluent end of the CZE capillary (50 μ m ID) and the pick-up end of the CF-FAB capillary (75 μ m ID) meet in a short segment of Teflon tubing (0.15 mm ID) placed in a plexiglass block consisting of two 90 degree intersecting passageways (1/16 in. ID). The other open passageway permits the introduction of the "flow-through" cathode and CF-FAB matrix. The flow rate differential between that in the CF-FAB capillary (5 μ L/min) and the CZE capillary (0.1 μ L/min) and the enclosure of the capillary juncture in the Teflon sleeve permits efficient transfer of CZE eluate to the mass spectrometer as shown in Figures 2 and 3. Figure 2 shows both the UV detector trace (upper) and the MS total ion chromatogram (lower) derived from a CZE/CF-FAB analysis of a synthetic peptide reaction mixture prior to purification of the desired product (the major peak). Direct comparison of the resolution recorded by the UV and MS detectors is difficult because of the differences in their physical characteristics and operating dynamics. Nevertheless, if one regards the chromatograms as an indication of 'apparent' resolutions as recorded by these detectors, then the profiles indicate that this apparent resolution is quite similar and that no major band broadening has occurred as a consequence of transfer in the interface. A similar result is shown in Figure 3 for the CZE/CF-FAB analysis of a tryptic digest of 40 pmol of recombinant human growth hormone. Again the UV trace and the total ion chromatogram show very similar profiles.

CF-FAB mass spectrometry was performed with a Finnigan MAT 90 high resolution instrument equipped with an ION Tech b11NF saddle field gun using Xe to create energetic atoms. The CF-FAB probe used was the Finnigan-MAT Bioprobe. The mass spectrometer was generally operated at a resolving power of 1500 and an accelerating voltage of 4.7 KV. The magnet was scanned at approximately 10 sec/decade. CsI was used to calibrate the instrument.

We conclude that CZE separations in combination CF-FAB analysis coupled through the interface described here is a powerful, high resolution, and sensitive tool that can be exploited by the protein chemist to assess protein/peptide structure.

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Fig. 1

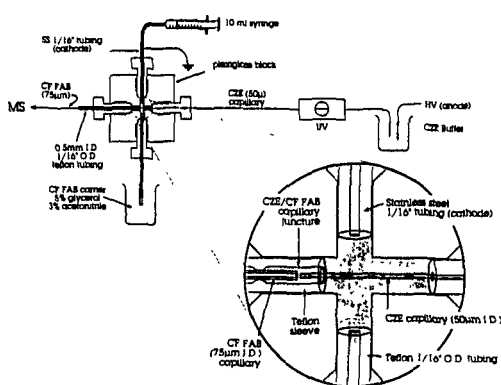


Fig. 2

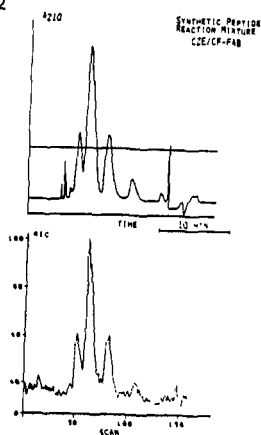
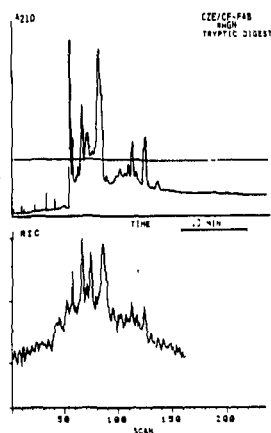


Fig. 3



SENSITIVITY ENHANCEMENT OF PARTICLE BEAM LC/MS.

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INTRODUCTION: Particle Beam (PB)-LC/MS are transport-enrichment interfaces that allow analytes contained in high flow (1 mL/min) effluent from a liquid chromatograph to be introduced into low pressure ion sources using conventional EI, CI, and FAB ionization. The first PB-LC/MS interface was referred to as the MAGIC-LC/MS¹.

Sensitivity with PB-LC/MS is dependent upon the efficiency of several processes; including, 1) efficiency of solute particle transport, 2) efficiency of solvent removal, 3) and efficiency of solute vaporization.

OBJECTIVES: The following studies are part of a continuing effort to evaluate the operating parameters that are critical to the performance of particle beam interfacing. It is the object of these studies to improve the sensitivity of particle beam sample introduction by optimizing these critical parameters. 1) Study the effects of nozzle/skimmer separation on quantitation, source pressure, and solute transport efficiency. 2) Study the effect of ion source contamination on sensitivity.

EXPERIMENTAL (One):

Condition A-	Mobile phase-none
	Flow rate- 0.00
	Flow rate nebulizer gas- 2 Liter/min
	Gas composition- Air
Condition B-	Mobile phase composition- 50/50 MeOH/H ₂ O
	Flow rate- 0.35 mL/min
	Flow rate nebulizer gas- 1 Liter/min
	Gas composition- Helium

RESULTS: The most sensitive performance has been observed to be at or near the minimum of the air curve. This is slightly outside the separation associated with the minimum pressure curve for condition A. No aerodynamic explanation for these observations is available at this time; however additional studies with flow versus separation may lead toward a physical explanation. In order to attain optimal performance, it may be necessary to change N/S separation depending upon the flow and solvent conditions.

The observed curvature of the analytical plots (Figures 2) are explained by lower momentum associated with the lower concentrations. This curvature was observed at much higher concentration with earlier designs. Possibly minimizing turbulence in the momentum separator will shift the drop-off to lower masses. Another solution to this mass transport problem has been the use of a less volatile matrix in the mobile phase, resulting in particle size being independent of solute concentration.

EXPERIMENTAL (Two): This experiment demonstrates the significant effect of source contamination on both response and background. A clean, well-tuned systems was subjected to repeated injections of phenylthiazine for 150 injections of 10 ng. The signal-to-noise was then measured as a function of increasing source contamination. The replacement of the ion region with a replaceable ion source repeller assembly was performed after 150 injections.

RESULTS: Experiment two measured the response of the mass spectrometer after repeated injections of phenylthiazine (10ng injected). After 150 injections the signal-to-noise was significantly reduced. Replacing the ion source repeller assembly with a clean volume reestablished the initial performance.

¹ Willoughby, R.C. and Browner, R.F. Anal. Chem. 56/14, 2626 (1984).

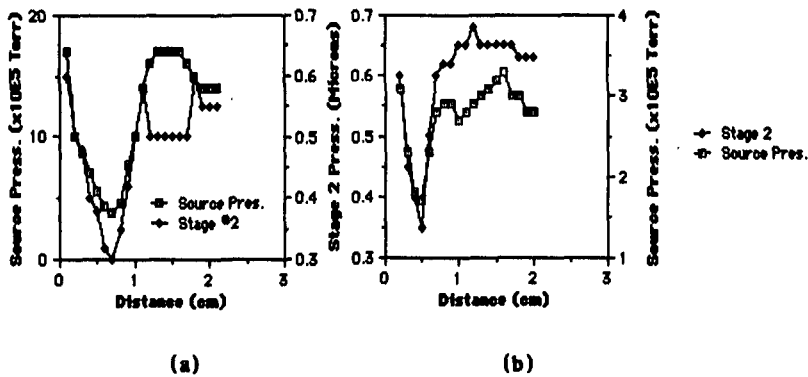


Figure 1. a) Effect of N-S on stage two and ion source pressure. Results for Condition B. b) Effect of N-S on stage two and ion source pressure. Results for Condition A.

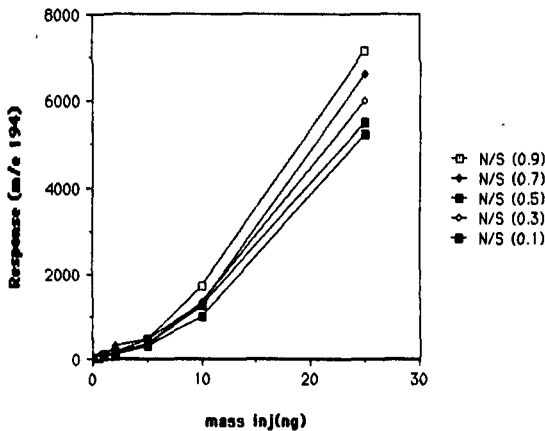


Figure 2. Linearity study of Caffeine response at various nozzle/skimmer separations.

PARTICLE BEAM LC/MS BY FAB

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Continuous flow FAB has demonstrated the ability to accomplish real time chromatographic separations coupled with sensitive fast atom bombardment ionization. However, problems such as the necessity for microbore LC systems and the associated low flow rates and clogging problems make continuous flow FAB a relatively difficult technique for routine analysis.

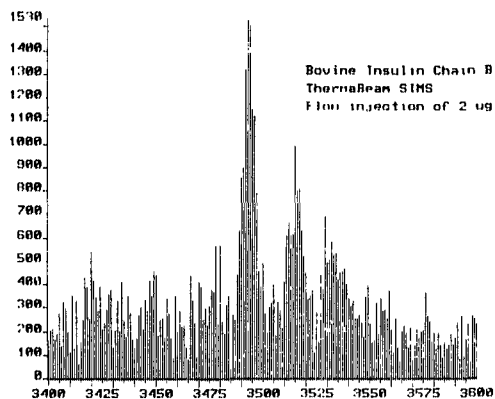
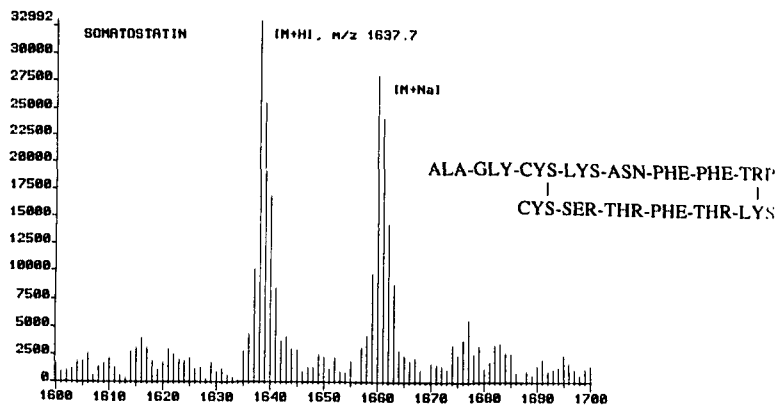
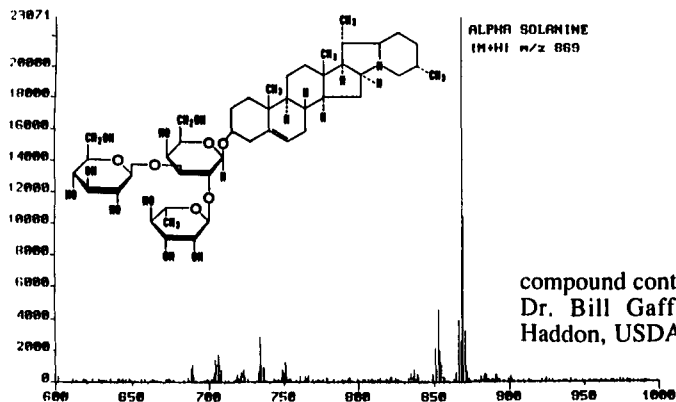
Particle beam introduction of the analyte through ThermoBeam™ (TAB) allows for the use of standard chromatographic systems at high flow rates (e.g. 0.5-2.0 mL/min.) reducing the need for microbore LC systems and their associated problems.

The matrix compound may be added to the system in a number of ways such as: post column addition, addition through the FAB target, or addition through flow injection. This flexibility of matrix addition makes it possible to perform a wide variety of experiments while maintaining a high flow rate of analyte into the mass spectrometer. Matrix addition through the TAB interface is possible utilizing concentrations in the range of 0.01-1.0%.

Higher matrix concentrations are possible through direct introduction through the target and the flow injection technique.

A cesium ion source (ANTEK, Palo Alto, CA) was used to generate the primary ion beam. The energy of the primary ions were 10 kV. Other sources of fast atoms or ions were tried: e.g. saddle field gun, capillaritron gun, but the cesium source produced the best results. The sensitivity of TAB/FAB has been demonstrated to be good, e.g. 5 ng of erythromycin (m.w. 733) yields a sodiated adduct of m/z 756 with a signal to noise ratio well over 10:1.

Flow injection experiments were performed with TAB/FAB on other compounds such as: benzo-[a]-pyrene glucuronide, somatostatin, alpha solanine, and bovine insulin chain B.



DETERMINATION OF ALKYL BENZENE SULFONATES BY CAPILLARY ZONE ELECTROPHORESIS - MASS SPECTROMETRY

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The objective of this research is to develop capillary electrophoresis - mass spectrometry (CE-MS) for determination of petroleum related compounds. In the present study, CE-MS was applied to the determination of alkylbenzene sulfonates, compounds commonly used as surfactants and detergents in the petroleum industry. The interface design under evaluation was first introduced by Ewing¹ and incorporates coupling of a CE capillary column to a continuous flow fast atom bombardment (fFAB) capillary.

Capillary electrophoresis separation of homologous alkylbenzene sulfonates was first developed off-line. Detection of eluting bands was accomplished by removing the polyimide coating from a section of column, thus forming a quartz window for UV detection. This arrangement enabled determination of CE separation efficiencies without band broadening due to extra column effects. Column diameters of 50 μ m gave the best resolution, while 75 μ m columns plugged less frequently and gave better detection. Figure 1 shows a typical separation achieved for selected alkylbenzene sulfonates.

CE-MS separation of alkylbenzene sulfonates is presented in Figure 2. Separation efficiencies are reduced compared to results shown in Figure 1 for the same compounds. Band broadening is probably due to extra column effects (dead space, diffusion and turbulence) at the coupling, laminar flow and diffusion in the fFAB capillary, differences in the anodic and cathodic buffers, and overloading. However, under-resolved peaks in conventional CE are easily deconvoluted by CE-MS, as demonstrated by the mass electropherograms for C-9 and C-10 alkylbenzene sulfonates.

Figure 3 presents mass spectra corresponding to the respective sulfonates of Figure 2. The organic anion of each sulfonate salt predominates, and is observed well above the background.

CE provides an alternative to ion-pairing HPLC for separation of ionic compounds such as alkylbenzene sulfonates. The combination of CE and MS appears most promising and constitutes a powerful technique in separation and characterization of petroleum related compounds. Future work will include refinement of the CE-MS interface and extension of the method for characterization of other compounds.

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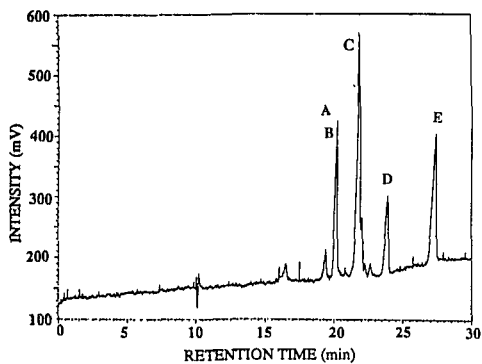


Figure 1. CZE-UV separation of alkylbenzene sulfonates: A, Decylbenzene sulfonate; B, Nonylbenzene sulfonate; C, Butylbenzene sulfonate; D, Ethylbenzene sulfonate; E, Benzene sulfonate.

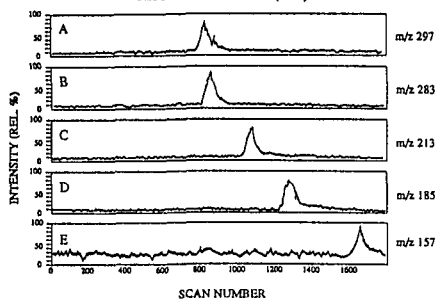


Figure 2. Mass electropherogram of the sulfonates as obtained by CZE-MS.

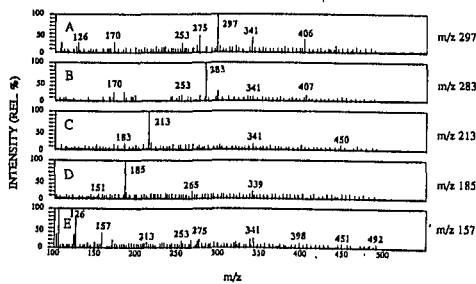


Figure 3. Mass spectra of the sulfonates as obtained from the separation of Figure 2.

**Capillary Zone Electrophoresis and Capillary Liquid Chromatography
Coupled with Sector Mass Spectrometry Using
Coaxial Continuous Flow FAB Interfaces**

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Capillary zone electrophoresis (CZE) and capillary liquid chromatography (CLC) have proven to be extremely useful tools for the analysis of nonvolatile and/or thermally labile compounds such as biomolecules. The high separation efficiencies that can be obtained with these microcolumn separation methods facilitate the separation of very complex mixtures, such as biological extracts. While LC remains the most widely used separation method with mass spectrometry, the distinctly superior separation efficiency of CZE over LC is attracting the attention of an increasingly large number of researchers. CZE has demonstrated separation efficiencies in excess of 1,000,000 theoretical plates of separation efficiency in less than 20 minutes (1) and in excess of 100,000 theoretical plates in less than 1 minute (2).

The development of "continuous flow fast atom bombardment" (CF-FAB) interfaces by Caprioli, Fan and Cottrell (3) has provided an MS interface that is particularly well suited for the analysis of biomolecules. Our research groups have therefore been pursuing the coupling of CLC (both open tubular liquid chromatography (OTLC) and packed microcapillary liquid chromatography) and CZE with sector mass spectrometry using CF-FAB. The very low flow rates of the CZE and CLC microcolumns (25 to 50 nL/min) places stringent requirements upon the design of the CF-FAB/MS interface. Therefore, an interface was designed (4) which uses a pair of coaxial fused silica capillary columns to independently deliver the microcolumn effluent and the FAB matrix solution directly to the face of the FAB probe. An evaluation of different FAB matrix solutions has revealed (5) that a solution of glycerol in water (25 % v/v) at a flow rate of approximately 1 μ L/min is optimum for most experiments. This coaxial CF-FAB interface offers the following advantages: independent optimization of the composition and flow rates of the two liquid streams; preclusion of any deleterious effects of the polar, viscous FAB matrix upon the separation process; and minimal band broadening.

The coaxial CF-FAB system has been used to interface both CZE and CLC with a VG ZAB 4F four sector mass spectrometer. The combined systems have been used to analyze several classes of biomolecules, including peptides. They have been successfully used to acquire on-the-fly FAB/MS spectra (Figure 1), and, with CLC, MS detection limits of 500 attomoles have been obtained. High resolution chromatographic separations have been obtained using packed microcapillary columns (50 μ m id packed with 10 μ m C8 and C18 particles) (Figure 2). The capabilities of the ZAB 4F have been exploited to obtain on-the-fly collisionally activated dissociation (CAD) MS/MS spectra of picomole to femtomole levels of biomolecules (Figure 3), which yield abundant structural information. With CZE this interface has proven (6) to be capable of acquiring MS and MS/MS spectra from low femtomole amounts of peptides while maintaining high (hundreds of thousands of plates) electrophoretic separation efficiencies (Figures 4-6).

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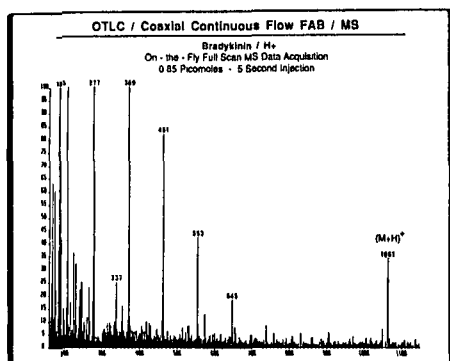


Figure 1

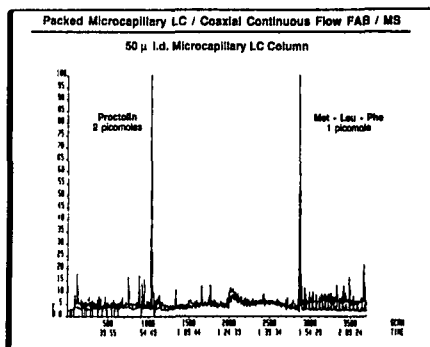


Figure 2

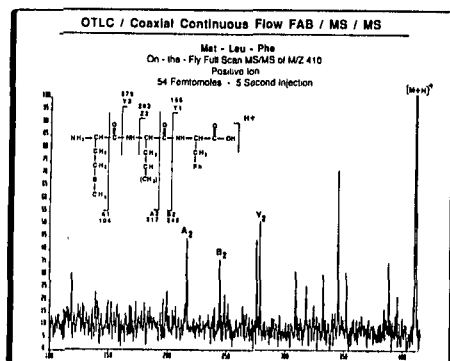


Figure 3

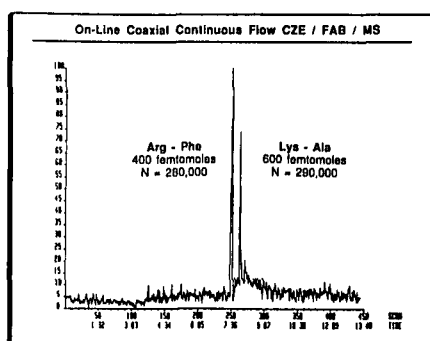


Figure 4

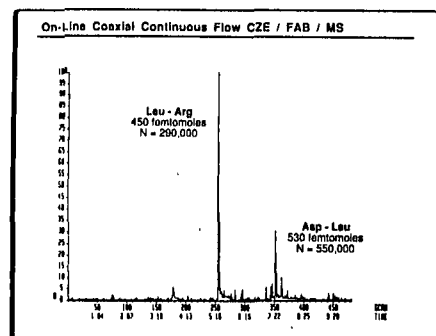


Figure 5

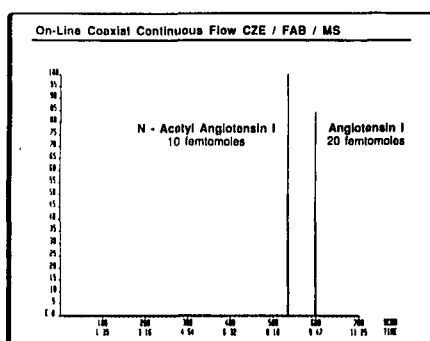


Figure 6

CAPILLARY ZONE ELECTROPHORESIS-MASS SPECTROMETRY OF PEPTIDES AND PROTEINS

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Capillary zone electrophoresis (CZE) is attracting extensive attention as a fast, high resolution analytical and micro-preparative separations technique for systems of biological interest. In zone electrophoresis, a column is filled with a single electrolyte having a specific conductivity. The mixture of substances to be separated is applied as a narrow band to the head of a buffer filled column in a band whose width is much less than the length of the column and at a concentration too low to affect the buffer conductivity. An electric field is then applied across the length of the column and the individual substances migrate and separate according to their net electrophoretic velocities. Zone electrophoresis carried out in small diameter (< 100 μ m) fused silica capillaries is a relatively new approach to the high resolution separation of aqueous samples. Very small volume samples (picoliter range) with separation efficiencies on the order of 10^6 theoretical plates for amino acids have been achieved (1). The method can be further enhanced by the dynamic combination of detection sensitivity and selectivity offered by mass spectrometry (MS). The on-line marriage of mass spectrometry to CZE is accomplished by an atmospheric pressure electrospray ionization source interface. Electrospray ionization (ESI) is rapidly growing as an ionization method of choice for nonvolatile biomolecules since the pioneering experiments of Malcolm Dole (2) and later by John Fenn (3). Fenn's group first demonstrated that ESI is a "soft" ionization technique, yielding little or no fragmentation, capable of producing intact multiply charged molecular ions for proteins with molecular weights (MW) exceeding 30 kDa from highly charged liquid droplets at atmospheric pressure through a field-assisted ion desorption process (4). Our research efforts have demonstrated that proteins with MW's greater than 100 kDa can be analyzed using a conventional quadrupole mass spectrometer with an upper m/z limit of only 1700.

Previous CZE-MS experiments have demonstrated the feasibility to analyze amino acids and small peptides. However, the CZE separation of larger protein systems in untreated fused silica capillaries has often resulted in broad, tailing peaks due to Coulombic attraction and adsorption of analyte on active sites to the negatively charged silica wall. Separations performed in buffered systems with the pH above the isoelectric point (pI) of the proteins allows both the proteins and the capillary wall to be negatively charged and mutually repulsive to greatly improve the resolution (5), as demonstrated in Figure 1. Subpicomole CZE-MS separations of myoglobins and smaller peptides can be easily performed in this manner, with over 125,000 theoretical plates obtained. CZE protein separations can also be achieved through the use of very low pH aqueous phosphate buffers, lower than the pI of the analytes (6). Preliminary CZE-MS results have been obtained for a mixture of horse and whale myoglobins and a variety of cytochrome c proteins at pH 2.4.

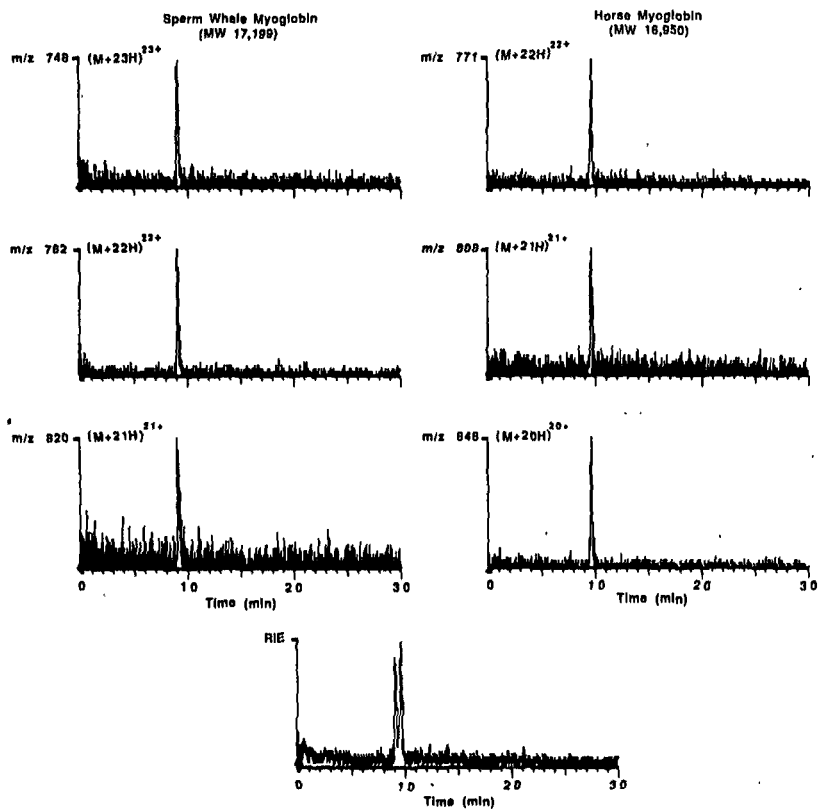
We thank the U.S. Department of Energy, Office of Health and Environment Research, for support of this research under Contract DE-AC06-76RLO 1830. *Pacific Northwest Laboratory is operated by Battelle Memorial Institute.

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100 cm x 50 μ m capillary
20 mM TRIS/HCl, pH 8.3
30 kV separation potential
2 pmol Injection



ANALYSIS OF TRYPTIC DIGESTS BY ON-LINE CAPILLARY ZONE ELECTROPHORESIS
TANDEM MASS SPECTROMETRY, Eric, Huang, and Jack D. Henion, Drug Testing and
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Recent interest in novel separation techniques suggest new alternatives to HPLC tryptic "mapping". Capillary zone electrophoresis (CZE), for example, promises separation efficiencies in excess of several hundred thousand plates in fairly short analysis times. If this technique is interfaced on-line with a tandem mass spectrometer (CZE/MS/MS) analysis of tryptic digests could provide very high separation power plus molecular weight and possibly sequencing information all within an analysis time of approximately 20 minutes. We have recently applied our CZE/MS/MS system(1) using atmospheric pressure ionization and the ion spray interface(2) to the characterization of tryptic digests of known proteins to evaluate its analytical potential.

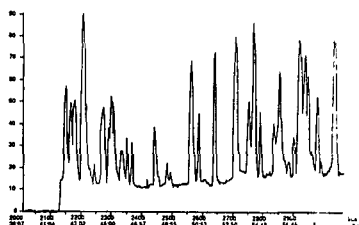
The ion spray LC/MS interface(2) was connected directly to the CZE system. The effluent from the CZE column passed through the ion spray LC/MS interface held at 3kV which produced charged droplets directed in the region of the ion sampling orifice of a TAGA 6000E tandem triple quadrupole mass spectrometer. The inlet end of the CZE system was held at 30kV while a buffer of either 10mM or 20mM ammonium acetate diluted with acetonitrile(50:50, v/v) and pH-adjusted with ammonium hydroxide or acetic acid. The separation column was a 100um x 100cm untreated fused-silica capillary while hydrostatic injections were utilized. A variety of proteins were digested with trypsin and prepared for analysis in the conventional manner.

Figure 1 is a total selected ion current profile for a SIM CZE/MS analysis of a tryptic digest of myoglobin. Singly- and doubly charged-ions characteristic of the peptides present were monitored with a buffer pH of 6.9 to obtain this ion current electropherogram. Our experience is that high separation efficiencies are obtainable by CZE/MS of such mixtures, but not without considerable difficulties. One must be careful to avoid overloading the capillary column with too much sample and pH control is critically important. There were many cases where most of the sample components eluted in one narrow region of the electropherogram at a given pH. A small change in the pH would then dramatically effect the separation such that some of the components did not elute or eluted too quickly. Thus, careful attention to the CZE technique and the experimental conditions must be observed.

We have analyzed a variety of different tryptic digests including hemoglobin and myoglobin and find that CZE/MS is a potentially exciting analytical technique especially once the vagaries of the capillary electrophoresis process are better understood. In our experience the ion spray interface for coupling the CZE system to the atmospheric pressure ionization system is analytically rugged and routine to implement.

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TOTAL SIM OF TRYPTIC DIGEST MYOGLOBIN
(pH 6.9)



LC/MS DETERMINATION OF LEUCINE-AND METHIONINE ENKEPHALIN IN EQUINE CEREBROSPINAL FLUID(CSF), Jack Henion and Wolfgang Muck, Drug Testing and Toxicology, Cornell University, 925 Warren Dr., Ithaca, NY 14850.

Understanding the various functions of the endogenous "opioid system" (opioid receptors and endorphins/enkephalins as natural ligands) can only be accomplished by highly sophisticated analytical techniques capable of detecting trace quantities of target peptides in biological fluids and tissues. Different approaches have been described which emphasize the key role of mass spectrometry as a vital part for unambiguous identification and confirmation. The often applied analysis of collected HPLC fractions by FD-MS or FAB-MS, however, is tedious and labor intensive.

As preliminary work for investigations related to pain relief phenomena in the horse and the possible abuse of enkephalins at race tracks, a method for the determination of leucine- and enkephalin (LE & ME) in equine cerebrospinal fluid (CSF) has been developed. The analysis scheme is similar to that outlined by Desiderio et al.(1). (D-Ala²)-leucine enkephalin is used as the internal standard (IS). Sample enrichment is based on liquid-solid extraction on C18-material after protein precipitation with acetonitrile. Reversed-phase micro LC (1mm x 100mm, C18 with 3 μ m particles, acetonitrile/trifluoroacetic acid) with mass spectrometric detection via an ion spray LC/MS interface and a commercially available atmospheric pressure ionization (API) triple quadrupole mass spectrometer provided the required selectivity and sensitivity for monitoring pg-levels of the two pentapeptides. The tandem mass spectrometer was run under selected reaction monitoring (SRM) conditions using m/z 120,136,278, and 556(LE) resp. 574(ME) and 120, 136, 292, and 570 for the IS.

Capillary zone electrophoresis(CZE) has received considerable interest due to its potential as a suitable analytical technique for the separation of complex mixtures containing biopolymers. The availability of our recently developed CZE/MS interface(2) allowed us to quickly change to another separation technique (100 μ m x 100cm fused-silica, acetonitrile/20-30 mM ammonium acetate (50/50) using hydrostatic injection) under the same on-line MS conditions and provided comparative studies to get clearer evidence of the capability and feasibility of CZE/MS confronted by this real-world bioanalytical problem.

The results showed that the limited sample volume for CZE(5-15nL) is a practical shortcoming in that the injected sample concentration must be at least in the low ppm range in order to introduce enough sample for detection by the APIMS. In contrast, the micro LC/MS system could accommodate several microliters of sample and can detect low ppb levels in the injected sample. Thus the CZE/MS system is not as well-suited for trace analysis and should be used where sample limitation is not a problem. However, aside from this restriction both approaches are a viable means of analyzing biological samples of the type described herein.

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THE ON-LINE COMBINATION OF CAPILLARY ISOTACHOPHORESIS WITH MASS SPECTROMETRY

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Capillary zone electrophoresis (CZE) has been demonstrated to have tremendous potential for high-resolution separations of ionic compounds of biological interest. The small capillary diameter ($<100\text{ }\mu\text{m}$) used in CZE and the need to keep the ionic strength of the sample two orders of magnitude less than that of the buffer place rather stringent limits on the amount of sample that can be injected. Isotachopheresis in a capillary format (CITP) is a complementary scheme that overcomes these limitations (1). The CITP/MS combination has several attractive features. First, there are no fundamental limits on the sample size that can be introduced. Second, CITP can result in the concentration of samples that are injected in dilute solutions. Third, once the separation is developed the sample elutes continuously allowing efficient use of detector time. Fourth, the ideal peak shape in CITP is sharp sided and flat topped. This is an ideal peak shape for mass spectrometric detection. The scan speed of most mass spectrometers is not challenged and MS/MS analysis is facilitated (2,3).

Electrospray ionization (ESI) has been shown to be an effective means of ionizing labile biopolymers from solution. In this work we present the results of an examination of two systems using CITP coupled to mass spectrometry by an ESI interface of our design (2).

Two mass spectrometers were used in these studies. The first was a single stage quadrupole. It was assembled from Extrel components and the vacuum system was designed for an atmospheric pressure inlet. An rf only set of rods precedes the analysis stage to aid in efficient transport of ions. The second was a Sciex 6000E triple quadrupole. The instrument was used with our ESI interface. The electrophoresis was done in a $100\mu\text{m}$ i.d. fused silica capillary column. DB-17 and OV-1 coated columns were used. The effect of treating the inside of the column is to eliminate electroosmosis and reduce peptide or protein binding to the surface. To minimize the total time, once the steady state has been reached the sample was flow migrated from the column by the application of a small pressure head.

An equimolar mixture of the small proteins bradykinin (Mr 1060) and Angiotensin (Mr 1296) was prepared from standards obtained from Sigma. The leading buffer was 0.01M ammonium acetate titrated to pH 4.9 with acetic acid. The sample was prepared at 10^{-4}M each in the leading buffer. The trailing buffer was 0.01M Ala^+ titrated with $\text{Ba}(\text{OH})_2$ to pH 7.1. The column was 2.5 M long with a DB-17 stationary phase. The column was initially filled with the leading electrolyte and then a sample band 64 cm long was loaded by pressurizing the sample reservoir. The column was transferred to the trailing buffer reservoir and the separation was developed at 58 kV off line for 15 minutes. The electrospray was then started. The sheath was methanol and the sample was eluted with voltage applied under a modest pressure head.

The separation reached steady state with two components separated into their own bands. The presence of a valley between the two components indicated the presence of impurities of intermediate mobility. The single ion chromatograms demonstrated the multiple charging which is so characteristic of ESI. Spectral scans taken over the mass range of interest during the elution of each of the major components showed clean spectra. A spectral scan taken in the valley between the bands for two components indicated small amounts of poorly characterized impurities of intermediate mobility.

An enzymatic digest of glucagon was prepared as an example of a less well characterized mixture. Since the enzyme was not of high purity the cleavages are not well characterized. It consisted primarily of trypsin and chymotrypsin. Trypsin is highly specific cleaving only Lys- and Arg-bonds. Chymotrypsin is less specific and is in fact a family of enzymes. Thus although a weak digest of glucagon by trypsin should lead to only four products, the presence of other enzymes considerably complicated the possible product mixture.

The leading buffer was 0.01M ammonium acetate titrated to pH 4.6 with acetic acid. The sample was made up in the leading buffer to a concentration of 10^{-4} M. The trailing electrolyte was 0.01M acetic acid. The column was 0.75 M long with a OV-1 stationary phase. The column was initially filled with the leading electrolyte and then a sample band was loaded hydrostatically. The column was transferred to the terminating electrolyte and the development was started. The ESI was used continuously from the very beginning with a methanol sheath. The voltage was manually ramped to 10 kV over a period of 9 minutes. After 30 minutes the electrolyte reservoir was raised 5 cm to elute the separation from the column. In a separate experiment an on column UV detector operated at 210 and 280 nm was used to monitor the separation under identical conditions.

Single ion plots for the most prominent peaks seen in the spectra show the bands in the beginning and end of the sample are more fully developed while those in the center still exhibit mixed band characteristics. Most, but not all of the ions could be correlated with expected digest fragments. As would be expected the single amino acid residue Arg is present and has the highest mobility amongst the sample components. The next band to elute is also well characterized and correlates with the T1 (2+) fragment. Two mixed bands correlating with the T2⁺ and T4-478 (2+) fragments appear next. The next two bands are again well developed but do not correlate with any expected major digest fragment. The last band to elute is again well characterized and correlates with the T4-1049 (2+) fragment.

The relatively long period of time over which a peak elutes in CITP makes it an attractive candidate for MS/MS analysis. Such an analysis was done on the leading and trailing bands of the sample. They gave structurally informative results that can be used to confirm the tentative identifications.

The combination of CITP with mass spectrometric detection is a promising, but still developing technique. The interface to mass spectrometry does not detract from the separation power of this technique. There is considerable variation in the sensitivity of ESI for the various fragments in a typical digest. Although most of the expected fragments were detected, some of them were not. For those fragments that are detected the sensitivity is generally good. The CITP format is ideal for MS/MS operation. We are confident that the CITP/MS combination will continue to develop in importance.

We thank the U. S. Department of Energy, Office of Health and Environmental Research, for support of this research under Contract DE-AC06-76RLO 1830. *Pacific Northwest Laboratory is operated by Battelle Memorial Institute.

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CHROMATOGRAPHY AND MASS SPECTROMETRY OF DOMOIC ACID, A MARINE NEUROTOXIN^{*}

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We have reported previously on the identification of domoic acid as the neurotoxin responsible for the outbreak of amnesic shellfish poisoning following the ingestion of cultivated blue mussels harvested from a localized area of Prince Edward Island.¹ Subsequent work has led to the development of routine methodology² for the quantification of this toxin in a variety of marine matrices including seawater, plankton and shellfish tissues by HPLC with either UV diode array detection or fluorescence detection, the latter using a fluorenylmethoxycarbonyl (Fmoc) derivative. The present work describes an evaluation of mass spectrometry-based methods for the confirmation and determination of domoic acid in complex matrices.

Gas chromatography-mass spectrometry

Domoic acid is a polar, non-volatile and thermally labile molecule and therefore requires chemical derivatization to enable its analysis by GC or GC-MS. We have developed a rapid extraction and clean-up procedure for complex marine matrices to facilitate the preparation of an N-trifluoroacetyl-O-tert-butyltrimethylsilyl derivative of the toxin. The mixed reaction approach overcomes the problem of incomplete silylation of the amine functionality observed in earlier work using only the silyl reagent.³ The derivatization is fast and proceeds under the necessary mild conditions required by this labile molecule.

The resulting DA-TFA-TBDMS derivative (Figure 1) is stable and is suitable for GC-MS. The EI mass spectrum shown in Figure 1 is notably clean, with a high proportion of the ion current being carried by the ion $[M-tBu]^+$ at m/z 692. This abundant ion at high mass is well separated from any interfering reagent species and is ideal for single ion recording (SIR). Reaction mixtures were injected directly into a GC-MS system and a typical separation of a contaminated mussel extract is shown in Figure 2. In the SIR mode we were able to detect low nanogram levels of domoic acid.

Liquid chromatography-mass spectrometry

We have adopted two approaches to the coupling of our routine LC methodology (Figure 3) for domoic acid to the mass spectrometer:

Continuous flow fast atom bombardment: initial results using flow injection seemed promising with low picogram sensitivity available in the full scan mode with as little as 1 nanogram required to obtain a recognizable spectrum for identification. Although we were able to obtain a chromatographic separation of the toxin using a packed fused silica capillary column coupled directly to the flow FAB probe (flow rate 3 μ L/min), we were not able to resolve any of the isomers known to be present nor detect any other components of this complex mixture. Problems with maintaining a stable ion current during a gradient were compounded by the loss of column performance due to the glycerol matrix in the mobile phase.

Ion spray mass spectrometry: we have recently reported encouraging results in the use of this technique for the analysis of several marine neurotoxins including domoic acid.⁴ In continuing this work we have been able to achieve excellent results for the gradient LC-MS analysis of mussel and plankton extracts for this toxin. A selected mass chromatogram of the protonated molecular ion m/z 312 is shown in Figure 4. Chromatographic integrity is well-maintained, and we were able to confirm the presence of numerous isomers and derivatives of domoic acid. The clean mass spectra produced (Figure 5) gave invaluable molecular weight information, while the MS/MS capability of the triple quadrupole MS provided useful structural information.

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• NRCC No. 30279

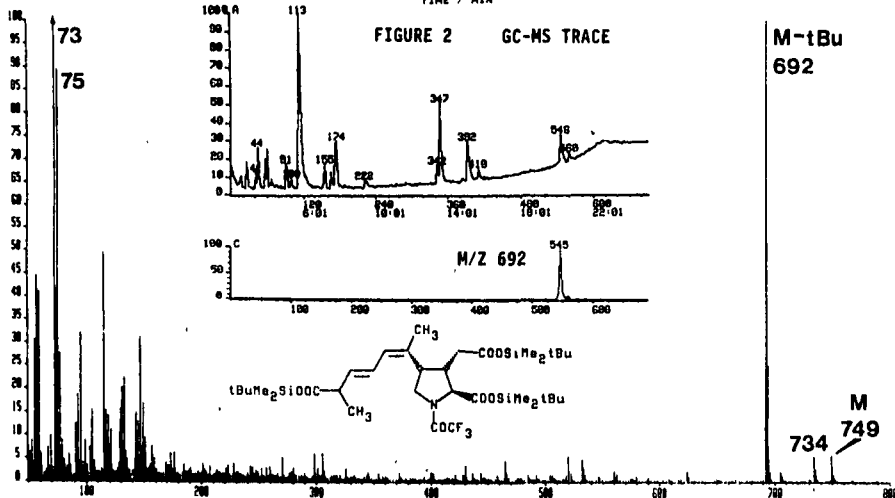
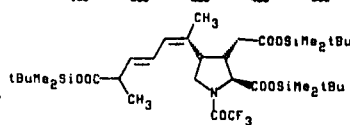
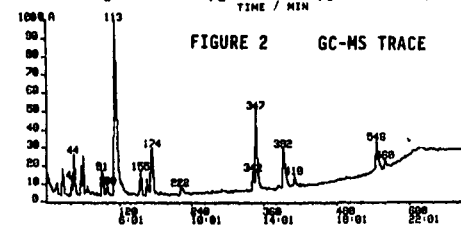
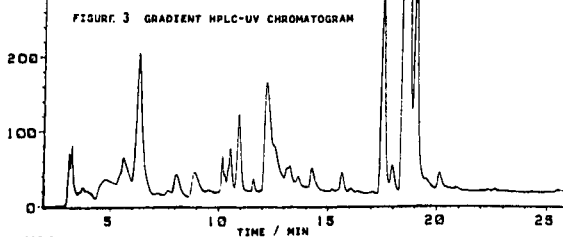
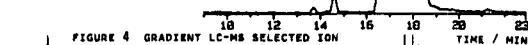
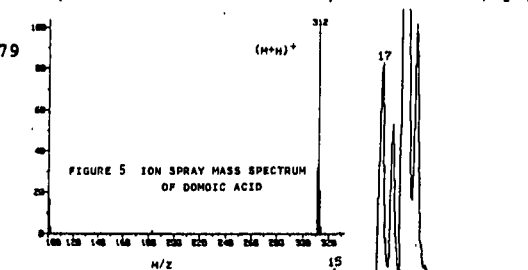


FIGURE 1

EI MASS SPECTRUM OF DA-TFA-TBDMS DERIVATIVE (2)

Applications of Packed Column Supercritical Fluid Mass Spectrometry
Using A Thermospray Ion Source

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Introduction

Supercritical fluids are recognised as good mobile phases due to their low viscosity, high diffusivity and increased analyte solubility relative to liquids. The coupling of supercritical fluid chromatography (SFC) to a mass spectrometer therefore combines these chromatographic properties with a very specific, selective and sensitive detector.

The purpose of this study was threefold. Firstly to determine if it was possible to connect a supercritical fluid chromatographic system to a mass spectrometer via a Vestec thermospray interface. Secondly to obtain qualitative data to help in the identification of metabolites of agrochemicals and thirdly to endeavour to evaluate the SFCMS system for quantitative analysis.

The compound chosen to evaluate the system was Hexaconazole (1) a triazole fungicide. Putative metabolites of hexaconazole were also analysed (2-4).

Experimental

All experiments were performed on a Finnigan MAT 4600 mass spectrometer using a Vestec thermospray interface. No modifications were made to the interface. The SFC system was home made and comprised of two Gilson HPLC pumps, with a cooling jacket around one of the pump heads. A rheodyne 7037 pressure relief valve was used as a backpressure regulator. The discharge mode of ionisation was used for all experiments.

Results and Discussions

The total ion current chromatogram obtained from the SFCMS analysis of Hexaconazole and three putative metabolites is shown in figure 1. A good separation was achieved resulting in the partial separation of the diastereomers of diol (3) (peaks 3 and 4). The mass spectra obtained from Hexaconazole and diol 3, which is representative of the other diols are shown in figure 2. The spectra are very simple and only protonated molecular ions are observed.

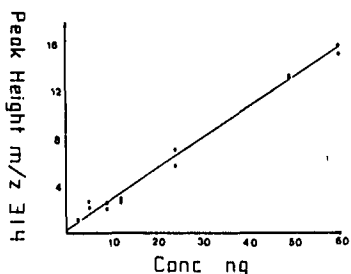
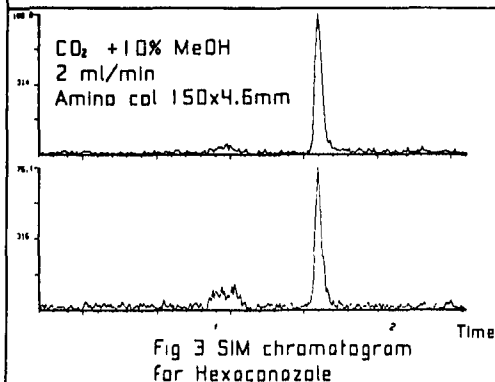
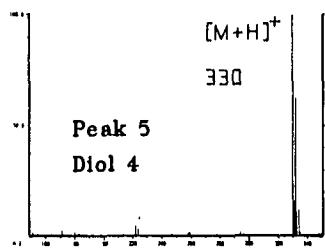
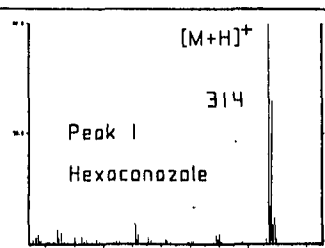
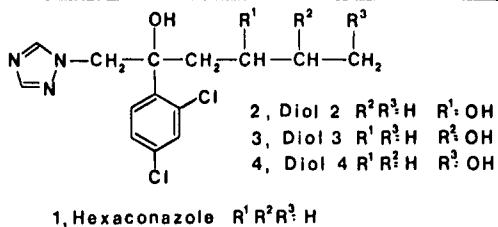
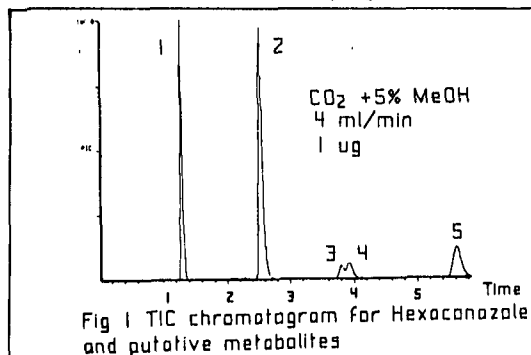
Selected ion monitoring (SIM) data were obtained for hexaconazole (1) using ions m/z 314 and m/z 316. A limit of detection of 2.4 ng injected on column was obtained (Figure 3). The reproducibility of the system was determined by making six replicate injections of hexaconazole (24 ng). A coefficient of variation (cv) of 9% was obtained. The use of an internal standard would help to improve this figure.

A calibration line relating the peak height of m/z 314 with the concentration of hexaconazole was linear in the range 2.4 ng to 60 ng injected on column (figure 4). The regression characteristics were slope, 25 intercept, 497 with a correlation coefficient of r , 0.993.

Conclusion

1. A Vestec thermospray source can be used for coupling a SFC system to a mass spectrometer.
2. Good qualitative data can be obtained with simple CI type spectra producing protonated molecular ions.
3. The system can be used for quantitative analysis with or without an internally standardised method. The use of an internal standard will improve the accuracy and precision of the method.
4. The system is easy to set up and use and this is shown by the fact that the above work was carried out in one week.

Acknowledgement:- The authors would like to thank Mr Graham Kilpatrick from Inter-ion Limited for the kind loan of the Vestec thermospray interface.



**HIGH TEMPERATURE VAPORIZATION EFFECTS
IN PARTICLE-BEAM MASS SPECTROMETRY**

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Particle-beam mass spectrometry (developed from MAGIC-LC/MS) has proven very effective in its ability to generate classical spectra from any compound amenable to the direct insertion probe analysis. This makes the technique valuable for a wide range of compounds ranging from high to intermediate volatility. Recent studies have also demonstrated that compounds of low volatility may also be readily vaporized using fast atom bombardment (FAB). However, while fast atom bombardment has great utility for many compounds, it is functionally more complex than standard EI particle-beam mass spectrometry. Additionally, FAB generated mass spectra are not readily searched using standard library procedures.

As stated above, the limiting factor for many methods is the inability to achieve vaporization of the sample, in such a state that is representative of the original structure. The goal of this study is to better understand the interaction of the particle beam and the heated ion source. All previous efforts have been directed toward the interactions role in detection limits and sample transport, but not on its affect on the vaporization of the sample and the sample integrity.

Once we have transported the sample to the ion source it becomes necessary to vaporize it. The problem being that some compounds do not go easily into the vapor state. This volatility problem exists because of the instability of many molecules at the temperatures required for their evaporation. The energy added to break surface bonds is distributed instead toward the decomposition reaction.

For us to limit the decomposition reaction, we must affect a change in the relative rates of the two competing processes, decomposition and volatilization. Basically, it is considered that heating an unstable, nonvolatile compound can be viewed as causing a kinetic competition between decomposition and volatilization, where decomposition is favored since the activation energy for the breakage of intramolecular bonds in these compounds is less than that required for the breakage of intermolecular bonds.

One way for us to affect a change in the relative rates and enhance the yield of intact molecules over fragments produced by surface decomposition processes, is to increase the rate at which energy is deposited into the sample. If the rate of evaporation of a neutral fragment is determined by an activation energy for decomposition, which is lower than the parent molecule evaporation activation energy, then the arrhenius plots of relative ion abundance vs. $1/T$ must intersect at some value of T . The fragment specie produced with its lower activation energy may be strongly favored at the lower temperatures but if the parent molecule can survive temperatures above the intersection of the respective plots, then it will be more abundant in the gas phase at the higher temperature.

One major detrimental factor in the process is multi-layer adsorption. the affect of multi-layer adsorption is to setup a temperature gradient from the molecules on the surface of the heating source out to the molecules at the surface. This causes an "overheating" past the temperature needed for efficient evaporation of the underlying molecules to achieve evaporation of the surface molecules.

In our study we looked at both the temperature and concentration effects on the loss of water from cholesterol. Often cholesterol is used as a measure of the thermal efficiency of a system. When cholesterol is subjected to thermal energy, the solid sample undergoes the degradative loss of water comparative to the amount of thermal energy it incurs. Therefore the ratio of the M^+ to the $M^+ - 18$ ions is a useful measure of the thermal efficiency of a system.

The results of our experiments are shown in figure one. The major overall trend is less thermal degradation with lower concentrations. This is apparently true due to the lessen affect of multilayer adsorption. For the two greater injection amounts of 1.0 and 0.5 ug, increasing the temperature only increases the amount of thermal degradation opposite the projected trend. We suggest that given the temperature gradient with the multi-layer adsorption, increasing the temperature only increases the

amount of "overheating" and therefore the amount of thermal degradation. As we lower the concentration of the injections, we do not see the apparent trend for the ratio to decrease with temperature. Hopefully, this suggests that we are getting closer to a mono-layer effect and show increase volatility.

We were even able to see the effect of concentration on individual chromatographic peaks. As the first particles reach the ion source, the ion ratios are good, but as amount increases reaching the ion source the ratios decrease, as seen in figure two.

In conclusion, we have seen that particle beam has the ability to enhance spectra if enough energy is imparted to the analyte and the analyte is present in low enough concentration to approach a mono-layer effect. Another benefit not discussed in this poster is that of the increased rate of desorption. If we can lower the activation energy, we also increase the resolution of the system.

TEMP. AND CONC. EFFECTS ON 386/368 ION RATIO

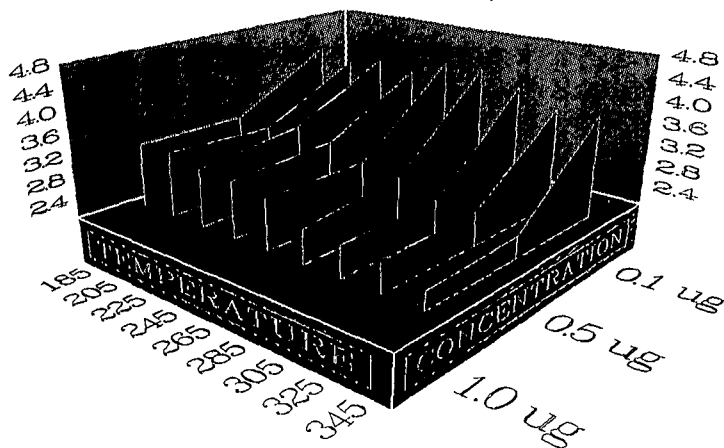


FIGURE 1

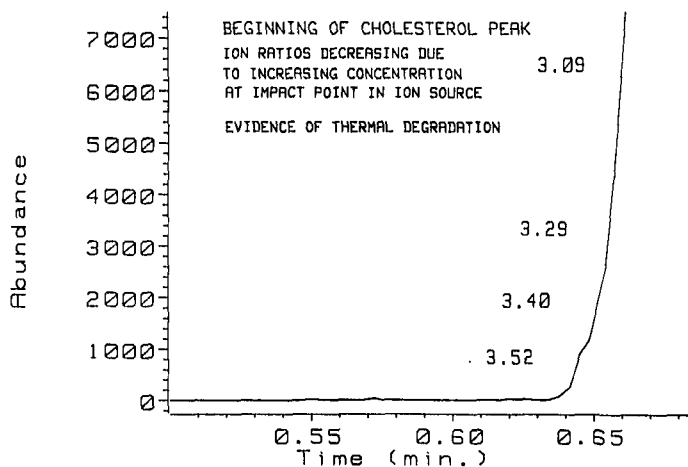


FIGURE 2

IN VITRO ANALYSIS OF ANTIMETABOLITES
IN BLOOD PLASMA BY MAGIC LC/MS

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Atlanta, GA, 30332-0400}

The major desire in chemotherapy is to achieve a constant drug level in the patient over a long period of time. This level must be controlled above the toxicity level of the cancerous cell and below the toxicity level of the host. Methods of antimetabolite (a major class of chemotherapy drugs) introduction include giving one bolus, whose concentration would degrade with time. Using this method, high concentrations occurring shortly after introduction could be determined and lower concentrations extrapolated. This gave a somewhat reasonable method for determining the concentration of the anticancer agent, but the introduction method gives varying amounts of the agent in the system instead of constant known concentration.

The current method of choice is to administer the agent by continuous infusion. In this manner, dilute solutions are continuously pumped into the patient and a constant cancer agent level is attained. Determining the actual level of this agent is compounded by the differing enzyme activity from patient to patient and day to day which breaks down the agents to inactive byproducts. There is a need to be able to attain low level detection of these antimetabolites in the blood plasma without time consuming extractions.

The goal of this study is to demonstrate the ability of MAGIC LC/MS to provide quantitative detection of these antimetabolites at low levels of concentration. Currently we now use a production model particle beam interface produced by the Hewlett Packard Co. which was developed from the MAGIC LC/MS interface developed at the Georgia Institute of Technology.

Experimental Setup and Procedure:

Instrumentation:

Hewlett Packard 5988 Quadrapole Mass Spectrometer
Hewlett Packard 59880A Particle Beam Interface
Hewlett Packard 1090 Liquid Chromatograph
Hewlett Packard Hypersil ODS 3um column, 60 x 4.6mm

Chemicals obtained from Sigma Chemical Co.

Uracil #U-750
Thymine #T-0376
5-Fluorouracil #F-6627

The elution was isocratic using Methanol at a flow rate of .6 mL/min. The peak eluted just after the void volume of the column but the chromatography is necessary to remove many mass interferences.

Extraction Procedure:

1. Obtain 1 mL of blood plasma.
2. Add 3 mL of phosphate buffer (pH + 6.85).
3. Extract with diethyl ether/isopropanol, (80/20) three times.
4. Pool organic phases and mix well.
5. Concentrate to dryness under nitrogen.
6. Dissolve residue in 100 uL methanol and homogenize (sonicate).

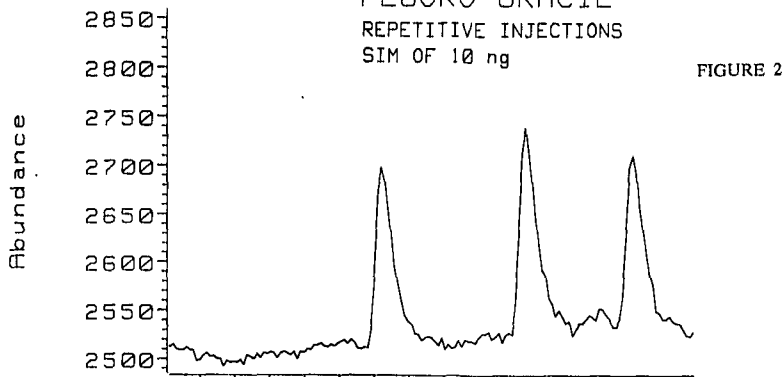
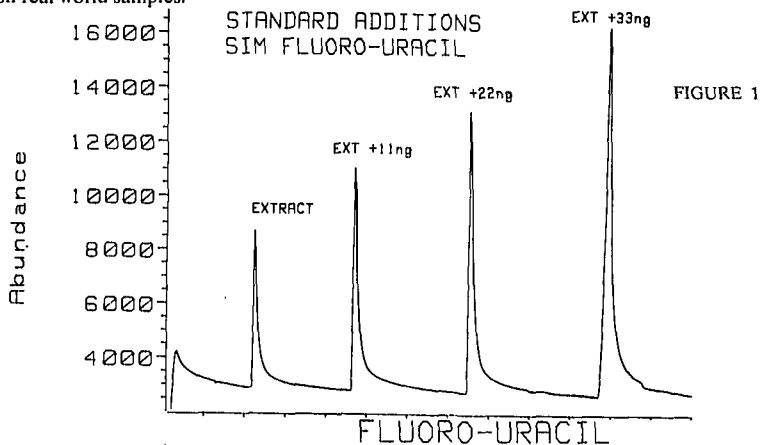
Note-Extreme care must be taken during the extraction process so that no aqueous phase is taken with the organic phase. This would introduce mass interferences damaging the ability to achieve quantitation. The extraction normally gives better than 80% recovery.

Apparently, with the extraction and the chromatographic cleanup, the pyrimidines of interest elute from the column in one narrow band. Coupling this with their excellent adeptness for either chemical ionization using isobutane or electron impact ionization, quantitation is achievable as seen in figure one.

Fluorouracil is the antimetabolite which gives all other methods trouble. The quantities present in patients receiving the chemotherapy drug by continuous infusion range from 10 to 40 ng per mL of blood plasma. Using isobutane as a CI gas, we could detect (3 to 1 ratio) as low as 3 ng in one mL of blood plasma. Part of the sensitivity study is shown in figure two.

Thymine and Uracil are present at levels from 100 to 800 ng/mL of blood plasma and ionize easily giving improved signal over the Fluorouracil and quantitative results.

In conclusion, we have seen that Particle Beam LC/MS has the ability to provide quantitative detection of these antimetabolites at the levels present in chemotherapy patients. The extraction is simple and can easily be run with limited discomfort to the patient due to the small amount of plasma needed (as compared to 5 mL needed for other analysis). As always, the process using the mass spectrometer is fast and accurate allowing the physician to quickly react to any major fluctuations in the antimetabolites concentration, thus obtaining a constant level below the toxicity level of the patient and above that of the cancerous cell. Presently, we are collaborating with the University of Alabama, Birmingham Pharmacology Department to run a series of plasma samples to validate our findings on real world samples.



AEROSOL TRANSPORT STUDIES IN PARTICLE BEAM MASS SPECTROMETRY

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Improvement of detection limits is a constant goal of all types of LC/MS interfacing. In the case of particle beam mass spectrometry (based on MAGIC-LC/MS), the aim is to reduce detection limits from typical values in the low nanogram range to values in the middle to upper picogram range. One way to partially accomplish this is to increase the efficiency of vaporization of the sample in the ion source area of the mass spectrometer. This paper will address the problem of aerosol transport in the interface by describing a series of carefully controlled experiments in which test compounds are injected through the interface and collected in a specially designed chamber, configured to simulate closely the physical environment in the ion source region of the mass spectrometer.

Experiments will be described in which a variety of commonly-used mobile phase solvents (e.g., H₂O, MeOH, CH₃CN) are examined, and operating parameters such as the liquid flow rate are varied. The mass of collected sample is determined by HPLC with UV detection, and by use of suitable test compounds, it will be possible to make absolute comparisons between sample transport parameters for the solvents tested.

The transport efficiency improves as the polarity of the solvent decreases: water, being highly polar, has poor transport efficiency (approximately 2%), whereas hexane, the least polar solvent tested, also yielded the best transport efficiency. Decreasing viscosity of the solvents did not necessarily predict improved transport efficiency, however. Common reversed-phase organic solvents, such as methanol, acetonitrile, and THF had comparable transport efficiencies, usually around 10 to 15%. Without exception, the transport efficiency was better for fast mobile-phase flow rates (0.7 to 1.0 mL/min) than for slower flow rates.

Two similar compounds, benzophenone and 2,2',4,4'-tetrahydroxybenzophenone (THB), were used as the test compounds for these studies. When both analytes were used to test a solvent, the benzophenone transport efficiency is 2-4% less than that of THB. It is suspected that this decrease could be due to the sublimation of benzophenone in the collection chamber. If this proves to be the case, another substituted benzophenone will be used in future studies.

FAST ATOM BOMBARDMENT: BROADENING THE CAPABILITIES OF PARTICLE-BEAM LC/MS

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Combining an HPLC with a mass spectrometer has been a problem of major concern to analytical chemists since the introduction of the GC/MS combination. Particle-beam mass spectrometry (derived from MAGIC-LC/MS) has the ability to generate searchable quality electron impact (EI) and chemical ionization (CI) spectra for a wide range of compounds. Generation of such spectra is accomplished only because of the efficient removal of solvent from the analyte before it reaches the mass spectrometer. The analyte leaves the interface in the form of a narrow particle beam which is flash vaporized in the heated ion source region. Compounds which produce high enough vapor pressures may be ionized using EI or CI processes.

A great number of compounds found in HPLC effluents are too involatile to produce high enough vapor pressures for EI and CI processes. These types of compounds require special ionization techniques (*i.e.* laser desorption, and fast atom bombardment (FAB)). This paper addresses the use of FAB as an alternate ionization process for particle-beam LC/MS. Figure 1 shows the experimental set-up. A drop of an appropriate matrix is placed on a probe tip inside the ion source region of the mass spectrometer. The particle beam created from the HPLC effluent by the interface strikes the matrix and is instantaneously ionized by the FAB gun producing standard FAB spectra.

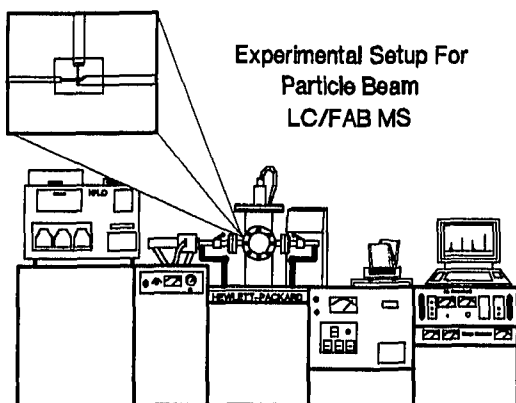


Figure 1

Prior to any chromatography it is necessary to check the compounds of interest to be sure they will generate FAB spectra. This is not unlike standard HPLC with UV detection where one checks to make sure the compounds absorb in the UV. Figure 2 is an ion chromatogram of some azo dyes which were run on the system with no column in place. Spectra were obtained and determined to be of good quality. In a later experiment the dyes will be separated and analyzed using Particle beam LC/FAB-MS.

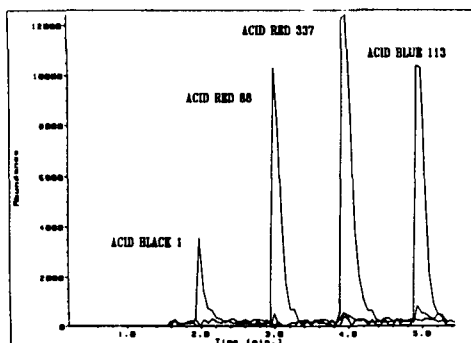


Figure 2

A mixture of two pharmaceuticals was separated and analyzed using the particle beam system. Figure 3 shows the ion chromatogram of the mixture. Note that the two baselines are due to the fact that the matrix produces a high ion count at m/z 181 which corresponds to the $(M+H)^+$ of aspirin. On the other hand at m/z 328 which corresponds to $(M+H)^+$ of Naloxone, there is very little ion production from the matrix, thus a lower baseline. Chromatographic conditions for the run were 94% methanol, 1% acetic acid, and 5% water, a flow rate of 1 mL/min on a Whatman Partisil C-8 column.

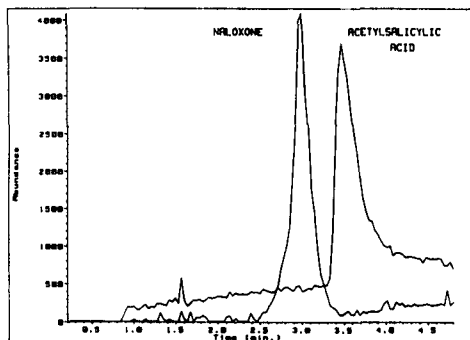


Figure 3

While this first chromatogram is simple and of little practical use, it is exciting in the fact that a new technique has been proven to work and the applications of this technique are unlimited.

LC/API/MS BY PARTICLE BEAM LIQUID CHROMATOGRAPHY

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Introduction. Atmospheric pressure ionization (API) sources for mass spectroscopy have been developed by several groups. The impetus for the development of API-MS can be traced to the biomedical community, whose spokespersons express an apparently limitless need to press ever lower the minimum detectable amount in samples of numerous complex organic materials. More recently, the analysis of trace quantities of compounds in air for air pollution studies; and in gases for the semiconductor industry has caused a resurgence in the interest of API-MS.

The upsurge of LC/MS in the last few years has caused the need for lower detection limits. While most LC/MS techniques have been limited to low nanogram levels there have been some groups that have pushed these levels down to the low picograms levels.

Objectives. It was the purpose of the present studies to explore the state of the art of API/MS and to demonstrate the feasibility of API spectra with four different designs. Design A is commercially available design from Extrel Corp (Ketkar and Siegel). Design A is currently used for monitoring the composition of air and effluents from incinerators. The other three designs (Designs B, C and D) are prototype designs. Design B was designed to study the transport of ions and charged particles through a momentum/ion separator. Design C was an adaption of Designs A and B to resolve the shortcomings of Design A (tailing) and Design B (lack of response). While Design D was an adaption of the electrospray developed by Yamashita and Fenn at Yale University; and ionspray developed by Bruins et al. at Cornell University.

Results. Table I is a summary of our results. Design A yielded the best results but it was hampered by extensive tailing of the peak. The most dramatic results were obtained with Design D. Spectra of multiply charged ions were detected for gramicidin S, mellitin, and substance P. Figure 1 is the full scan profile spectrum of mellitin, similarity reported by Covey et al.

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Table I.

<u>Ionization Method</u>	<u>API Interface</u>	<u>Analytes/Sensitivity*</u>
Corona Discharge	Design A (TAB)	Caffeine 500 ng (SIM)
	Design A (TSP)	Atenolol 1 ng (SIM)
	Design B (TAB, TSP)	No Ions Detected by MS
	Design C (TAB)	Adenine 700 ng
		Atropine 200 ng
		Caffeine 200 ng
		Cotinine 1 ug
	Design C (TSP)	Caffeine 200 ng
		Cotinine 500 ng
Electrospray	Design B	Caffeine 50 ng (SS)
Ion Spray	Design D	Atenolol 100 ng
		Caffeine 200 ng
		Cephalosporin C 900 ng
		Cotinine 800 ng
		Cramicidin S 200 ng
		Mellitin 1 ug
		Substance P 500 ng

*All data has been taken with Flow Injection and Full Scan, unless otherwise as noted.
SIM: Single Ion Monitoring. SS: Steady State introduction of sample (ng/min). TAB: ThermoBeam™ Nebulizer. TSP: Thermospray Nebulizer.

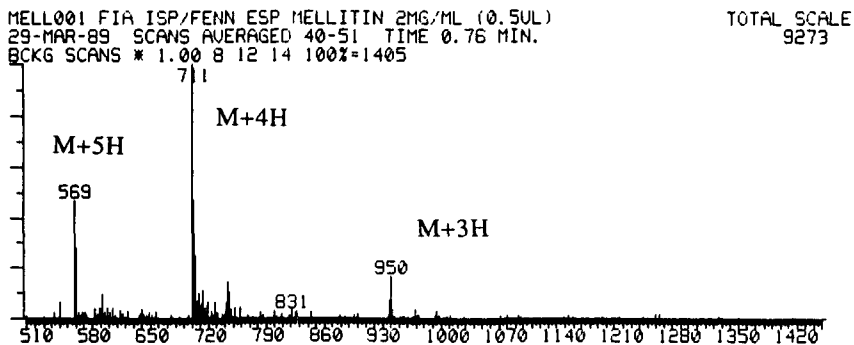


Figure 1. Ion-spray spectrum of mellitin (average molecular weight 2846.4)

OPTIMIZATION AND EVALUATION OF PARTICLE BEAM HPLC/MS PERFORMANCE FOR THE ANALYSIS OF PESTICIDE COMPOUNDS AND METABOLITES

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Introduction

Nine pesticides and related compounds were chosen for study using a particle beam (PB) HPLC/MS interface in order to explore analysis capabilities with this new and promising instrumental technique. The analytes represented a wide range of chemical classes that have poor gas chromatographic performance properties. These compounds included a phenyl urea, organophosphates and carbamates, a dinitroaniline, sulfur and oxygen heterocyclics, and several sulfones and sulfoxides. Our objectives were to determine optimal PB HPLC/MS instrumental parameters (such as mobile phase pH, composition and flow rate, nebulizer position, helium flow rate, and desolvation and MS source temperatures), to evaluate qualitative and quantitative data collected under optimum conditions, and to compare PB results with those generated with a thermospray HPLC interface.

Experimental

All PB HPLC/MS experiments were performed using a HP Particle Beam interface and HP 5988 MS system with a Waters HPLC system. A Vestec thermospray interface, Finnigan 4500 MS, and Waters HPLC system were used for the thermospray experiments.

The compounds studied were difenoxuron, fenamiphos sulfone, aldicarb, fenamiphos sulfoxide, thiobencarb, aldicarb sulfone, trifluralin, atraton, and plantvax. For the optimization experiments, only four of these compounds were used. All optimization experiments were performed with sample loop injections (no HPLC column). Full scan spectra were then collected with those instrumental parameter settings considered optimal.

Results

Most interface parameters investigated were found to be mobile phase dependent instead of analyte dependent. The best instrument response to all of the test compounds was obtained with methanol as a mobile phase. Other organic solvents investigated were acetonitrile, isopropanol and tetrahydrofuran. Increasing water content in a methanol mobile phase caused a decrease in the detected ion current. The signal intensity decreased approximately 15% over the range when changing buffers from 0.1 M acetic acid to 0.1 M sodium acetate to 0.1 M sodium hydroxide in 50% methanol/water. Methanol was used as the mobile phase for all instrument parameter optimization. An optimum HPLC mobile phase flow rate was determined to be about 0.6 mL/min with methanol.

The optimal nebulizer setting was somewhat compound dependent; however, all the compounds showed two optima in the regions corresponding to a capillary tip position approximately 0.5 mm inside and 0.5 mm outside the nebulizer nozzle tip. The best signal intensity was produced with a helium flow into the nebulizer such that the instrument inlet flow gauge reading was approximately 30 psi. A desolvation chamber temperature setting of about 550 °C produced the best results, but the optimum MS source temperature appeared compound dependent.

Particle beam electron impact-type spectra (see Figure 1) showed logical fragmentation patterns, and molecular ions were detected for all compounds except aldicarb and aldicarb sulfone. The spectra compared well with references from the NBS Mass Spectral Library. When the PB interface was operated under CI conditions (see Figure 2), the spectra showed $[M+H]^+$ and $[M+20]^+$ ions as well as large characteristic fragments similar to those seen with thermospray.

A calibration curve was constructed with twelve (12) injections of aldicarb sulfone at each of five calibration levels ranging from 20 ng and 500 ng. Linear regression analysis of the calibration data showed a r^2 value of 0.994. Triplicate injections at the 20 ng level had a RSD of 7%.

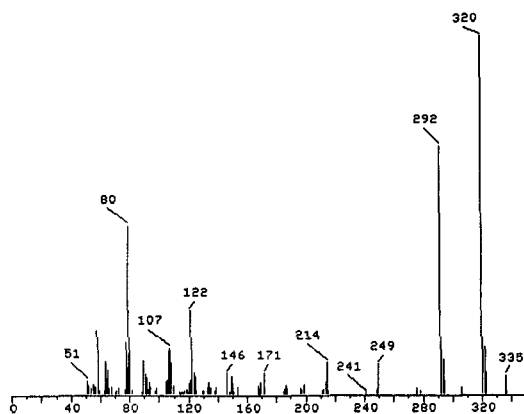


FIGURE 1 - FENAMIPHOS SULFONE ELECTRON IMPACT MASS SPECTRUM

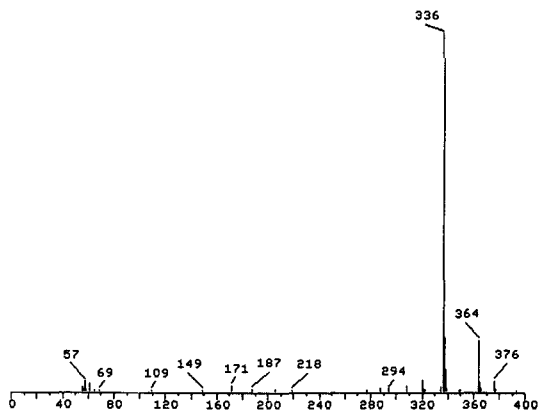


FIGURE 2 - FENAMIPHOS SULFONE METHANE CHEMICAL IONIZATION MASS SPECTRUM

THE APPLICATION OF PARTICLE BEAM HPLC/MS TO THE ANALYSIS OF DERIVATIZED AND
UNDERIVATIZED PROSTAGLANDINS

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Prostaglandins are a group of fatty acids that modulate a broad range of physiological functions. As such, they may be involved in a number of pathological conditions. The measurement of the prostaglandins by MS has been primarily accomplished through the use of GC/MS with negative ion chemical ionization (NCI) (1). This technique offers high sensitivity, but requires extensive sample derivatization (esterification, silylation and oximation) prior to analysis. Underivatized prostaglandins have been studied by desorption CI/MS (DCI/MS) (2,3). However, the potential for variations in sample handling make it unsuitable for use in quantitation.

The current study examines the use of the Particle Beam(PB) HPLC/MS as a tool to allow the measurement of prostaglandins via HPLC. Unlike GC, HPLC does not require extensive derivatization to effect separation of involatile compounds. In addition to observing the positive CI (PCI) and NCI MS of the underivatized prostaglandins PGE₂, PGF_{2a}, TXB₂, and TXB₂-o-methyl acetal, a series of corresponding methyl esters and pentafluorobenzyl esters were prepared. These derivatives were purified by normal phase HPLC and examined by PB/HPLC/MS.

Experimental:

All spectra were acquired using a HP 5988A LC/MS system equipped with a PB interface and RTE-A data system scanning from 250-600 amu. A mixture of Methane:Ammonia 99:1 was used as reagent gas for both PCI and NCI at a source pressure of 0.5 torr. HPLC was performed by a HP 1090L equipped with an autoinjector. A hypersil SI (3uM particle, 60x4.6 mm) column was used for all separations with a mobile phase of CH₂Cl₂:MeOH 100:0 to 70:30 in 15 minutes. Methyl esters were prepared by reacting 100ug of a given prostaglandin in 0.5ml of ethereal diazomethane for 5 min and then evaporating. Pentafluorobenzyl esters were prepared by reacting the prostaglandin with 50uL of a 1:3 solution of pentafluorobenzyl bromide in ACN and 50uL of a 1:4 solution of diisopropylethylamine in ACN for 5 minutes at 40 C, followed by evaporation. All residues were dissolved in CH₂Cl₂.

Results and Discussion:

Figure 1 shows the PCI spectrum of PGE₂ in the molecular ion region. The PCI spectra of the prostaglandins were found to resemble those obtained by DCI earlier, suggesting that rapid volatilization was occurring in the ion source. Source temperature was found to influence the observed abundance of NH₄⁺ adduct ions vs elimination ions. Also, total sensitivity to the prostaglandins increased at higher source temperatures. In general, limits of 0.5 to 2 uG and 0.1 to 1uG were observed for the free acids and methyl esters respectively. The use of NCI in scan mode, or PCI with selected ion monitoring (SIM) mode allowed 10x greater sensitivity over PCI in scan mode. Figure 2 shows the results for the NCI MS of PGE₂ pentafluorobenzyl ester in both scan and SIM mode. These derivatives yielded the best sensitivity with better than 10:1 s/n at 1 ng. Because chlorinated solvents are known to compete with solute in the resonance electron capture mechanism of NCI (4), it is expected that greater sensitivity may be obtained via the use of nonchlorinated solvents in either normal or reverse phase.

These results demonstrate the feasibility of using PB/HPLC/MS to reduce the number of derivatization steps (one instead of three), and therefore the time, needed to analyze prostaglandins at physiologically useful levels vs GC/MS. Studies on the application of this technique to urinary metabolites are in progress.

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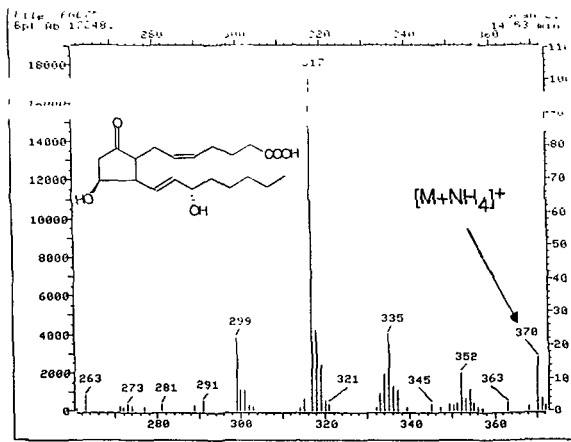


FIGURE 1

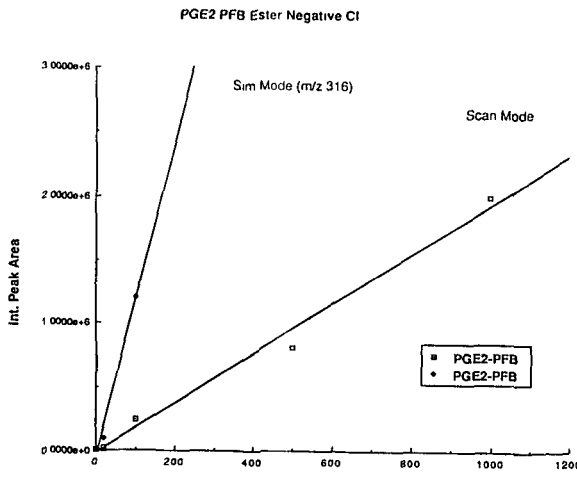


FIGURE 2

Characterization of Flavors and Aromas by GC/MS and Particle Beam LC/MS.

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INTRODUCTION

The characteristic flavors and aromas of fruits and fruit products are the result of complex mixtures of organic compounds. Because flavor components occur in natural products as complex mixtures, several sample handling techniques have been developed in order to analyze citrus constituents by using mass spectrometry. Components of high volatility (b.p. < 125 °C) and intermediate volatility (b.p. 125-225 °C) have been easily identified by using GC-MS. They are usually separated on conventional GC and volatile enough to be introduced into the source of a mass spectrometer at normal operating temperatures. Components of citrus flavor fractions that do not distill below 100 °C under high vacuum are generally regarded as nonvolatile components. Nonvolatile components are usually separated by crystallization, distillation, or some form of liquid chromatography or thin-layer chromatography. Their mass spectra are determined off-line with a solids probe. Use of a solids probe often requires heating the sample above normal source operating temperatures which can result in decomposition of the desired component or volatilization of an impurity to give an erroneous mass spectrum. (1)

OBJECTIVES

The objectives of this study were to (i) simplify the existing methods for analysis; (ii) develop on-line techniques for faster more accurate qualitative results using electron impact ionization; and (iii) evaluate major and minor components that were previously difficult because of thermal degradation or lack of volatility. Emphasis will be placed on LC/MS because of the associated problems of introducing high molecular weight components into the ion source.

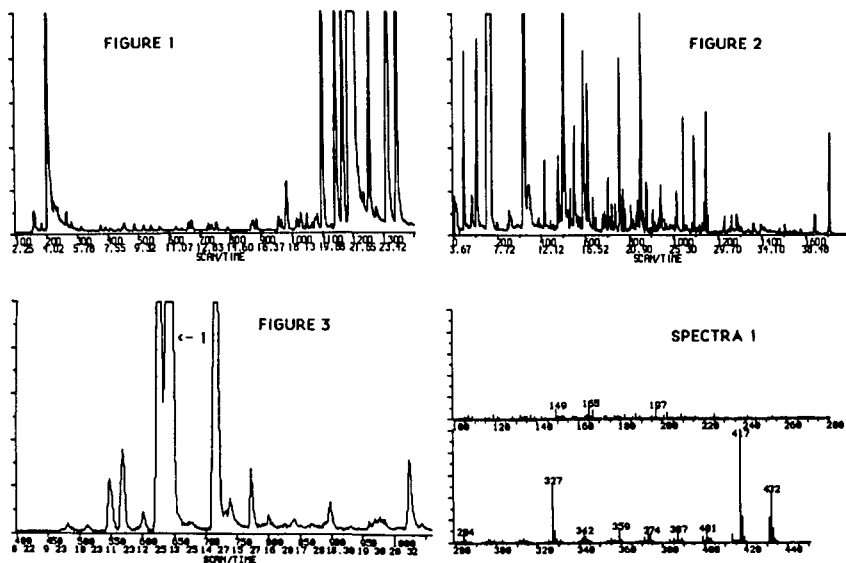
EXPERIMENTAL

This analysis involved using the EXTREL 400-2 Mass Spectrometer equipped with both a GC and a ThermoBeam™-LC/MS interface in the on-line mass spectral analysis of Oil of Orange (McCormick & Co., Inc.). The GC/MS analyses involved the techniques of purge and trap (Tenex/silica gel) of 5 microliters of the oil in 5ml of water onto a 1% SP-1000 on carbopack B column; and the injection of 1 microliter of the concentrated sample (5ml reduced to 1ml by warming) onto a SPB-5 (30m) capillary column. The LC/MS analysis was accomplished by injecting 10 microliters of the previously concentrated sample onto a C-18 (4.6mm) reverse phase column. A gradient elution program using methanol and water was used at a flow rate of 0.5ml/min.

RESULTS

The GC/MS runs show typical compound rich chromatograms (Figures 1. Purge and Trap; Figure 2. Splitless Injection, 1ul) that have been well characterized(2).

Figure 3 shows the total ion current for the LC/MS run. Spectrum 1 shows an example of a nonvolatile component of orange extract that eluted in 25 minutes (3). This component along with some others in Figure 3 did not elute in either of the GC/MS analyses. Previous techniques used to identify this component involved size exclusion HPLC (300-400 MW fraction) with further separation using reverse phase HPLC and final analysis using a solids probe introduction to a mass spectrometer (4).



This study concludes that by using LC/MS in conjunction with GC/MS techniques a broad range of volatile and nonvolatile components can be studied from flavors and fragrances in a relatively short period of time. These techniques compliment each other with regard to qualitative results.

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DEVELOPMENT OF AN INTERNAL STANDARD AND A
PERFORMANCE TEST MIXTURE FOR PARTICLE BEAM LIQUID
CHROMATOGRAPHY/MASS SPECTROMETRY SYSTEMS

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Several types of particle beam interfaces for high performance liquid chromatography/mass spectrometry (HPLC/MS) have been described. These interfaces permit a new approach to the development of a broad spectrum HPLC/MS methods for the simultaneous identification and measurement of non-gas chromatographable toxic organic environmental pollutants. Most of the work done to date has involved the use of flow injection analysis (FIA) for the evaluation of these interfaces. While FIA is a valuable technique for the rapid injection of compounds through the LC/MS interface and into an electron impact mass spectrometer, the performance data obtained by this process probably has little relevance to the performance obtained by real HPLC/MS. The performance observed with FIA is compromised in real LC/MS by the HPLC column, necessary ionic strength additives and pH buffers in the mobile phase, and variable solvent compositions resulting from gradient elution.

Factors which affect the overall response of an analyte are the operating parameters of the interface and the analyte itself. Caffeine has been proposed as a test substance for the optimization of the particle beam HPLC/MS interface operating parameters. Adjustable operating parameters for particle beam interfaces include: the position of the capillary transfer line with respect to the entrance to the desolvation chamber, the temperature of the desolvation chamber, the temperature and flow rate of the nebulization gas, and the composition of the mobile phase.

We have studied these parameters on two particle beam HPLC/MS systems: the Hewlett-Packard Particle Beam LC/MS and the Extrel Thermabeam LC/MS system. The operating parameters were varied and the integrated signal intensities of the ions for caffeine and sixteen nonvolatile compounds (see Figure 1) were measured. The compounds in this mixture were chosen to elute within a reasonable time from a reversed phase C-18 HPLC column using gradient elution of acetonitrile-water (0.01M NH_4OAc) solvent system at 0.4 ml/min. A generally good correlation was established with FIA between caffeine signal intensities and the intensities of compounds in the test mixture as long as the same mobile phase composition (e.g. 100% acetonitrile) was used for each compound. However, as the composition of the mobile phase changes, as in gradient elution, the optimum parameter settings for some compounds, in relation to caffeine, changes. Because each compound will elute under different mobile phase composition in real HPLC/MS, FIA optimization with caffeine may or may not give the parameter settings which will give optimum response for all compounds in a mixture. Compounds with volatilities different from caffeine may have maximum response at different settings. A compromise setting must be used. Therefore, through the column optimization was performed for the mixture by varying the operating parameters over several runs. By observing the performance of the whole mixture, an optimum setting which maximizes the response of all compounds in relationship to each other, can be found.

This mixture can also be used to test the performance and continuing operation of individual system components. Degradation of the HPLC column may result in peak broadening and loss of resolution between close eluting compounds. This results in loss of sensitivity for some compounds. The desolvation chamber temperature can be critical to signal reproducibility for certain compounds (i.e. late eluting compounds and volatile compounds). If the temperature fluctuates during a chromatographic run, the relative standard deviation (RSD) of the response for these compounds increases. It is also important to test the instruments ability to produce reproducible standard electron impact mass spectra. A gas chromatographable compound, decafluorotriphenylphosphine (DFTPP), routinely used in GC/MS methods to check tuning on mass spectrometers, was included in the mixture. While this compound is not ideally suited to LC/MS due to its volatility, the electron impact spectra meets EPA ion abundance criteria.

We have also investigated the use of particle beam HPLC/MS systems for quantitative analysis. Calibration curves were generated for all sixteen compounds and response factors were calculated using dichlorobenzidine-D₆ as an internal standard. Most of the compounds exhibited linear calibration curves and response factors with RSDs in the 10-30% range. However, coeluting compounds have been found, in some cases, to affect the performance of each other by either enhancing or reducing their response relative to their response when run individually. Therefore, the use of labelled internal standards, which coelute with their non-labelled counterpart, may not be possible.

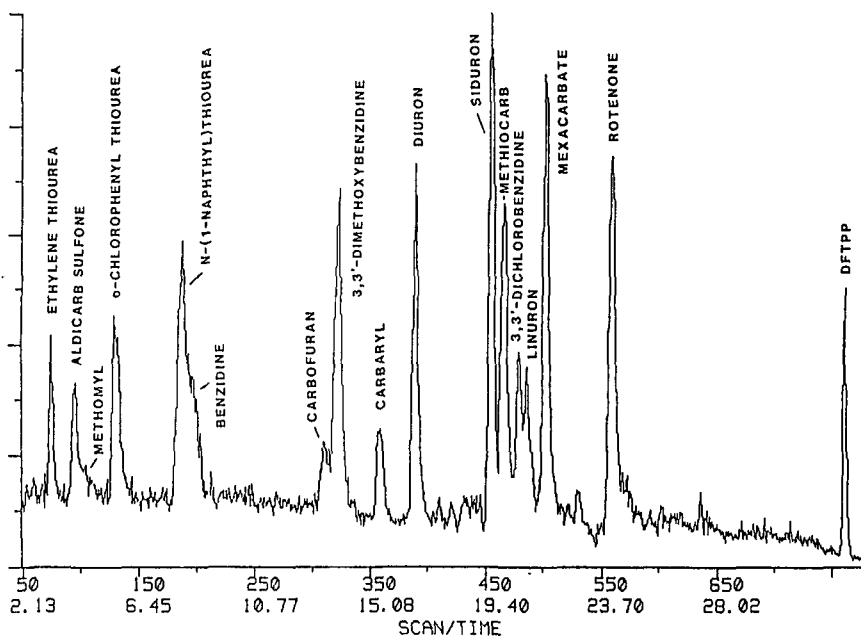


Figure 1. Total ion chromatogram of compounds in the test mixture.

Various Aspects of Ionspray Mass Spectrometry/Mass Spectrometry
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Ionspray (IS) or nebulizer-assisted electrospray is a relatively new ion generation technique. It is also a potentially excellent method for interfacing liquid chromatography to mass spectrometry.

One of the unique features of IS is its relative ease in generating multiply charged ions. Macroions containing some twenty charges have been reported (1,2). Yet, small triply or quadruply charged ions that exist in solution are frequently not seen by IS. In an attempt to establish if a correlation exists between ion evaporability and charge to size ratio, we have surveyed some thirty anionic species containing multi-carboxylic, sulphonic and/or phosphoric groups. These include: (A) homologs of straight chain dicarboxylic acids, (B) benzenecarboxylic acids containing two to six carboxylic groups, (C) EDTA and CDTA, (D) ADP and ATP, and (E) azo dyes containing three to four acidic groups.

Ionspraying was carried out in a solution of 70/30 methanol/water containing 1 mM ammonium acetate at pH 9.0. According to pKa data, all acidic groups in the species studied are expected to be ionized in solution. Yet, the ionspray spectra showed significant presence of ions containing undissociated acidic groups indicating that these less highly charged ions are preferentially evaporated. Further, for the more highly charged ions, evaporability is apparently a function of ion size, for example, no ions more highly charged than two were seen when benzenhexacarboxylic acid was sprayed, whereas the triply and quadruply charged ions of Ponceau S (ion mass for the 4- ion, 668.6) were intense when the azo dye was introduced. For ions of similar elemental composition, a reasonable indicator of ion size is ion mass. The main result of the survey is summarized in Fig 1 where ion charge is plotted against mass for ions that were observed and for ions that were expected but not observed. For singly and doubly charged ions, all ions that were expected were observed. For the triply and quadruply charged ions, it is quite apparent that a minimum mass or size has to be satisfied for successful ionspraying. For the types of ions that were surveyed, the cut off point is about 450 amu for triply charged and about 700 amu for quadruply charged ions. These results agree qualitatively with Born's theory of ion solvation. A larger ion may be viewed as more able to stabilize or spread its charge. For a relatively large quadruply charged ion like Ponceau S, the sulphonic groups are well separated to the extent that ion evaporation may be considered as simultaneous evaporation of four singly charged species, whose evaporation is almost always observed.

On the practical side, we have successfully applied IS MS/MS without prior chromatographic separation to the rapid determination of tributyltin (TBT) in a sediment certified reference material for trace metals, PACS-1 (NRCC). TBT is the active ingredient in marine anti-fouling paint and is toxic to shellfish. TBT was first extracted from the sediment with iso-octane or n-butanol, diluted with methanol containing 1 mM ammonium acetate, flow-injected into the ionspray interface, and quantitated by selected reaction monitoring of the daughter/parent pair of m/z 179/291 (a daughter ion spectrum of m/z 291, the most intense isotope of TBT^+ , is shown in Fig 2 (3)). The minimum detectable amount of TBT was about 5 pg Sn absolute or 0.2 μ g Sn/g sediment. PACS-1 was found to contain 1.29 ± 0.07 μ g Sn/g sediment of TBT. Fig 3 shows a standard additions run of PACS-1 extracts.

References

- (1) T.R. Covey, R.F. Bonner, B.I. Shushan and J.D. Henion, Rapid Commun. Mass Spectrom. 2, 249 (1988).
- (2) J.A. Loo, H.R. Udseth and R.D. Smith, Rapid Commun. Mass Spectrom. 2, 207 (1988).
- (3) K.W.M. Siu, G.J. Gardner and S.S. Berman, Rapid Commun. Mass Spectrom. 2, 201 (1988).

Captions

Fig 1: Ion charge versus mass: ●, ions observed; x, ions expected but not observed.

Fig 2: Collision induced dissociation of m/z 291 from TBT. Bu = butyl.

Fig 3: Flow injection analysis of PACS-1 extracts: Determination by using standard additions method.

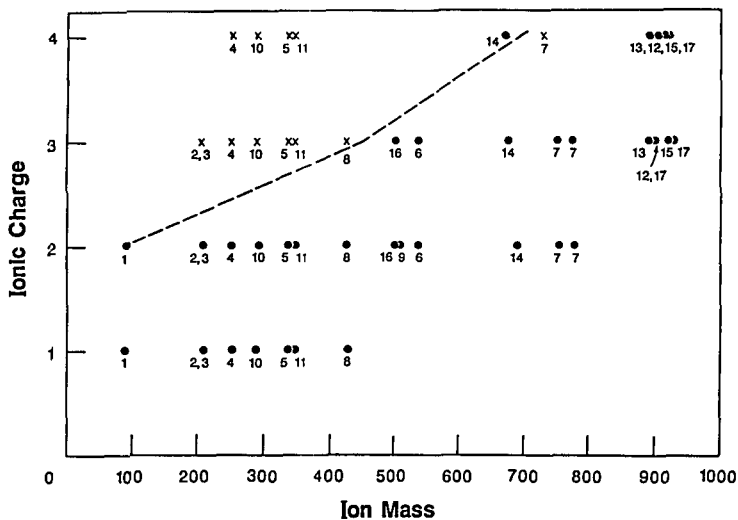


Fig 1

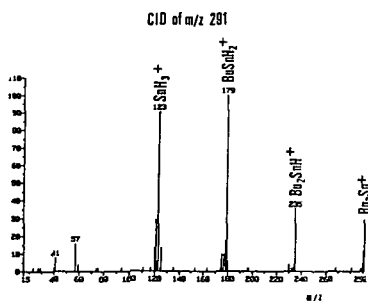


Fig 2

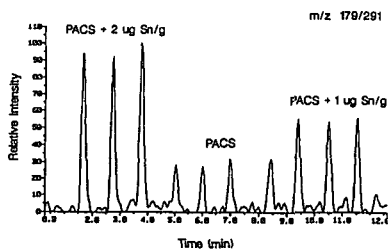


Fig 3

ION-SPRAY MASS SPECTROMETRY FOR THE ANALYSIS OF MARINE TOXINS*

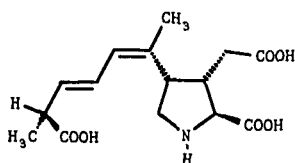
M.A. Quilliam, S. Pleasance, B.A. Thomson¹ and G.J. Scott¹

Atlantic Research Laboratory, National Research Council of Canada, 1411

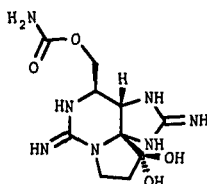
Oxford St., Halifax, N.S., Canada, B3H 3Z1;

¹Sciex, Division of MDS Health Group Ltd., 55 Glen Cameron Road, Thornhill, Ontario, Canada, L3T 1P2.

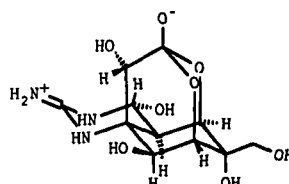
Marine organisms produce many toxic metabolites, some of which are responsible for numerous cases of seafood poisoning each year. Such toxins include domoic acid (responsible for ASP, amnesic shellfish poisoning¹), saxitoxin (a member of a family of compounds responsible for PSP, paralytic shellfish poisoning²), tetrodotoxin (the pufferfish toxin³) and okadaic acid (one of the compounds responsible for DSP, diarrhetic shellfish poisoning²).



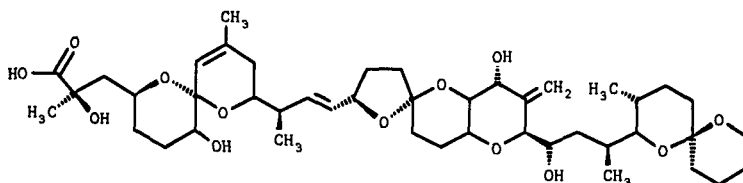
Domoic Acid (DA)



Saxitoxin (STX)



Tetrodotoxin (TTX)



Okadaic Acid (OKA)

The most commonly used technique for detecting marine toxins in seafood is the AOAC mouse bioassay. Although this method has the advantage of being non-selective and thus all-inclusive for protection of the public, it is recognized that bioassay suffers from considerable variability and gives little information on toxin composition. Instrumental methods of analysis offer the potential of automation as well as greater selectivity and sensitivity. However, most marine toxins are polar, thermally labile compounds that are difficult to analyze by conventional methods (e.g., GC and GC/MS). High performance liquid chromatography is a preferred method of analysis, but still requires chemical derivatization for UV or fluorescence detection since most marine toxins do not have significant chromophores. Mass spectrometric methods for DA, PSP and TTX have been investigated, with fast atom bombardment (FAB) ionization being useful as a means of structural confirmation at moderate sensitivity.⁴⁻⁷

Atmospheric pressure ionization (API) is recognized as a technique which provides high sensitivity for the analysis of polar organic compounds. The SCIEX® "IonSpray™" system is a very promising API technique based on nebulizer-assisted electrospray.⁸ The ion formation mechanism in both ion-spray and electrospray, a similar technique⁹, is ion evaporation (field assisted desorption of preformed solute ions from micro-droplets of solution)¹⁰ although nebulization also plays a role in ion-spray.⁸ The technique can be used with flow rates from a few to

several hundred $\mu\text{L}/\text{min}$ and thus can be used on-line with HPLC as well as for direct sample introduction. The objective of this study was to investigate the possibility of using ion-spray mass spectrometry and LC-MS to detect trace levels of marine toxins. A preliminary report of this work has been published.¹¹

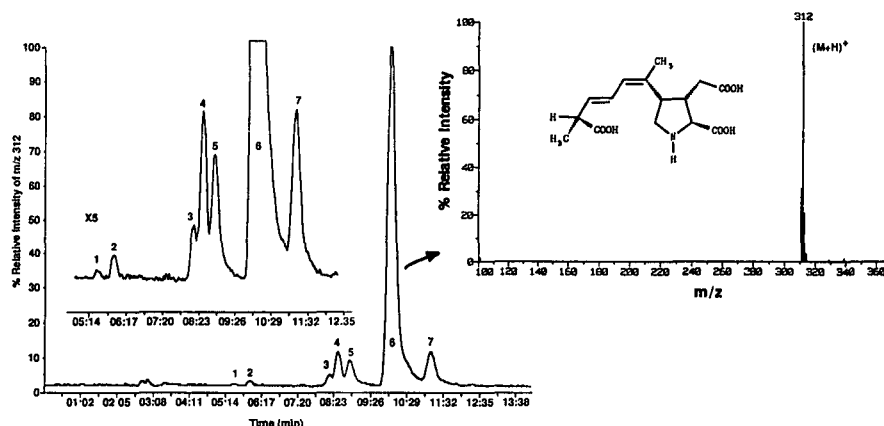
Three of the toxins (DA, STX and TTX) gave positive ion spectra with abundant protonated molecular ions and no significant fragmentation. Both DA and OKA gave negative ion spectra with $[\text{M}-\text{H}]^-$ ions. Tandem mass spectrometry (triple quadrupole) provided useful fragment ion spectra. Detection limits for flow injection analyses with selected ion monitoring were determined to be 30 pg for STX, 100 pg for DA and 200 pg for TTX. Insufficient material was available for such measurements with OKA. Combining liquid chromatography with ion-spray mass spectrometry was very easy and allowed the determination of DA and some of its isomers and derivatives in toxic shellfish tissue extracts (Figure 1).

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*NRCC No. 30278

Figure 1: Positive ion-spray LC-MS analysis of a toxic mussel tissue extract after clean-up with C18 solid phase extraction. Peak 6 in the reconstructed mass chromatogram (m/z 312, MH^+) is domoic acid (74 ng injected) and the other numbered peaks are DA isomers. The inset shows the very simple mass spectrum acquired at the apex of peak 6. Conditions: 200 $\mu\text{L}/\text{min}$ 10% acetonitrile in water with 0.1% formic acid; 2 μL injection volume; 25 cm x 2.2 mm I.D. 5 μm Vydac 201TP column.



A (VIRTUALLY) NON-AEROSOL VAPORIZATION INTERFACE
FOR LC/MS AND LC/MS/MS

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A novel capillary LC/MS interface vaporizer has been developed which involves the condensation of high pressure steam on the surface of a low thermal mass metal capillary. The process of condensation deposits substantial energy to the capillary equivalent to the latent heat of vaporization of the working fluid (steam), resulting in rapid vaporization of the aqueous LC eluent within the capillary. Virtually complete eluent vaporization is obtained at flow rate of 1.0 ml/min, at a working fluid temperature of 160 °C. The high latent heat of vaporization of water that often limits many LC/MS interfaces is used in reverse by this interface, improving energy coupling to the aqueous LC eluent and reducing the temperature necessary for continuous LC eluent vaporization.

The prototype version of the vaporizer, shown in Figure 1, consists of a 21 cm length of thin wall 6.35 mm o.d. nickel tubing containing approximately 1 ml of water as the working fluid. A nickel capillary (0.60 mm o.d., 0.35 mm i.d.) coaxially traverses the length of the chamber serving as the transfer line from the LC column as well as the vaporization cavity. The outer surface of the capillary is in contact only with the vapor and liquid phase of the working fluid. The nickel chamber is heated with insulated resistance wire wrapped around and along the length of the chamber. A copper jacket between the resistance heater and the nickel chamber improves temperature uniformity of the nickel chamber and provides additional thermal ballast for the working fluid.

The vaporizer is currently interfaced with a TAGA 6000E tandem mass spectrometer. The vaporized LC eluent is diluted with 10-30 liters/minute of laboratory air prior to ionization at atmospheric pressure. A 22 mm o.d. glass transfer line carries the diluted vapor to the point of ionization. The glass transfer interface is wrapped with heat tape. Since vaporization is virtually complete prior to dilution with air, the transfer interface to the TAGA APCI source is maintained at sufficient temperature to prevent cold spots which may result in solute or solvent condensation.

The vaporizer can readily accommodate fluctuations in eluent flow as well as water:methanol and water:acetonitrile gradients at constant operating temperature. The equilibrium vapor phase of the working fluid condenses predominately along the outer surface of that portion of the capillary containing liquid eluent, with little condensation (energy coupling) occurring along that portion of the capillary containing vapor. Gradients and flow fluctuations rapidly result in an increase or decrease of the effective region of working fluid condensation without any need to modify the temperature of the vaporizer.

The measured temperature at atmospheric pressure of the eluent vapor 2 mm beyond the exit of the capillary is shown in Figure 2 for 100% water at various flow rates as a function of working fluid temperature. A rise in eluent vapor temperature is observed following virtually complete vaporization within the capillary. The actual temperature of the eluent vapor may be slightly higher than that indicated in Figure 2 since the jet core near the exit of the capillary is less than 1 mm in diameter, and may contain entrained cooler air at the surface.

Figure 3 illustrates a negative ion LC/MS chromatogram obtained by selected ion monitoring on the TAGA from injection of 20 µl of a 50 ppm methanol solution containing the indicated explosives. The ions monitored were m/z 46 (NO₂⁻) and m/z 62 (NO₃⁻) resulting from apparent NCI autodetachment in the TAGA APCI ion source. The column used was a 6.3 mm Dupont Zorbax C-18 eluted with a water:methanol gradient at a flow rate of 1 ml/min from a Varian 5000 LC pumping system. The working fluid temperature was held constant at 160 °C throughout the analysis. Periodic fluctuations in the m/z 46 chromatogram suggest pump pulsing effects, however, evidence of pump pulsing effects on the interface are absent from the m/z 62 chromatogram.

Initial experiments with this vaporizer suggest that it provides a very efficient method of vaporizing aqueous LC eluent at conventional flow rates of 1 ml/min without exposing the vapor to excessive temperature. The vaporizer operates at constant temperature for isocratic or gradient elution. Further work is under development to improve sensitivity with the TAGA. Versions of the interface are under development for use with a Thermospray ion source as well as in a particle beam configuration.

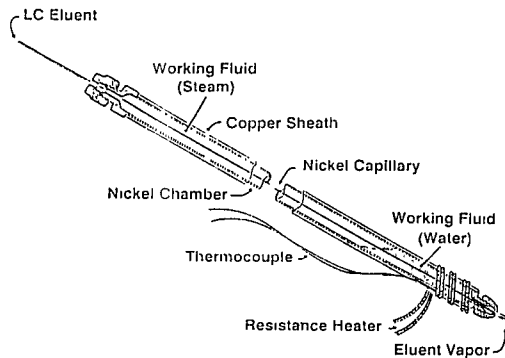


Figure 1. The Prototype Vaporizer

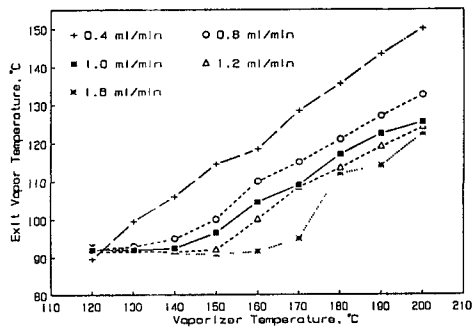


Figure 2. Exit Vapor Temperature for 100% Water

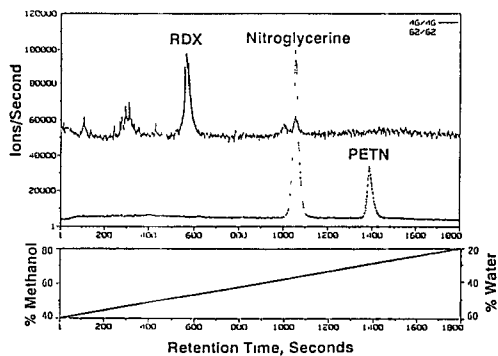


Figure 3. Negative Ion LC/MS Chromatogram from 20 μ l of 50 ppm Explosives Standard, 1 ml/min, 160 $^{\circ}$ C

Atmospheric Pressure Spray Ionization for Liquid Chromatography/Mass Spectrometry (LC/MS)

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Liquid Chromatography/Mass Spectrometry (LC/MS) is one of the most important analytical methods, and various types of LC/MS systems have been reported. The authors have previously developed an atmospheric pressure ionization method for LC/MS, and have continued a study for an ionization technique suitable for LC/MS. During this study, we discovered a new ionization method, that is, atmospheric pressure spray (APS) method^{1,2)}. The ionization principle is shown in Figure 1. In this method, mobile phases containing samples at a flow rate of 1ml/min are nebulized into small droplets under atmospheric pressure by the capillary which is heated up to above 400°C. Produced droplets are introduced into a drift region located between two electrodes with apertures. A voltage can be applied to this region in order to break cluster ions. Generated ions in this region are brought to a mass analyzing region.

Although APS method is a kind of thermospray method operated under atmospheric pressure, obtained APS mass spectra and operating conditions are very different from those of thermospray method. The main characteristics of APS method are as follows:

(1) Cationized molecules can be obtained for nonvolatile compounds with high cation affinities, while protonated molecules for nonvolatile compounds with high proton affinities; (2) Intense molecular ion species can be observed with few fragment ions; (3) Highly sensitive detection can be achieved for saccharides and their related compounds; (4) APS method combined with a collision induced dissociation technique at the drift region can produce many fragment ions which are very useful for identification; (5) A good stability of ion intensities can be obtained.

Figure 2 shows an APS mass spectrum of globoside, which is one of glycolipids. Since the proton affinity of this compound is not so high, abundant protonated molecules cannot be observed. However, in APS method, intense cationized molecules resulting from the binding of cations and oxygen atoms of globoside can be observed. Figure 3 shows the result of a saccharide mixture analysis from soy bean containing glucose, sucrose, raffinose and stachyose. These results reveal that LC/MS using APS method is very promising as an ionization method for LC/MS.

SHEET DIRECT INSERTION MEMBRANE PROBE FOR SELECTIVE INTRODUCTION OF
ORGANIC COMPOUNDS INTO A MASS SPECTROMETER

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Membrane introduction mass spectrometry is a simple yet sensitive technique for monitoring volatile organic compounds in aqueous media. Membranes used in these inlets have typically been mounted outside the mass spectrometer, however, exterior mounting can lead to poor response times, memory effects, and analyte dilution due to transfer effects. To overcome these problems two different types of direct insertion membrane probes (DIMP) have been built. One probe uses a capillary membrane (C-DIMP) [1], the other a sheet membrane (S-DIMP) [2], and both allow the transfer of the liquid analyte solution directly to the heated ion source of a mass spectrometer. We present here, the S-DIMP and its performance.

A schematic of the S-DIMP is shown in Figure 1. The probe incorporates a sensor and heater for temperature control of the membrane interface. The membrane used in these studies was a dimethyl-vinyl-silicone polymer (ASTM: VMQ Dow Corning, thickness 0.254 mm). MS/MS data was acquired using a Finnigan TSQ-4500 mass spectrometer.

Knowledge of the effects of temperature, flow rate, and matrix are all essential for optimum use of the S-DIMP. Figure 2 shows the ion current from m/z 78 obtained from passing an aqueous solution containing 120 ppm benzene through the probe. At least a 10 fold increase in the m/z 78 ion current is observed due to the increase in permeation rate of benzene with temperature. The signal decreases near 100°C due to the vaporization of the solution at the probe tip which causes a dilution of the analyte. Working at higher solution/membrane temperatures, below the vaporization point, is thus preferred.

Optimization of flow rate is also important in obtaining high mass transfer. A continuous monitoring experiment shows a 25 % improvement in sensitivity of THF m/z 72 at 2.1 ml/min versus 0.6 ml/min while segmented flow analysis shows increased sensitivity at low flow rates.

The chemical matrix of the solution can also have an effect on the permeation rates, ionization efficiencies, memory effects, and membrane properties. The effects of matrix compounds on permeation rates and ionization efficiencies were studied using aqueous solutions of ethanol/acetoin (%/%). EI measurements for solution A (Figure 3a) (concentrations of ethanol/acetoin in solution A (%v) : 0.1/0.1, 0.5/0.5, 1/1, 5/5, 10/10) and CI measurements for solution B (concentrations of ethanol/acetoin in solution B (%v) : 0.1/10, 0.5/5, 1/1, 5/0.5, 10/0.1) show that at higher concentrations (5-10 % V), ethanol enhances the permeation rate of acetoin. However, the permeation rate of ethanol appears to be unaffected by ethanol. CI measurements for solution A (Figure 3b) show that the signal of both compounds in a mixture is lower at high concentrations than for pure solutions. This is attributed to high analyte partial pressures in the ion source causing insufficient protonation. Memory effects are most troublesome when analyte concentrations are low compared to concentrations of matrix compounds. Full membrane recovery between injections and excellent reproducibility for S-DIMP is shown in Figure 4.

Figure 5 shows a typical CI mass spectrum of *Bacillus polymyxa* fermentation broth. The main constituents of fermentation are identified as ethanol m/z 47, acetoin m/z 89 and 2,3 butanediol m/z 73 (MH-H₂O*) by comparison of their daughter spectra with standards.

As shown in Table 1, the S-DIMP has also proven to be a sensitive device for detecting volatile organics in aqueous environmental monitoring.

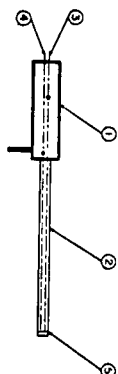
Our results show that the S-DIMP is a valuable device for reaction monitoring/control and environmental analysis since it allows on-line quantitation (ppb level) and identification of unknown compounds when used together with MS/MS.

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* Present address: Finnigan MAT, 355 River Oaks Parkway, San Jose, CA 95134.

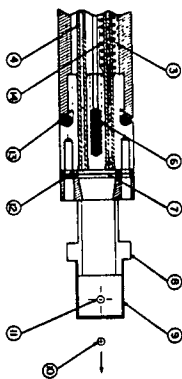
** On leave from Technical Research Center of Finland, Chemical Laboratory, Biologinkuja 7, 02150 Espoo, Finland.

SHEET DIRECT INSERTION MEMBRANE PROBE



(1) Probe handle, (2) Probe barrel, (3) Inlet conduct, (4) East conduct, (5) Probe tip

CROSS SECTION OF THE PROBE TIP



(6) Inlet conduct, (7) East conduct, (8) RTD sensor, (9) Membrane, (10) Ion volume in the ion source block, (11) C Reagent orifice, (12) Ion beam, (13) Electron beam, (14) Heating coil

FIGURE 1

CHANGE IN ION ABUNDANCE OF BENZENE (m/z 78) DUE TO A CHANGE IN THE S-DIP TEMPERATURE.

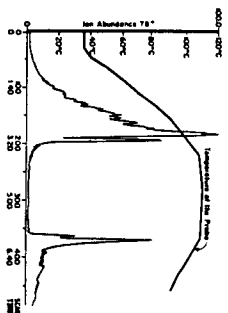
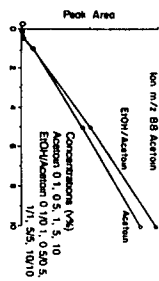
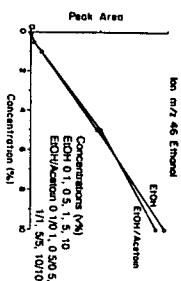


FIGURE 2

EI MEASUREMENTS FOR SOLUTION A



CI MEASUREMENTS FOR SOLUTION A

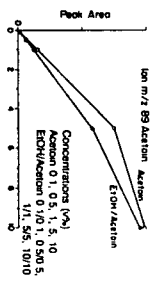
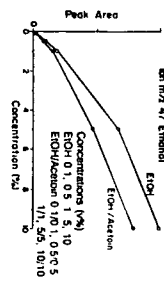


FIGURE 3

SEPARATED FLOW ANALYSIS AT 50 °C FROM ACETONE FOR 1.0, 2.5, 5.0 AND 10 V% SOLUTION IN WATER.

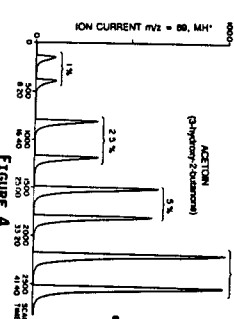


FIGURE 4

CI (NEGATIVE) MASS SPECTRUM OF A BACILLUS PASTEURII FERMENTATION SAMPLE.

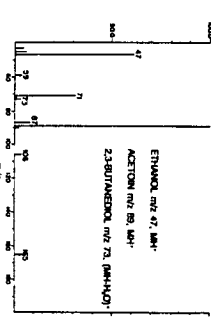


FIGURE 5

COMPOUND	IONIZATION	PAEATION	MRB IONS	DETECLIMIT
BENZENE	EI	78	52, 39, 75	10 ppb
CHLORO-	EI	112	77, 51, 105*	10 ppb
BENZENE	CI, ME	113	77, 51, 105*	10 ppb
DICHLORO-	EI	84	49, 50*	100 ppb
METHANE	CI, ME	85	49, 50*	200 ppb
2-PROPANONE	CI, ME	58	43, 51, 50*	500 ppb
TETRAHYDRO-	EI	72	42, 41, 70*	500 ppb
FURAN	CI, ME	73	55, 31, 66*	80 ppb
TOLUENE	EI	91	65, 41, 95*	20 ppb
	CI, ME	93	77, 51, 90*	10 ppb

ME = METHANE, * = BACKGROUND ION

TABLE 1

Mass Spectral Determination of Pancuronium Bromide and Vecuronium Bromide in Biological Fluids Utilizing the Moving Belt LC/MS Interface.

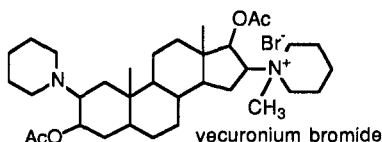
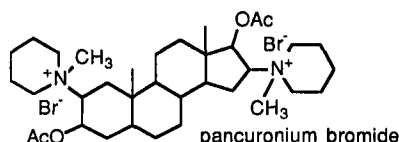
Timothy B. Baker and Paul Vouros

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Pancuronium bromide and vecuronium bromide are neuromuscular blocking agents used in anesthesiology in conjunction with general anesthetics. Accurate determination of these drugs in biological fluids is required for pharmacokinetic and pharmacodynamic studies.

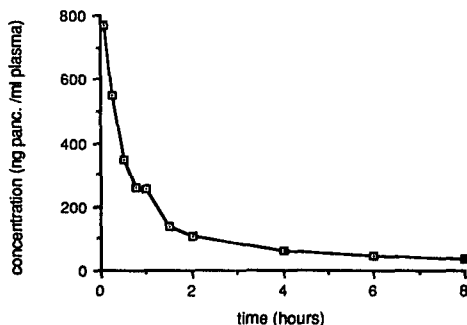


Previously reported assays for these compounds and their deacetylated metabolites involve the use of TLC [1], HPLC [2], GC [3], and direct insertion probe mass spectrometry [4]. The objective of this work was to develop an assay for these compounds which could be characterized by improvements in specificity, sensitivity, and sample throughput.

This method is based on syringe application of sample extracts to the moving belt surface, thermal desorption, and single metastable transition monitoring in the chemical ionization mode. This thermal process results in dealkylation of the compounds to form a free base which is subsequently protonated via Cl. Compounds, such as pancuronium and vecuronium, that are thermally dealkylated to form the same free base have identical spectra and can not be distinguished.

The most abundant metastable ion in the B/E spectrum of pancuronium is a result of the loss of acetic acid from the protonated free base (ie m/z 543 $\rightarrow$ m/z 483). Monitoring this single metastable transition provides an added degree of specificity over single ion monitoring and eliminates chemical interferences that may occur in the assay. As an example, plasma extracts contain an interference at m/z 543 which can be eliminated by monitoring the metastable transition. The analogous acetic acid loss m/z 549 $\rightarrow$ m/z 486 is monitored for the hexadeuteriated internal standard.

The assay described here has been utilized to determine the rate of post-surgical clearance of pancuronium from the plasma of human patients who are suffering from traumatic burns.



The assay has also been used to determine post-operative levels of pancuronium in urine. An eight hour urine composite for a typical patient was found to contain 1.4 ug/ml of pancuronium.

The simultaneous analysis of the deacetylated metabolites can be performed via similar metastable transition monitoring. Mono-deacetyl analogs can be determined as a unit via monitoring of the transition m/z 416 $\rightarrow$ m/z 356. The bis-deacetyl metastable transition m/z 374 $\rightarrow$ m/z 356 has similar analytical utility.

Chemical ionization and B/E spectra of the drugs d-tubocurarine, metocurine, and gallamine have been obtained using the same conditions required for compounds of the pancuronium series. This indicates the potential for the use of this assay to determine other neuromuscular relaxers in biological fluids.

The methods described here will find utility in a continuation of this work which will utilize the demonstrated detection capabilities of this method in conjunction with an HPLC separation of these compounds.

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Collision Induced Dissociation Studies of Transition Metal Oxide Clusters

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Introduction: The interest in transition metal oxides derives largely from their use as catalysts for the oxidation of olefins and other unsaturated molecules, and as semiconducting, dielectric or insulating materials in electronic applications. Selective ion mass spectrometric techniques such as tandem mass spectrometry or FTMS are well suited to the study of metal oxides in the gas phase, especially in conjunction with process environments where complex mixtures of ionic species must be analyzed. We present here preliminary results for the ion production, collisional activation, and ion dissociation of the metal oxide clusters, $(MO_3)_n$, $M=Mo, W$, where $n=1-13$ for $(MoO_3)_n$ and $n=1-8$ for $(WO_3)_n$.

Experimental: A Finnigan triple stage quadrupole mass spectrometer (TSQ-70) was operated in negative ion mode with 1200 mTorr argon source pressure, temperature of 800°C, and 1 mTorr collision cell with argon target gas. Fourier transform mass spectra were acquired on a Nicolet FTMS-2000 dual cell spectrometer with an argon source pressure of 5×10^{-6} Torr and an analyzer pressure of 2×10^{-8} Torr. Samples were applied to a platinum or rhenium filament from a methanol suspension, and ions were produced by increasing the filament current until sufficient signal intensity was obtained.

Discussion and Conclusions: Clusters to $n=13$ for $(MoO_3)_n$ and $n=8$ for $(WO_3)_n$ were observed to the upper mass limit of the TSQ spectrometer. A TSQ spectrum for MoO_3 is shown in Figure 1. The base peak in the spectra of both oxides was $(MO_3)_3$, while the monomer, MO_3 was barely observed. The intensities of the clusters for $n>3$ generally decreased with increasing mass, although different ionization and analyzing conditions yielded different relative abundances for MO_3 clusters. FTMS spectra acquired at a pressure 10^6 less than TSQ spectra exhibited only the dimer and trimer. A representative spectrum of MoO_3 is shown in Figure 2. The isotopic distribution patterns for the clusters were verified by comparison with calculated spectra of the same ions. The CAD behavior of the metal oxide clusters was also studied, and breakdown curves were generated from the TSQ spectra of $(MoO_3)_n$ in the 60-160 eV collision offset potential range. A dominant feature of the fragmentation pathway is loss of neutral trimer, as opposed to simple loss of monomer. This behavior suggests that the clusters have more complicated bond energetics than would be expected from loosely associated gas phase aggregates. The overall fragmentation scheme is shown in Figure 3.

Acknowledgements: We are grateful to the Chemistry Department, University of Texas, and the Robert A. Welch Foundation.

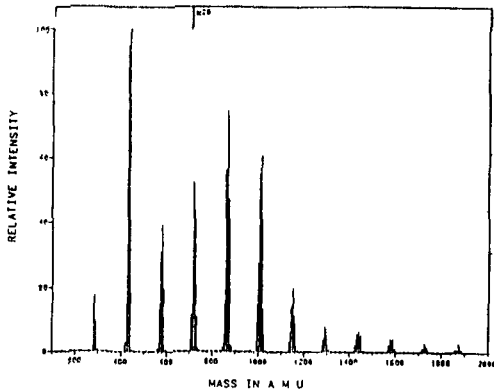


Figure 1 TSQ spectrum of MoO₃.

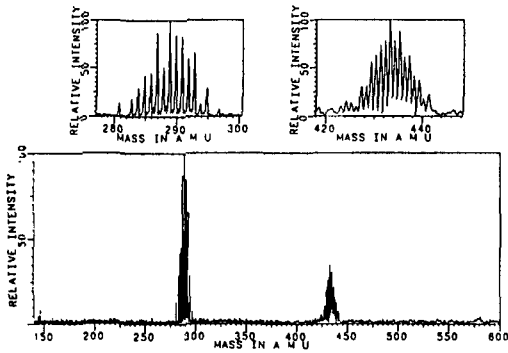


Figure 2. FTMS spectrum of MoO₃.

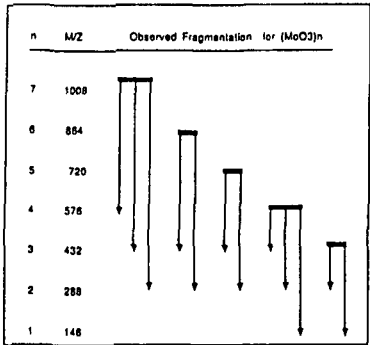


Figure 3.

CLUSTER ION FORMATION BY DIRECT LASER VAPORIZATION AND PARTICLE BOMBARDMENT

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Two useful methods for the formation of cluster ions are direct laser vaporization (DLV) and particle bombardment (with keV ions or atoms). However, the effectiveness of these methods is highly dependent on the material from which ions are formed. For example, DLV of metals (1) seldom produces anything beyond atomic ions or small clusters. By contrast, particle bombardment can produce high mass cluster ions from either metals or salts (e.g. CsI)(2). The situation is largely reversed in the case of graphite. DLV can produce very high mass clusters ($n > 100$, where n is the number of atoms in the cluster)(3), whereas particle bombardment only produces small clusters (up to $n=5$). The work described here considers possible mechanisms of cluster ion formation based on mass spectrometric studies of ions produced by DLV and particle bombardment.

The materials used in these studies were either metal foils or pressed pellets of single- or multi-element powders. Ions were produced by DLV with a Nd:YAG laser (532 nm or 1064 nm, 50 mJoule/pulse) or by particle bombardment with 8 keV atoms or ions. Mass analysis was performed with a VG ZAB-2F double focussing magnetic sector instrument, a Finnigan TSQ-70 triple quadrupole mass spectrometer, or by a Naval Research Laboratory constructed time-of-flight (TOF) instrument. The latter employs a modified Wiley-McLaren source and a Mamyrin-type electrostatic mirror.

As noted by Freiser and co-workers (1), DLV of polished Ag produces only Ag^+ . By contrast, the FAB spectrum of Ag produces Ag_n^+ to at least $n=11$. The differences here may result from the fact that Ag does not strongly absorb visible light, and the resultant laser induced plasma does not contain sufficient ions and neutrals to allow significant cluster formation. By contrast, DLV of Ag pressed in a graphite pellet produced not only carbon cluster ions, but nearly equal abundances of Ag^+ , Ag_2^+ , and Ag_3^+ , as well as Ag_3C_2^+ and Ag_3C_4^+ (figure 1). These results indicate that the graphite matrix allows enough energy deposition to produce a fairly dense plasma, in turn allowing formation of large abundances of higher mass Ag clusters. Particle bombardment of Ag in graphite produced considerably lower intensities of higher mass Ag cluster ions (compared to Ag alone under similar beam fluence). This may be due to the fact that some of the kinetic energy of the particle must go into breaking carbon-carbon bonds. Unexpectedly, mixed AgC species such as Ag_3C_2^+ , Ag_5C_2^+ and Ag_5C_4^+ were observed in the particle bombardment spectrum. These species suggest that ion-molecule chemistry in the seldedge may play a role in the formation of clusters by particle bombardment. Similar results were observed for Ta in graphite. DLV of Ta in graphite produced clusters of the form Ta_xC_y^+ , where $x=1-8$ and $y=0-14$. The presence of mixed TaC species with large numbers of carbon atoms supports the hypothesis that ion-molecule reactions are important. With particle bombardment of Ta in graphite, two types of clusters were observed: Ta_xO_y^+ ($x=1-8, y=0-9$) and Ta_xC_y^+ ($x=1-8, y=1-5$). Oxides would be indicative of a direct emission mechanism. TaC clusters are not expected from direct emission processes, however, and their appearance supports a partial role for ion-molecule chemistry in the seldedge region.

The role of energy deposition and the formation mechanism of cluster ions may be reflected in their kinetic energies. The kinetic energies of cluster ions have been studied using the TOF instrument by varying the delay time between the ion formation pulse (either the laser or a pulsed ion beam) and the extraction pulse. Plots of the arrival time of the cluster ions in the extraction region can be generated and from this information (and the distance the ions travel between formation and extraction), kinetic energies can be estimated. For DLV, plots of the square of ion velocity vs. the inverse of cluster mass, which yield a slope which is proportional to kinetic energy, are generally linear with cluster size. Typical values obtained are 2-4 eV. This suggests that the cluster ions are all formed in a similar environment, consistent with the formation of a plasma. The measured kinetic energies indicate a plasma temperature in excess of 10^4 K. This value is somewhat larger than expected. However, evidence from DLV of graphite shows that cluster ions are not always

characterized by a uniform kinetic energy (figure 2). For example, small carbon cluster ions ($n=1-3$) are characterized by a kinetic energy of roughly 7-8 eV, while larger clusters have energies on the order of 3-4 eV. This suggests that some component of DLV cluster formation may be influenced by a direct emission process. Studies currently underway with sputtered species suggest that kinetic energies decrease with increasing cluster size, and are similar to those produced in DLV. This may indicate that larger clusters are formed by a different process than smaller clusters.

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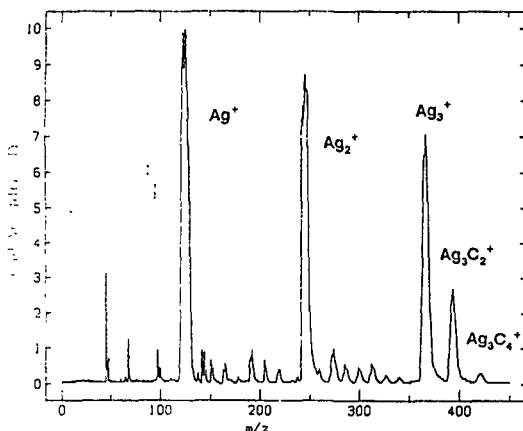


Figure 1. DLV/TOFMS spectrum of Ag in graphite.

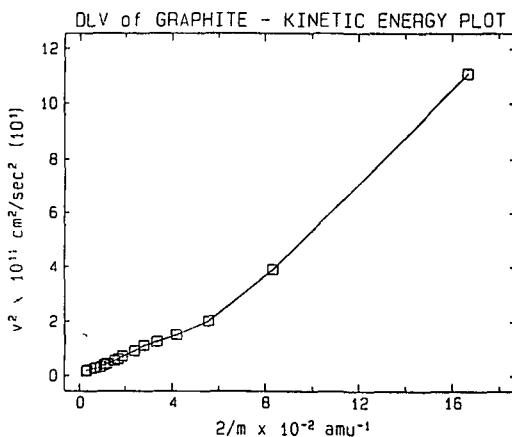


Figure 2. Plot of v^2 (where v is velocity) vs. $2/m$ (where m is mass) for carbon cluster ions generated by DLV.

FTMS STUDIES OF CARBON CLUSTER ANIONS

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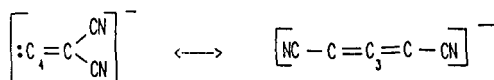
The chemistry and physics of covalently-bonded metal and semiconductor cluster ions is an extremely active area of research (1). Carbon clusters have been one of the most widely studied systems to date. The reactivity of small positive carbon cluster ions, C_n^+ ($n = 3-30$), have been well characterized in our laboratory (2). Collision-induced dissociation (CID) and ion/molecule reaction studies have provided structural information on these cluster ions, suggesting a linear to cyclic transition between $n = 9$ and 10. Evidence for structural isomers of the $n = 7-9$ cluster ions has also been observed by differences in reactivity and structural analysis of the product ions formed. This study is a detailed investigation of the corresponding carbon cluster anions using Fourier transform mass spectrometry (FTMS).

The distribution of carbon cluster anions produced by direct laser vaporization (DLV) of graphite is shown in Figure 1. The C_n^- ions observed ($n \leq 13$) is considerably smaller than for C_n^+ under similar conditions ($n > 200$ with $n = 7, 11, 15...$ being most abundant). The enhancement of the even cluster anions (Figure 1) is consistent with their higher electron affinity (3). The C_n^- distribution observed using molecular beam (MB)/supersonic expansion sources, however, extends to much larger n (3,4). Thus, larger C_n^- are stable species but are not formed by DLV and/or detected in the FTMS.

The predominant fragmentation observed in low-energy CID is loss of neutral C_3 . This is also the major fragmentation observed for C_n^+ (2) and in the photodissociation of C_n^- (4). There is competition between electron detachment and fragmentation for the smaller cluster anions ($n < 6$), while fragmentation of the cluster anions dominates for larger n ($n > 6$). This is consistent with the increase in electron affinity of C_n^- with cluster size (3). In a related photodissociation study of C_n^- formed in a MB source (4), different behavior was observed in which photodetachment begins to compete with fragmentation as n increases up to $n = 13$, above which photodetachment occurs exclusively. This was attributed to the predominance of cyclic isomers for larger n which have lower electron affinities (3,4). The combination of the CID and photodissociation results suggest that the anions detected in the FTMS consist exclusively of the linear isomers. The cyclic isomers may be formed by DLV but do not survive the long time-scale of the FTMS due to their low electron affinity. This would explain the truncation of the C_n^- distribution at $n = 13$ observed by DLV/FTMS.

The ion/molecule reactions of C_n^- are also quite different than for the cluster cations. For example, C_n^- are unreactive with D_2 , O_2 and small hydrocarbons, which react readily with the corresponding cations. The ion/molecule reactions of C_n^- with the highly electronegative molecules F_2 , HCN and C_2N_2 have been studied. The variation in rate constants with cluster size is shown in Figure 2 for C_n^- with HCN and C_2N_2 and also C_n^+ with HCN for comparison. The C_n^- rates decrease with increasing cluster size (higher electron affinity). Also, there is no dramatic change in rate which would suggest a structural transition as was observed for C_n^+ between $n = 9$ and 10 (Figure 2). No evidence for C_n^- structural isomers formed by DLV was observed, in contrast to the C_n^+ isomers observed previously (2) and isomers of C_{11}^- formed in MB sources (3). Differences in the product ions formed for the even (e.g. $C_n CN^-$ with HCN) and odd (formation of $C_n HCN^-$) carbon cluster anions suggest alternating cumulene (odd n) and polyacetylene (even n) structures for these species. Another difference between the cation and anion cluster reactions is the many higher-order reactions observed for the anions. For example, solvation with HCN occurs with the primary product ions $C_n CN^-$ to yield $C_n CN(HCN)_3^-$. CID results suggest that these HCN molecules are hydrogen bonded to the anion. Structural isomers of the product ion ($C_5(C_2N_2)^-$) formed in the reaction of C_5^- with C_2N_2 were detected by their reactivity: 79% of the product ions are reactive to

yield $C_5(C_2N_2)_2^-$ and 29% are completely unreactive. This behavior may be explained by the rearrangement of the postulated carbene insertion structure:



to a structure which contains no reactive carbene site.

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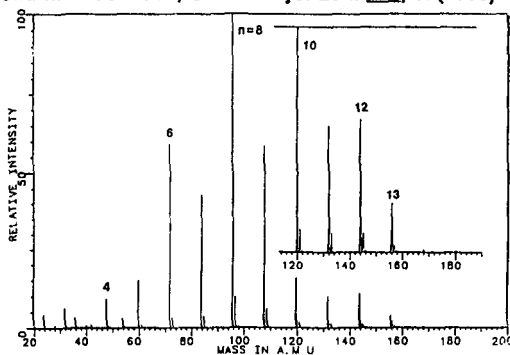


Figure 1 C_n^- Formed by Direct Laser Vaporization

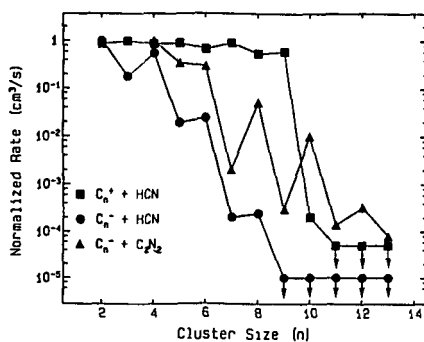


Figure 2 Reaction Rates vs. Cluster Size

DISSOCIATION AND REACTIVITY OF COBALT AND MOLYBDENUM OXIDE CLUSTER IONS

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Numerous processes employ metal oxides as catalysts for hydrocarbon oxidation. In particular, molybdenum and cobalt oxides are commonly used as industrial catalysts. Oxygen atom transfer involving oxometal groups (M-O) is a prominent mechanistic feature in these reactions. In order to gain insight into the intrinsic features of these reactions, ion/molecule reactions of cobalt and molybdenum oxide cluster ions with simple hydrocarbons have been investigated by Fourier transform mass spectrometry (FTMS). In addition, low-energy collision-induced dissociation (CID) has been employed to provide structural information.

Metal oxide cluster ions were produced by direct laser vaporization (DLV) of metal oxide pressed pellets (CoO , Co_3O_4 , CoMoO_4 , MoO_2 , MoO_3) using the 532 nm output of a Nd:YAG laser. DLV was found to be a convenient method for producing metal oxide cluster ions. Co^+ dominated the positive ion DLV spectra of CoO and Co_3O_4 , however Co_3O_4 also produced CoO^+ , Co_2^+ , and Co_2O^+ at levels sufficient for further study. The negative ion DLV spectra of both cobalt oxides (Figure 1) yielded CoO_2^- in greatest abundance, followed by Co_xO_x^- and $\text{Co}_x\text{O}_{x\pm 1}^-$, $x=1-10$. Changes in sample stoichiometry had minimal effects on cluster anion distribution, aside from the minor variations in oxygen enrichment or deficiency for ions with five or more cobalt atoms. Positive ion DLV on MoO_2 and MoO_3 yielded primarily Mo^+ , MoO^+ , and MoO_2^+ . DLV on MoO_3 also produced abundant $\text{Mo}_x\text{O}_{3x}^-$ ($x=1-4$), while MoO_2 produced predominantly $\text{Mo}_x\text{O}_{3x}^-$ ($x=1-4$) with minor amounts of $\text{Mo}_x\text{O}_{3x-1}^-$ and $\text{Mo}_x\text{O}_{3x-2}^-$ (Figure 2). Molybdenum-containing ions dominated the DLV spectra of CoMoO_4 , with some low abundance mixed metal oxide anion formation.

Small Co_xO_y^+ and Mo_xO_y^+ ($x=1,2$) yield M^+ and MO^+ under low-energy CID. Co_xO_y^- readily dissociate to produce the same anions which are most abundant in the DLV spectra (and are presumably the most stable species). In contrast, $\text{Mo}_x\text{O}_{3x}^-$ undergo little or no low-energy CID.

Ion/molecule reaction studies centered around $\text{C}_1\text{-C}_3$ hydrocarbons. No metal oxide anions were observed to react, with the exception of Co_2O_2^- which yielded the stable CoO_2^- as a major product in reactions with ethene and propene. The addition of oxo ligands increased the reactivity of Co^+ , Co_2^+ , and Mo^+ . The relatively weak $\text{Co}^+\text{-O}$ bond (84 kcal/mole (1)) is cleaved extensively during the reactions of Co_xO_y^+ with small hydrocarbons. Similar behavior has been previously reported in the reactions of FeO^+ (2) and CrO^+ (3) with hydrocarbons. Oxidative addition into C-H bonds dominates the Co_xO_y^+ reactions. Evidence exists for hydrocarbon oxidation, particularly in the reactions of Co_2O_2^+ with alkenes. In contrast, MoO_y^+ ($y=1,2$) reactions with $\text{C}_1\text{-C}_3$ hydrocarbons involve primarily dehydrogenation, with no oxygen incorporation into hydrocarbon ligands bound to the metal. The $\text{Mo}^+\text{-O}$ and $\text{Mo(O)}^+\text{-O}$ bonds are equally strong at 114 kcal/mole (1) and are rarely cleaved in these processes.

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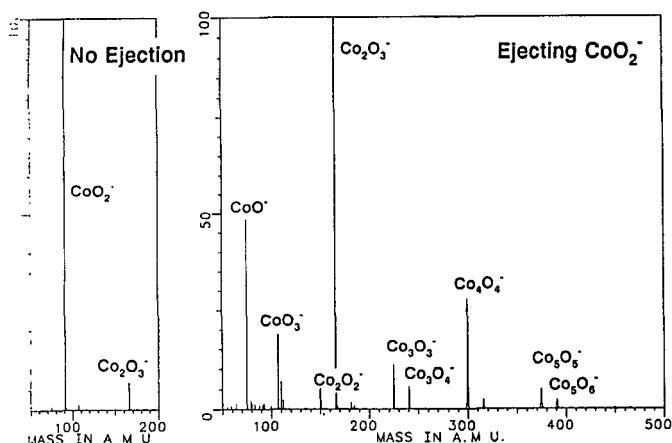


Figure 1. DLV/FTMS of Co_3O_4 , Negative Ions

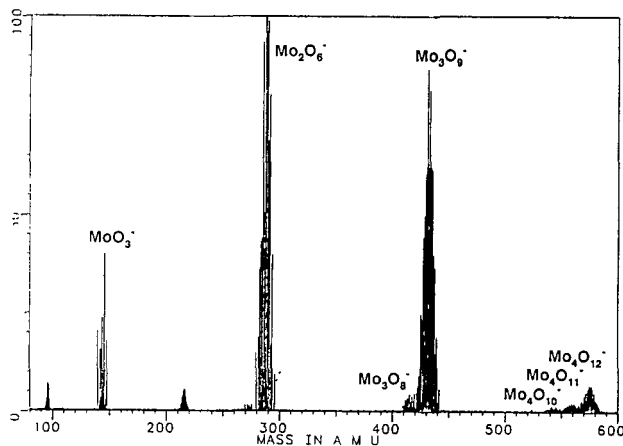


Figure 2. DLV/FTMS of MoO_2 , Negative Ions

STABILITY AND REACTIVITY OF BISMUTH CLUSTER IONS

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The study of clusters is important for an enhanced understanding of the nature of matter while having application in areas such as catalysis and development of new alloy materials. The characterization of cluster ions involves formation, mass-selection and either collision-induced dissociation or ion/molecule reaction. In this way some insight is gained into the structures, stabilities and reactivities of gas-phase cluster ions. The focus of this work is on clusters of the main group "semi-metal" bismuth. Both homogeneous bismuth clusters and heteroclusters of bismuth and antimony were studied. In addition to determination of cluster stabilities, the rates and products of cluster oxidation were measured, in order to compare with known condensed phase properties.

Cluster ions of bismuth (Bi_x^+) and of bismuth antimonide (Bi_xSb_y^+) were formed by sputtering (SIMS) in a triple quadrupole (TQMS) and sector (ZAB) mass spectrometers and by direct laser vaporization (DLV) in an FTMS. The details of bismuth cluster ion production by these methods are published [1]. This previous work revealed that the Bi_x^+ distribution has enhanced abundances at $x=3,5,7,10$ and 13 and anomalously low abundances at $x=6,9$ and 12, for $x=2$ to 14. The extent of unimolecular dissociation of each bismuth cluster ion was measured, and the aforementioned more abundant species showed less decomposition relative to that of the less abundant cluster ions in the distribution. The anomalies must arise from different cluster stabilities and, therefore, different rates of unimolecular decomposition.

The abundance distribution of Bi_xSb_y^+ heterocluster ions showed anomalies at the same cluster sizes as in the homogeneous cluster distribution, indicating that the cluster stability is determined by the number of atoms. Experiments with the FTMS were directed toward distinguishing between heterocluster ions formed by DLV (Figure 1) and neutrals (ionized by EI) produced in the laser-generated plasma (Figure 2). In addition to enhancement of the trimer species (as in the homogeneous cluster distribution), clusters with $(x+y)$ up to 5 are observed by DLV in Fig. 1 while the neutrals in Fig. 2 extend to only $(x+y)=4$. It is interesting to note that the two spectra are complementary in that the abundant species in one are nearly absent in the other. The Bi-containing heterocluster ions dominate the DLV spectrum in Fig. 1 because of lower ionization potentials while Sb-containing species are the more stable neutrals. This tendency is reflected in the products of dissociations of the heterocluster ions, where there are mostly Bi-containing fragment ions and Sb-containing neutrals lost. The heterocluster ion dissociations are characterized by production of a stable, odd-numbered cluster ion and loss of an atom, dimer or tetramer, as is the case for the homogeneous cluster ions [1].

The characteristics of cluster oxidation were investigated by (1) sputtering Bi_2O_3 , (2) oxidation of Bi_x^+ with NO_2 and (3) dissociation of Bi_xO_y^+ . Certain preferred Bi_xO_y^+ are formed by (1) and/or (2), with $(x,y)=(3,4)$, $(5,7)$ and $(7,10)$ for odd x and $(4,5)$ and $(6,8)$ for even x . In both sets, each ion arises from addition of Bi_2O_3 to the preceding species. The rates of oxidation by NO_2 for Bi_x^+ , $x=1$ to 5, are shown in Figure 3. The even-numbered cluster ions react 50 times faster than the odd-numbered species, and this is consistent with the greater stability of the latter cluster ions.

The dissociations of Bi_xO_y^+ consist of loss of Bi or BiO from the lower-oxidized species (number of O < number of Bi) and loss of BiO or Bi_2O_3 from the fully-oxidized clusters, which yield the next most stable oxide (e.g. $\text{Bi}_5\text{O}_7^+ \rightarrow \text{Bi}_3\text{O}_4^+ + \text{Bi}_2\text{O}_3$). The stable neutral Bi_2O_3 (and Bi_4O_6) represent the base units of bulk bismuth oxide. The structure of Bi_3O_4 , to which most of the oxides dissociate, most likely has a cyclic structure with bridging oxygen bonds, which would account for the enhanced stability. Such tetrahedral structures are well-known for other Group Va oxides (e.g. P_4O_6).

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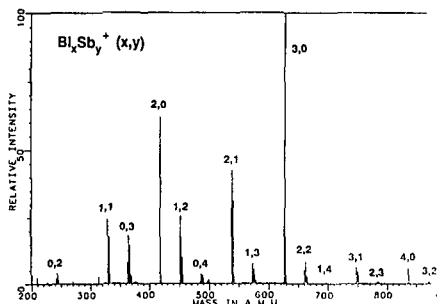


Figure 1. Bi_xSb_y^+ from DLV of BiSb

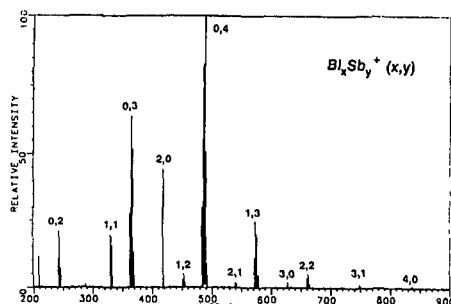


Figure 2. Bi_xSb_y^+ from EI of neutrals

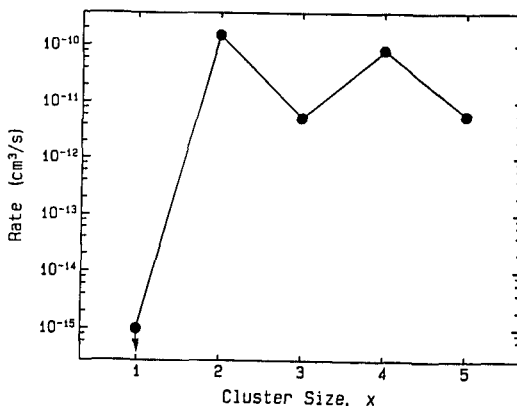


Figure 3. Rates of $\text{Bi}_x^+ + \text{NO}_2$ vs. Cluster Size (x)

FORMATION AND STRUCTURE OF OXO-METAL CLUSTERS IN THE
FAST-ATOM-BOMBARDMENT OF SIMPLE SALTS OF U, Eu AND RELATED METALS

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Introduction

The fast atom bombardment (FAB) positive ion mass spectrum of uranyl nitrate hexahydrate in aqueous solution contains a series of peaks of considerable intensity above the molecular ion peak. These singly-charged positive ions have the stoichiometry $[(UO_2)_xO_y]^+$ where y has values of -1 to 6 and ions of masses corresponding to x = 1 up to 14 have been observed. As the value of x increases, the intensities of the peaks in each cluster decrease, rapidly up to x = 4 and more gradually at higher values, and the most intense peak within a cluster occurs at higher values of y. This presumably reflects the need for a greater number of oxygen atoms to stabilise higher mass ions. For example, when x = 4, the most abundant ion is $[(UO_2)_4O]^+$, m/z 1096, whereas when x = 8, the dominant species is $[(UO_2)_8O_4]^+$, m/z 2224. (Fig. 1).

These clusters were observed when the salt was dissolved in a variety of solvents, including involatile solvents such as sulpholane, and under different conditions of pH etc., leading to moderate changes in relative intensities within a cluster. Similar spectra were also observed using continuous flow FAB (dilute aqueous solution) and a single crystal of uranyl nitrate hexahydrate.

Spectra obtained using Single Crystals of Uranyl Nitrate Hexahydrate

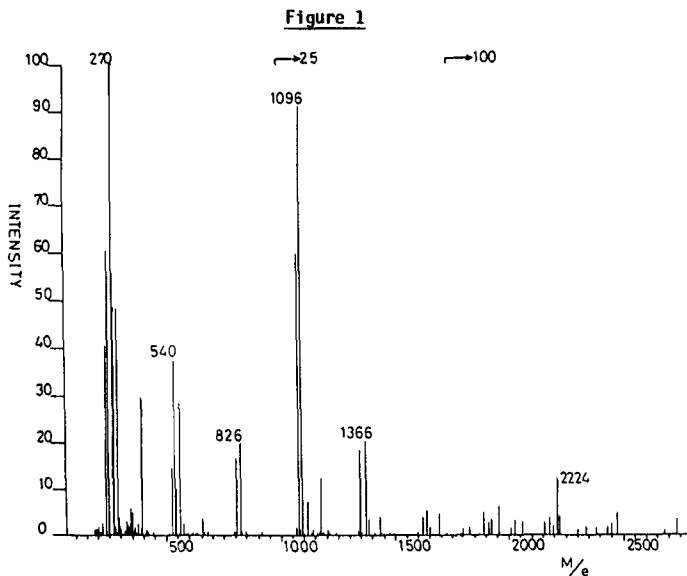
Although spectra given by crystals initially closely resembled those given by solutions of the salt, the relative intensities of the less oxygenated species, e.g. $[(UO_2)_{x-1}(UO)]^+$ increase with time. This presumably results from the loss of lattice water of crystallisation since on removal from the mass spectrometer, the sample showed no sign of melting or loss of crystallinity.

Solvent & Concentration Effects

The relative intensities of peaks in observed spectra were essentially independent of concentration variation over the range 10^{-4} to 10^{-1} mol dm⁻³ in water,

sulpholane, acetonitrile, dimethyl sulphoxide or 0.1 mol dm^{-3} nitric acid, with the exception that the peak at $m/z = 326$ was rather more prominent at the lowest concentrations. The changes in absolute intensities of all ions with concentration in water and 0.1 mol dm^{-3} nitric acid show two linear regions, a rapid rise at low concentrations followed by a more gradual rise at higher concentrations. In each case, however, the behaviour changed in the region of 0.01 mol dm^{-3} of UO_2^+ ions, and this may reflect a change in the extent of ionic aggregation in solution. Although the behaviour in 0.1 mol dm^{-3} acid in the region of 0.01 mol dm^{-3} UO_2^+ differs somewhat, presumably due to the increased participation of protonated species in ionic equilibria in solution, no additional ionic species were observed in the spectra of samples made up with a pH within the range 0.1 - 1.6. Preliminary studies have also been made of the corresponding europium nitrate FAB spectrum which shows similar behaviour, similar species have also been reported by Kahwa et al¹.

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FORMATION OF CARBON CLUSTER IONS FROM LASER ABLATION OF POLYMERS

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Many recent studies have examined the structures and reactivities of high mass even-numbered carbon cluster neutrals and ions (1), usually referred to as fullerenes. These species have been suggested to be important in systems involving flame and space chemistry (2). Most studies have emphasized the formation of fullerenes from ablation of graphite, although fullerenes have been observed by ablation of some polymers (3). These studies do not provide much information about fullerene formation in the presence of large amounts of hydrogen or heteroatoms, including reactive radicals, which could interfere with the formation of purely carbon-containing species. This lack of information limits the relevance of studies of fullerenes to problems such as soot formation.

We have observed that three very different types of materials produce fullerene ions by laser ablation (4). These materials are in addition to ablation of polyimide, which has been discussed previously (5). These materials illustrate the range of conditions under which fullerenes form.

Ions are formed by laser ablation with 266 nm radiation from a Nd:YAG laser (fourth harmonic), and are detected by Fourier transform ion cyclotron resonance mass spectrometry (Nicolet FTMS/2000). The IBM laser microprobe system, described previously (6), was used to position the sample and examine it via a light microscope.

The first polymer which was studied is a copolymer of ethylene and tetrafluoroethylene (ETFE, sold as Dupont Tefzel[®]), which is a saturated hydrofluoropolymer. The laser is observed to decompose the surface of the polymer to form a blackened layer, probably liberating HF. For the first 50-70 laser pulses near threshold power density, a fullerene distribution is observed. A sample mass spectrum is shown in Figure 1. With more laser pulses on a spot or higher power density, a low mass distribution is observed. Work is in progress to characterize the modified surface using Raman spectroscopy (7). These results may indicate the range of conditions of the modified surface for which fullerene formation is favorable.

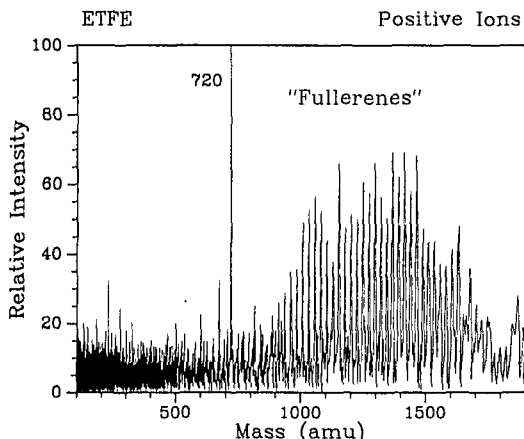


Figure 1: Positive ion mass spectrum of laser ablation of ETFE, averaging the first fifty laser pulses.

Another polymer for which fullerene ions are observed is polyphenylene sulfide (PPS). The fullerene distribution is observed initially, and extends above m/e 4000 amu and has a smaller m/e 720 peak than the ETFE spectrum. As the polymer blackens a lower mass distribution is observed, which is more useful analytically (8).

Finally, the ablation of a diamond-like carbon film also gives a fullerene distribution, which has a mass range intermediate between ETFE and PPS. The film is 40 nm thick and was determined to contain 10 at.% hydrogen. Ablation with one laser pulse of a thickness of approximately 20 nm produces a fullerene spectrum. Of the samples studied, this is the smallest quantity of ablated material for which fullerenes are formed. Since only one pulse is used and the film is very thin, surface formation of the fullerenes is very unlikely and gas-phase condensation of the ablation fragments to form the carbon clusters is the only reasonable mechanism of formation.

For all the spectra, comparisons were made to a reference fullerene distribution from ablation of graphite. There is <1-2 amu difference in the fullerene peak masses between the graphite spectrum and the sample spectra, and the spacing between the peaks is 24.0 amu (4). Therefore, the fullerenes never incorporate significant amounts of H or heteroatoms.

The most significant result from the study of these materials is that fullerenes can form in laser ablation plasmas in the presence of hydrogen and heteroatoms such as F, S, O, and N or reactive species containing them such as CN and HF. In addition, the heteroatoms are not incorporated in detectable levels. This evidence indicates that fullerenes may be able to form in real systems which do not consist of pure carbon plasmas. This conclusion is consistent with an observation of even carbon cluster ions in flames (9).

The formation in these different systems suggests that there may be a common mechanism involved. However, the mechanism may only occur for a limited range of plasma conditions, as illustrated by the ETFE results and the fact that fullerenes are not observed for most polymer ablation (8). Studies of laser ablation of other samples, such as DLC films with a range of hydrogen concentrations, may help to delineate the range of conditions. The mass range of the fullerene distribution may also indicate the kinetics of formation in the plasma. The resolution of these questions is potentially a fruitful area of study.

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TANTALUM CARBIDE CLUSTER IONS: PRODUCTION, DISSOCIATION AND REACTIVITY

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As an extension to our studies of carbon cluster ions (1), the formation, collision-induced dissociation (CID) and ion/molecule reactions of metal carbide cluster ions ($M_xC_y^+$) have been investigated using Fourier transform mass spectrometry (FTMS). Numerous studies using Knudsen effusion mass spectrometry have shown that the early transition metals most readily form M_xC_y species, typically with enhanced abundances for $y = 2$ and 4 (2). In this study, direct laser vaporization (DLV) (1-5 mJ/pulse, 532 nm) of solid samples containing tantalum and carbon were used to produce $Ta_xC_y^+$. Tantalum was chosen because it is monoisotopic and the $Ta_xC_y^+$ species produced are higher in mass ($m/z > 180$) than the abundant carbon cluster ions (C_n^+ , $n < 15$) also produced in the vaporization process.

PRODUCTION

The ion distributions for TaC_y^+ produced from different samples are shown in Figure 1. Both Ta/graphite and Ta/amorphous carbon samples produce TaC_y^+ with up to $y = 14$. Also, Ta/amorphous carbon mixture produces more abundant TaC_y^+ than the graphite-containing sample. Larger $Ta_xC_y^+$ with $x = 1-8$ and $y = 1-14$ are also produced by DLV. In contrast, DLV of bulk tantalum carbide (TaC, Figure 1) only produces TaC_y^+ with $y \leq 5$ in low abundance. This suggests that the large TaC_y^+ ions are formed by reactions of Ta and carbon species in the laser-generated plasma. In addition, the enhanced abundance for $y = 2$ and 4 (Figure 1) is consistent with the effusion mass spectrometry studies (2). Further evidence for the reactions in the plasma were obtained using labeled carbon. DLV of a 1:1 molar TaC/ ^{13}C amorphous carbon sample produces predominantly ($> 80\%$) $Ta^{13}C_y^+$ (i.e., with no ^{12}C from the bulk TaC). This is illustrated in Figure 2, where both the statistical and experimentally observed isotopic combinations for TaC_5^+ are shown.

DISSOCIATION

Low-energy CID of TaC_y^+ results in fragmentation by loss of neutral C_n species due to the higher ionization potentials of C_n (9-11 eV). For $y < 5$, the major fragmentation is loss of C_y to form Ta^+ . For larger TaC_y^+ , however, C_3 and C_{10} loss are the observed fragmentation channels. Neutral C_3 loss is also observed in the CID of C_y^+ and is predicted to be the lowest energy process (1). The loss of C_{10} for $y \geq 10$ is intriguing since the transition from linear to cyclic structures for neutral and ionic carbon clusters occurs between $n = 9$ and 10 . Thus, the TaC_y^+ species with $y \geq 10$ may have metallocene structures. Preliminary CID studies of $Ta_2C_y^+$ suggest the presence of bridging carbido ligands, e.g. Ta-C-Ta.

REACTIONS

The ion/molecule reactions of TaC_y^+ ($y = 0-14$) have been studied with D_2 , NO and C_1 and C_2 hydrocarbons. In general, TaC_y^+ reactions are quite different than the previously studied C_n^+ reactions (1), suggesting that the reactions are initiated at the metal ion center. The hydrocarbon reactions usually involve extensive and sequential dehydrogenation which is typical for the early transition metal ions. The carbon ligand in the TaC_y^+ reactions plays an active role in the ion/molecule reactions as shown in reaction 1 for CH_4 .



Labelled experiments with $^{13}\text{CH}_4$ have shown that this reaction channel to lose C_2H_2 results in total scrambling of all the carbons ($y+1$) in the reaction intermediate. This behavior is quite surprising and suggests the presence for both mobile hydrogens and possibly a metallacyclic intermediate in the reaction.

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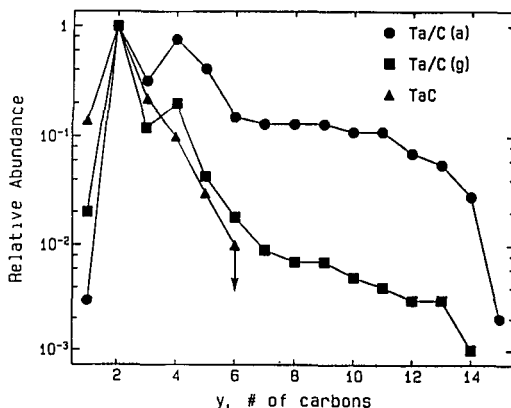


Figure 1. TaC_y^+ Distributions From Different Samples

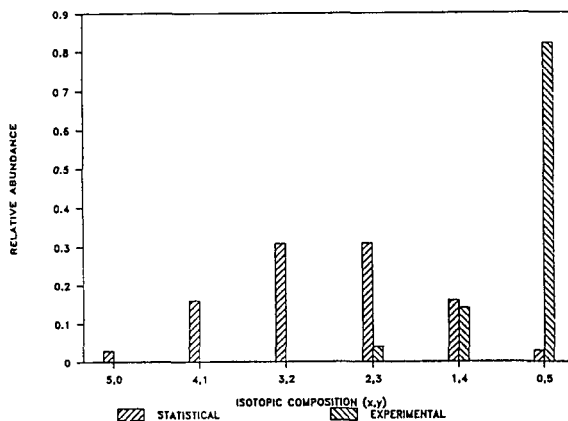


Figure 2. $\text{Ta}^{12}\text{C}_x^{13}\text{C}_y^+$ Distribution for TaC_5^+ from 1:1 $\text{TaC}/^{13}\text{C}$ (a) Mixture.

SAMPLE INTRODUCTION CHARACTERISTICS OF FLOW INJECTION WITH ICP-MS

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ICP-MS provides multi-elemental information, excellent detection limits, simple spectra and isotope ratio values. Sample introduction systems for ICP-MS typically employ pneumatic nebulization. There is currently great interest in the development of novel sample methodology for ICP-MS. Flow injection has emerged as a powerful and adaptable method of sample introduction for ICP-MS in the last few years. Flow injection(FI) allows rapid introduction of small volume samples with reduced interference, and makes possible sample introduction for different sample types, simple dilution, matrix modification and standard calibration. However, with ICP-MS, the major problem for interfacing FI derives from limitations in coupling the sample introduction system.

In this study a flow injection apparatus was connected to a Perkin-Elmer Sciex Elan ICP-MS system, where the ICP-MS acts as a multi-elemental detector for the flow injection system. Figure 1 is a schematic diagram of FI-ICP-MS system used in this work. Li, Co, Ba and Pb were the elements selected as models for investigation of sample introduction characteristics of FI-ICP-MS. The concentration for each element was in the range of 10 to 1000 ppb. These 4 elements covered a wide range of masses from 7 to 208. A Scott spray chamber with a coolant sleeve was used for organic sample introduction. When organic samples were introduced, the organic solvent vapor was condensed at 0 °C by the coolant sleeve. The ICP/MS operation parameter was set in multielement mode. The data obtained was transferred to a IBM PC/AT computer.

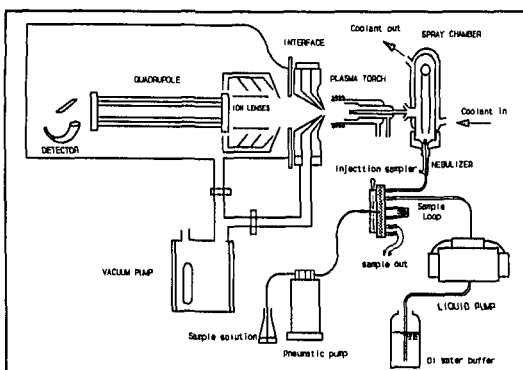


Figure 1. Schematic diagram of flow injection with ICP/MS.

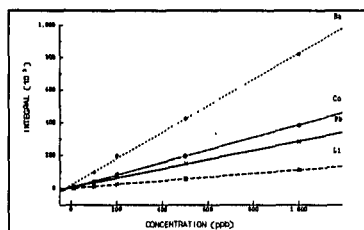


Figure 2. Peak integral responses vs. concentration of elements.

sample concentration for all selected elements, as shown in Fig. 2.

By carefully coupling the FI system with the ICP/MS, the sample injection system does not change the ICP/MS configuration. Optimum nebulizer flow rate remains the same as it used in normal conventional aspiration. For all the elements chosen in this study, the nebulizer flow is 0.9 - 0.95 L/min. The operating parameters can also be optimized independently, in the range as desired. One of the most important features of FI-ICP-MS is that the system requires only a small volume, typically 20 μ l to 100 μ l. This would be inadequate for a standard ICP/MS measurement with conventional nebulization. Both peak heights and the peak integral were used for quantitative analysis. It is observed, from the results obtained with Li, Co, Ba and Pb at various sample loop and liquid uptake flows, that peak area integral show a good linear response with

The combination of flow injection with ICP-MS provides an efficient sampling technique to ICP-MS with several important advantages. It readily allows operation with various types of sample solutions, such as samples with high content salt or organic samples. Fig. 3 shows a typical comparison of the salt effect resulting from a solution with high concentration of sodium by ICP/MS with conventional nebulization and FI-ICP-MS. It is clear that, in normal ICP/MS mode, samples with high content of salt will interfere with the performance of the ICP/MS, including altering plasma status, and it is difficult to recover the sensitivity of the instrument. After introducing a sample with 2000 to 5000 ppm Na for about 20 minutes, the sensitivity dropped rapidly (Fig. 3-A), the plasma status changed and the sensitivity can not be recovered. But with FI-ICP-MS, as shown in Fig. 3-B, it is possible to tolerate such types of sample solutions. In this study some experiments were also made using organic solvents. The mixtures of water/acetone and water/methanol in ratio from 100:0 to 0:100 were injected using the FI-ICP-MS system. It is interesting to observe that signal response increases when organic samples are introduced. This may be due to the evaporation of organic solvent from the aerosol droplets. Preliminary results indicate that organic samples can potentially be analyzed directly by FI-ICP-MS.

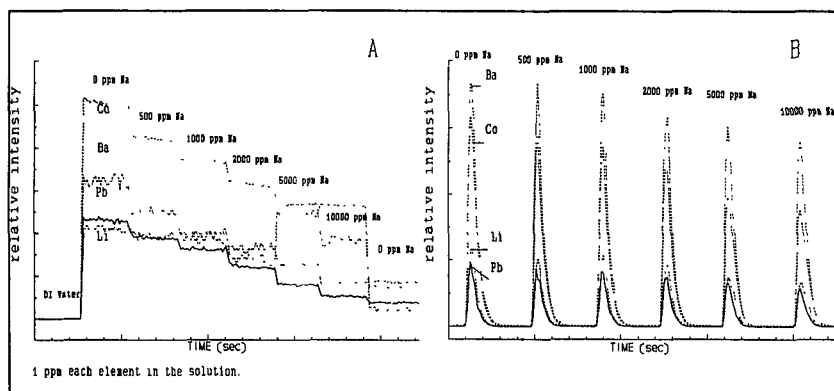


Figure 3. Influence of high content of sodium on signals. (A) Normal conventional nebulization; (B) FI-ICP-MS.

Flow injection was carried out at several sample dispersions. Dispersion may be optimized with various operating parameters, such as sample volume, sample flow rate and carrier flow rate. Signal transient time is dependent on the uptake flow rate. Memory effects in FI-ICP-MS are minimized principally through the role of exponential dilution, $C_t = C_0 \cdot \exp\{-ut/V\}$, where C_0 is the initial concentration estimated at the outlet of spray chamber, u is nebulizer flow rate, V is spray chamber volume, t is time, C_t is the concentration at the time "t". By careful choice of parameters, return to baseline may be achieved only 25 seconds after reaching the peak signal. This allows FI-ICP-MS to achieve rapid sample throughput, without loss of accuracy. To date, studies have shown great promise using synthetic mixtures. The real test of the system will, however, require the use of samples of diverse matrix configuration. Such work is currently underway.

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COLLISION-INDUCED DISSOCIATION SPECTRA OF POLYAZA RUTHENIUM COMPLEXES

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INTRODUCTION: Complexes of the type $\text{Ru}^{\text{II}}(\text{bpy})_2\text{L}(\text{PF}_6)$ were investigated using fast-atom bombardment tandem mass spectrometry. The ligands are various types of polyaza compounds being investigated to determine the structure and conformation of various ruthenium complexes depending upon ligand denticity and geometry. The low energy collision-induced dissociation (CID) spectra provide confirmation of the structures and details about fragmentations of the complexes. Doubly charged ruthenium complex mass spectra ions were observed arising from a nitrobenzylalcohol matrix and were collisionally dissociated.

EXPERIMENTAL: Fast-atom bombardment (FAB) was used to obtain the mass spectra of the ruthenium complexes. An energetic beam of 8 keV xenon atoms was used to desorb the sample from a nitrobenzylalcohol (NBA) matrix. A VG 70-SEQ tandem mass spectrometer was used to analyze the samples and obtain the low energy CID mass spectra. The collision gas (argon) pressure was adjusted to reduce the primary ruthenium complex ion intensity to 40-50 percent of the original intensity. The collision energy of the ions was adjusted to range from 20 - 50 eV (laboratory coordinates).

RESULTS AND DISCUSSION: The FAB mass spectrum of $[\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})_2)(\text{PF}_6)_2]$ (bdmt = 3,3',5,3"-bis(dimethylene)-2,2':6,2"-terpyridine; bpy = 2,2'-bipyridine) is shown in Fig. 1. Examination of the isotope cluster at m/z 699 in the mass spectrum in Fig. 1 reveals that the following ions are formed $[\text{Ru}^{\text{II}}\text{bdmt}(\text{bpy})_2]^+$ and $[\text{Ru}^{\text{II}}\text{bdmt}(\text{bpy})_2\text{H}]^+$. The formation of $[\text{Ru}^{\text{II}}\text{bdmt}(\text{bpy})_2]^+$ presumably arises from a reduction during the sputtering process. Abundant doubly charged ions were observed in the mass spectrum. The CID of $^{102}\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})_2)^{2+}$ is shown in Fig. 2.

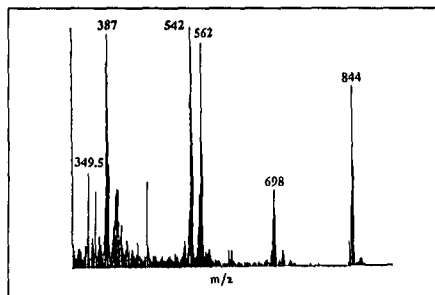


Fig. 1: FAB of $[\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})_2)(\text{PF}_6)_2]$

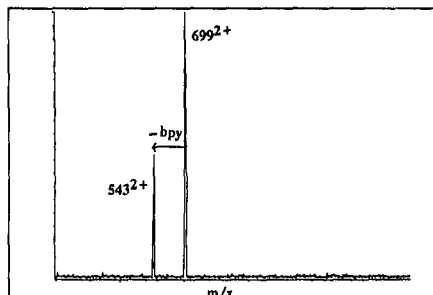


Fig. 2: CID of $^{102}\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})_2)^{2+}$

The loss of a bipyridine and a PF_5 could result in a fluoride transfer to a vacant site on the metal center (Fig. 3). The CID of $^{102}\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})\text{F})^+$ (Fig. 4) shows the loss of a bipyridine ligand as well as the loss of HF, leaving the ruthenium still with a formal oxidation state of +2.

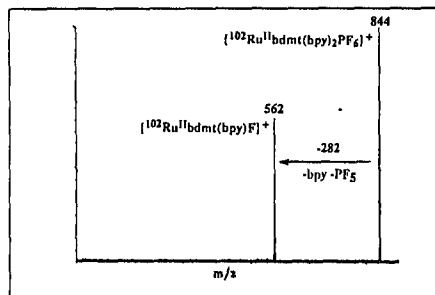


Fig. 3: CID of $^{102}\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})_2)(\text{PF}_6)^+$

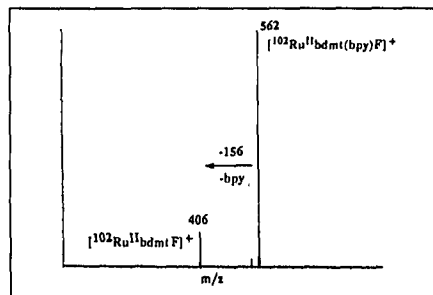


Fig. 4: CID of $^{102}\text{Ru}^{\text{II}}(\text{bdmt}(\text{bpy})\text{F})^+$

The complex $[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})](\text{PF}_6)_2$ (tbd = 2,3,5,6-tetrapyridyl-1,4-diazine) has two pendant pyridyl groups and therefore would be expected to undergo the losses of these groups via a simple C-C bond cleavage. These losses were observed in both the FAB and CID spectra (Fig. 5). It was suspected that the terpyridine was bidentate (like

$[\text{Ru}^{\text{II}}(\text{bdmt})(\text{bpy})_2](\text{PF}_6)_2$ and therefore had a pendant pyridyl group. Examination of the FAB mass spectra (Fig. 6) revealed the loss of 79 ($\text{C}_5\text{H}_5\text{N}$) from m/z 647 and this was observed in the CID spectrum as well. Proton NMR studies of $[\text{Ru}^{\text{II}}(\text{tby})(\text{d-bpy})_2](\text{PF}_6)_2$ has confirmed that the terpyridine is bidentate. The FAB mass spectra of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})](\text{PF}_6)_2$ shows the following ions. The CID spectrum of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})-\text{H}]^+$ shown in Fig. 5 shows the loss of one and two pyridyl groups as well as the loss of terpyridine and one and two peridyl groups. The FAB mass spectra of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2](\text{PF}_6)_2$ shows the following ions. The CID spectrum of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2]^+$ (m/z 647) shown in Fig. 6 shows the loss of a pyridyl group as well as the loss of a bipyridine and a terpyridine. The predominant loss of a bipyridine suggests that terpyridine becomes tridentate and hence becomes more tightly bound.

m/z	Stoichiometry
868	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})\text{PF}_6]^+$
723	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})]^+$
722	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})-\text{H}]^+$
645	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})-\text{C}_5\text{H}_4\text{N}]^+$
567	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})-2\text{C}_5\text{H}_4\text{N}]^+$
489	$[\text{Ru}^{\text{II}}(\text{tpd})-\text{H}]^+$
412	$[\text{Ru}^{\text{II}}(\text{tpd})-\text{C}_5\text{H}_4\text{N}]^+$
334	$[\text{Ru}^{\text{II}}(\text{tpd})-2\text{C}_5\text{H}_4\text{N}]^+$
335	$[\text{Ru}^{\text{II}}(\text{tpy})]^+$

m/z	Stoichiometry
792	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2\text{PF}_6]^+$
647	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2]^+$
568	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2-\text{C}_5\text{H}_5\text{N}]^+$
490	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})]^+$
414	$[\text{Ru}^{\text{II}}(\text{bpy})_2]^+$
335	$[\text{Ru}^{\text{II}}(\text{tpy})]^+$
323.5	$[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2]^{2+}$
258	$[\text{Ru}^{\text{II}}(\text{bpy})]^+$

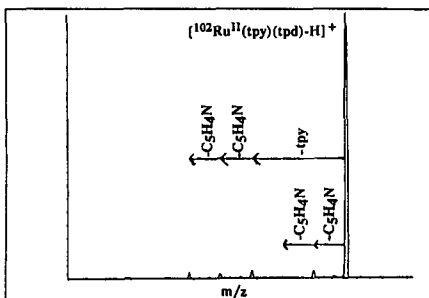


Fig. 5: CID spectrum of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{tpd})-\text{H}]^+$

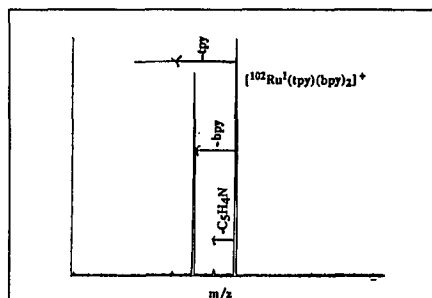


Fig. 6: The CID spectrum of $[\text{Ru}^{\text{II}}(\text{tpy})(\text{bpy})_2]^+$

SUMMARY: The highest mass peak observed for these ions corresponded to $[\text{Ru}^{\text{II}}(\text{L})(\text{bpy})_2(\text{PF}_6)]^+$. The predominant fragment ions in the CID spectra correspond to losses of intact ligands and PF_6 . An F^- transfer was observed from the PF_6 counter ion to the metal when the ruthenium complex became less than six-coordinate. Low-energy CID of the doubly charged ions was observed to yield the simple loss of intact ligands.

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PURITY OF 1% SiH₄ AND 1% SiCl₂H₂ IN H₂:
RELATION TO DEPOSITED FILM PURITY

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An analytical technique has been developed, based upon a Fourier transform mass spectrometer, to evaluate the purity of 1% SiH₄ and 1% SiCl₂H₂ in H₂. The gas is introduced into the mass spectrometer via clean stainless steel lines and a variable leak valve. Chemical ionization using the hydrogen in the sample produces simplified mass spectra. The impurities are identified by accurate mass measurement of the detected ions and knowledge of the chemistry involved to produce the ions. Impurities are quantified by the formation rate of their corresponding ions rather than by standard addition techniques. Concentrations of exotic or unstable (in pure form) components are determined. Total oxygen in the gases varies from 20 ppm in clean SiH₄ samples to 4400 ppm in dirty SiCl₂H₂ samples. These concentrations translate into 0.2% to 44% oxygen relative to silicon on a per atom basis. Likewise, carbon content varied from <1 ppm to 200 ppm which translates into <0.01% to 2% carbon relative to silicon. Table I summarizes the results for 10 gas samples analyzed. Oxygen contamination was primarily due to siloxanes in silane samples or chlorinated siloxanes in dichlorosilane samples. Carbon was present in a variety of forms, including hydrocarbons, methylsiloxanes, carbon monoxide and carbon dioxide.

The oxygen and carbon content of films deposited from these gases are evaluated by SIMS for comparison with the gas purities measured. The films were deposited by Rapid Thermal Chemical Vapor Deposition (RTCVD) at substrate temperatures of 550°C to 900°C. Incorporation of carbon and oxygen into the films varies from near stoichiometric at 550°C to weakly dependent or independent of gas composition at 900°C. Although the best quality films are grown at the highest temperatures, low temperature film growth is desirable for applications where minimal diffusion is required. It is clear that gas purity is an important parameter for obtaining consistent, high quality films at lower temperatures.

TABLE I

OXYGEN AND CARBON CONTENT OF
1% SiCl₂H₂ AND 1% SiH₄ IN H₂

Sample	Concentration Relative to Silicon (atomic ppm)		Sample	Concentration Relative to Silicon (atomic ppm)	
	Carbon	Oxygen		Carbon	Oxygen
SiH ₄	3,100	4100	SiCl ₂ H ₂	11,000	35,000
SiH ₄	70	1,700	SiCl ₂ H ₂	4100	15,000
SiH ₄	5,900	21,000	SiCl ₂ H ₂	17,000	300,000
SiH ₄	17,000	42,000	SiCl ₂ H ₂	15,000	440,000
SiH ₄	5,100	5,000	SiCl ₂ H ₂	4300	29,000

APPLYING THE CONCERTED ISOTOPE PATTERN - EXACT MASS METHOD OF ESTABLISHING ELEMENTAL COMPOSITION

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The purpose of this presentation is threefold:

- I. to characterize the useful limits of the CONCERTED method of analysis
- II. to develop routine instrumental strategies for acquiring the isotope data
- III. to present software for performing a CONCERTED analysis of exact mass and isotope pattern data.

I. Three factors dictate the useful limits of all mass spectrometric methods for determining elemental composition:

1. the number of different elements comprising the analyte
2. the number of occurrences of the elements (i.e., the size of the compound)
3. the accuracy and precision of the mass spectral data

Figs 1 and 2 show the number of matches found with an exact mass analysis and a CONCERTED analysis as the number of different elements is increased and as the size of the sample is increased (while the other factors are held "constant"). For those compositions containing > 6 elements or having M.W. > 800 amu the specificity of the exact mass analysis decreases rapidly and becomes problematic while that for the CONCERTED analysis is significantly better (improvements range between 10 and 30 fold). In even more difficult cases (> 7 elements or M.W. > 1600 amu) the specificity of the CONCERTED method also begins to deteriorate rapidly. Thus, the CONCERTED approach may be of greatest utility for the analysis of compounds containing 6 or 7 different elements and having M.W. between 800 and 1600 amu.

Fig. 1 Average Number of Matches vs Number of Elements for Compositions from $C_{20}H_{40}$ ($N_2 O_2 S_2 Si_2 F_2 Cl_2$)

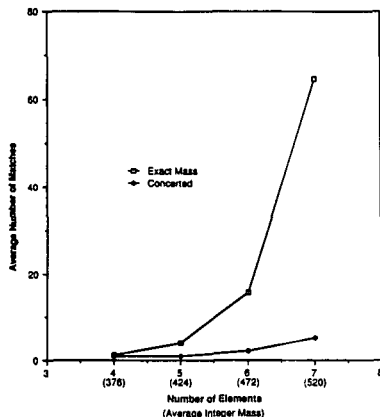


Fig. 2 Number of Matches vs n (Number of Occurrences) for $C_n H_1 S_n N_0.3n O_0.3n Si_0.1n$

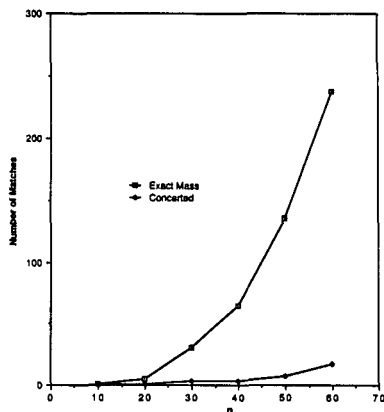


Fig 3 demonstrates how the number of matches found with a CONCERTED analysis depends on the precision of the isotope pattern moment data. Even poor isotope data can give significant improvements in specificity (a CONCERTED analysis using isotope data with a precision of $\pm 10\%$ produces 12 matches for the 7 element example while the exact mass analysis produces 65, see fig 1). The number of matches decreases monotonically with increasing precision. There are no clear breaks in the dependence; the selectivity continues to improve with increasing precision of the isotope data even at precisions of better than $\pm 1\%$.

Fig. 3 Average Number of Matches vs Deviation in M_2 for Compositions from $C_{20}H_{40}$ (N_2 O_2 S_2 Si_2 F_2 Cl_2)

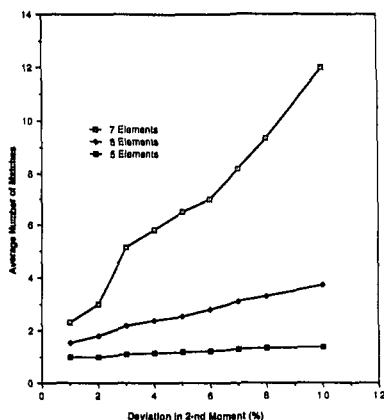
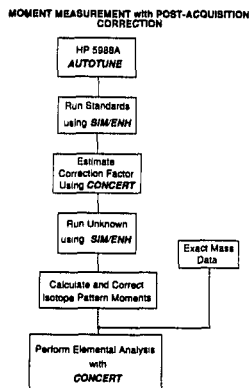


Fig. 4



II. The flow diagram in Fig 4 shows a routine procedure for obtaining second moment data with an HP 5988A GC/MS at a precision of about $\pm 3\%$ or better. This strategy uses AUTOTUNE, Selected Ion Monitoring (SIM), and a Post-Acquisition Correction of the data (to factor out systematic errors). The validity of the Post-Acquisition correction is demonstrated in Table I with data for four compounds with M.W.s of about 350 amu. With this particular instrument, the AUTOTUNE/SIM routine produces second moment data with an approximately constant absolute error, 0.027 ± 0.003 amu². We presume that the magnitude and nature of the systematic error will vary from one instrument to another; but, the relative ease, speed, and apparent accuracy of this strategy should make the initial effort of characterizing these systematic errors well worth while.

Table I

SYSTEMATIC ERRORS IN MEASURING MOMENTS

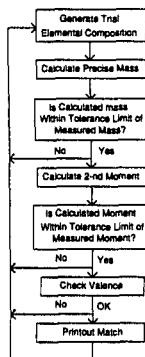
Composition	Mass	Second Moment ^a	Absolute Error ^b	Relative Error
$C_{21}H_{16}O_3$	316	0.2501	-0.022	-8.87%
$C_{18}H_{15}O_2S_3F$	378	0.7161	-0.027	-3.80%
$C_{18}H_{15}N_2O_4Cl$	358	0.9754	-0.031	-3.18%
$C_{18}H_{15}N_3OCl_2$	359	1.7198	-0.029	-1.69%

^a Average results for 2 or 3 analyses

^b Estimated error in M_2 $M_2(\text{measured}) - M_2(\text{calculated})$

Fig. 5

Flow Diagram for CONCERT Analysis



III. A program has been written in Microsoft BASIC for implementing the CONCERTED analysis once the exact mass and isotope data have been obtained. The program will run on any IBM compatible system, is menu driven, and provides the auxiliary support needed to analyze raw data. Fig 5 shows the basic outline of the CONCERTED analysis utilized by the program. The program may be obtained in both compiled and non-compiled forms on either 3.5" or 5.25" floppy disk by writing to the first author.

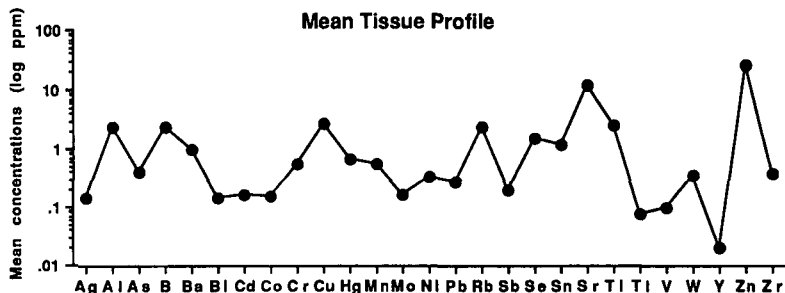
INORGANIC FINGERPRINT OF RAT TISSUES

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Most biochemical reactions enabling to maintain the integrity of living organisms require the presence of inorganic cofactors within a precise and narrow range of concentrations. Departing from those limits is often at the origin of pathological disorders. Surprisingly, in spite of the widely recognized biochemical importance of the inorganic cofactors and the availability of numerous independent data from very different techniques (e.g. Atomic Absorption and Neutron Nuclear Activation) in the literature, little is known on the "normal" inorganic content of living tissues down to the ppb level. This situation mainly results from the lack of a single analytical technique able to perform rapid and simultaneous determinations of the almost totality of the elements of the periodic table on the small quantities of tissue usually available in this particularly important domain of biomedical, toxicological and environmental research.

In this work, we profit of the quasi-simultaneous multielemental analysis capability of a new technique, the Inductively Coupled Plasma / Mass Spectrometer (ICP/MS), to establish the inorganic fingerprint of a series of ten major biological tissues (bone, brain, erythrocytes, heart, kidney cortex, kidney medulla, liver, lung, muscle, and plasma) from healthy male Sprague Dawley rats. Pieces of tissue (ca 250 mg) were digested in 1.50 ml of ultrapure nitric acid 8N inside a Parr microwave acid digestion bomb for 2 minutes at medium power in a standard microwave oven. Aliquots of solution were used for characterization and individual standard addition determination of elements. Rhodium (100 ppb) was added as internal standard. NBS 1577a bovine liver was used as reference. 43 elements were observed. Among this series, 27 elements in the ppb or low ppm range were determined, the others either are not currently measurable by ICP/MS (e.g. C, N, O, ...), or, due to their high concentrations, are more easily measured by atomic absorption spectroscopy (e.g. Ca, K, Mg, Na, ...).

Following discriminant analysis, principal component analysis applied on the previously defined correlation matrices was used to extract the existing relationships between the measured elements and the main discriminant sources of the different tissues. Finally, the "inorganic fingerprint" of each tissue was determined by reference to the average element profile of the studied tissues. Elements have been classified according to their normal deviate (Z) from the corresponding mean value of the tissues, as highly (+++/---, Z>6), moderately (++/--, 4<Z<6), and weakly (+/-, 2<Z<4) characterizing. A positive label expresses a concentration in excess to the mean element tissue profile while a negative label emphasizes a depletion.



Inorganic fingerprint of healthy male Sprague Dawley rat tissues

Bone			Kidney cortex			Lung		
	Average ppm	V. Test		Average ppm	V. Test		Average ppm	V. Test
Sr	120.788	+++	Cu	6.255	+++	Sn	0.295	---
Zn	133.012	+++	Cr	0.289	--	Al	1.035	--
Ba	4.829	+++	Sb	0.084	+	B	3.426	+
Co	1.230	+++	Al	3.545	+	Hg	0.210	-
Tl	8.404	+++	B	1.814	-	Zr	0.152	-
Ag	0.455	+++	Mo	0.292	+	Cu	1.017	-
Ni	0.877	+++	Ag	0.046	-	Se	1.044	-
Cr	1.043	++	Ni	0.148	-	Cr	0.373	-
Zr	0.791	++	Zr	0.226	-	Mn	0.161	-
Rb	0.586	-				Cd	0.071	-
Sn	1.803	++						
Cu	1.042	-						
Cd	0.288	+						
Brain			Kidney medulla			Muscle		
	Average ppm	V. Test		Average ppm	V. Test		Average ppm	V. Test
Bi	0.346	++	Tl	0.449	+++	Se	0.839	--
Sb	0.325	+	Hg	1.734	+++	V	0.044	-
Rb	0.993	-	W	0.645	++	Cd	0.057	-
Cd	0.271	+	Pb	0.586	++	Rb	3.800	+
			V	0.174	++	Mn	0.133	-
Erythrocytes			Rb	4.437	++	Ni	0.155	-
	Average ppm	V. Test	Mn	1.281	++			
			B	3.503	++			
As	10.333	+++	Sn	1.740	+	Liver		
Cr	0.890	++	Cd	0.288	+		Average ppm	V. Test
Al	1.778	-	Mo	0.301	+	Mo	0.518	+++
Mn	0.186	-	Bi	0.410	+	Mn	1.685	+++
Mo	0.052	-	Cu	4.310	+	Rb	3.832	+
			Cr	0.393	-	Ag	0.270	+
			Ni	0.522	+	Al	3.389	+
Heart			Plasma			V	0.064	-
	Average ppm	V. Test		Average ppm	V. Test	Sb	0.285	+
Se	2.853	+++				Tl	1.082	-
Sb	0.034	--	Rb	0.300	--			
W	0.182	--	B	1.402	--			
Cu	5.021	+	Mn	0.038	-			
V	0.147	+	Ni	0.126	-			
Cd	0.073	-	Cu	1.299	-			
Zr	0.230	-	Mo	0.042	-			

ELEMENTAL COMPOSITION OF MILK: FROM THE COW TO THE CONSUMER

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Milk is a known fundamental nutriment for mammals and, as such, for humans. It is a complexe mixture of elaborated organic and organometallic compounds. As such, its inorganic content represents a dietary supply of elements of prime importance in maintaining the integrity of life of superior organisms. However, although numerous studies on the most classical elements (Ca, K, Mg, Na, P, . . .) are available in the literature, there is very little, if any, compositional information on the totality of the inorganic elements contained in milk that has been carried out systematically on a single and modern analytical technique.

In this work, we took advantage of the quasi-simultaneous multielemental analysis and high sensitivity capabilities of a new technique, the Inductively Coupled Plasma / Mass Spectrometry (ICP/MS) to establish the complete inorganic content of milk along the different steps of its packaging from the cow's milk to the consumer's bottle.

In its standard configuration ICP/MS requires introduction of liquid samples through a nebulizer inlet system. To meet this requirement, an efficient methodology, based on the use of Parr microwave acid digestion bomb, has been optimized for direct and complete digestion of milk samples. The general conditions used are: 1 ml of milk sample + 1.25 ml HNO₃ + 0.25 ml H₂O₂ heated for 2 minutes in a standard microwave oven at medium power and allowed to cool for 15 minutes at room temperature.

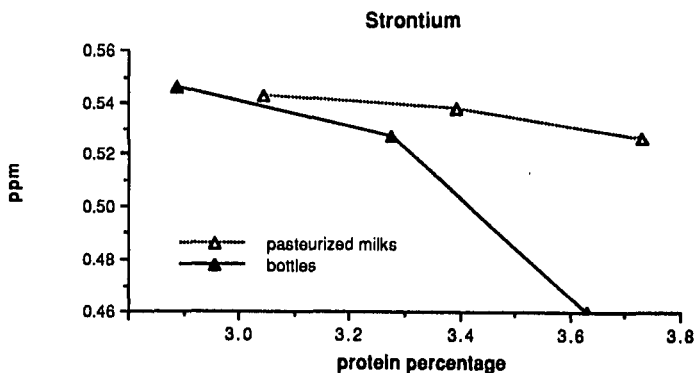
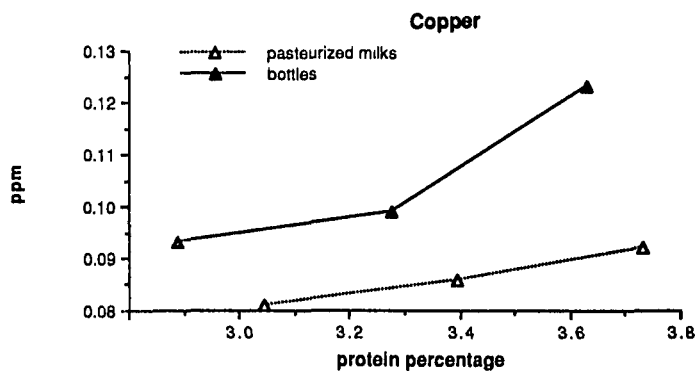
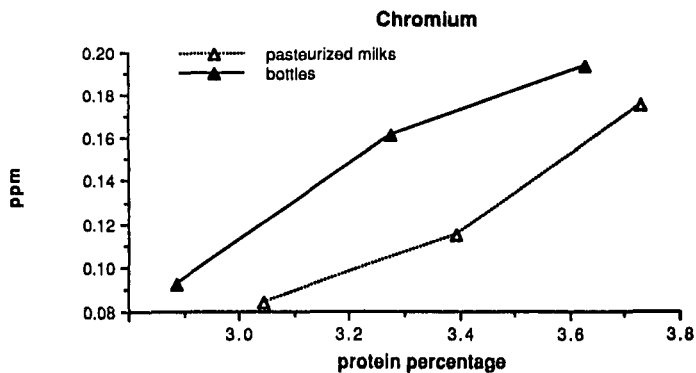
Besides the usual C, H, O, N and P elements, preliminary screening down to the ppb level allowed characterization of thirty (30) additional elements (Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sn, Sr, Ti, V, W, Zr and Zn) in each of the individual state of milk packaging from the cow's milk to the table of the consumer, including the various usual presentations: homogenized (HOMO), partly skimmed (2%), skimmed milks and pure cream. Due to the presence of interfering peaks (Fe, K) or because of their high concentrations (Ca, Mg and Na) some elements were determined by atomic absorption spectroscopy.

Characteristic relationships between specific elements of physiological importance and protein and/or fat content were found. This is especially the case for chromium and copper, which concentrations are proportional to the protein content, while Sr is inversely proportional to the same reference. This observation holds true when analysing cream where specific accumulation (e.g. Al, Cr, Cu, Mo) or depletion (e.g. Rb, Sr, Ti, W) of elements can be observed. This lets open the question of possible physiological nutritive consequences resulting from social habits when drinking a particular milk packaging.

Acknowledgements:

This work was supported by the Dairy Bureau of Canada.

Relationships between selected elements of possible physiological importance and protein content



THE DETERMINATION OF LOW-ABUNDANCE OXYGEN
IN FLUORINE GAS MIXTURES

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Oxygen is a problematic and ubiquitous impurity in commercially available fluorine, ranging from 0.2% to more than 2% abundance relative to fluorine. The oxygen content of fluorine gas mixtures used in chemical laser systems is a critical parameter. Oxygen is often added to the gas mixture to inhibit premature $H_2 - F_2$ reaction by breaking free-radical chain reactions; on the other hand, too much oxygen limits laser output. The purpose of this work is to develop an automated mass spectrometric methodology for real-time quantitative analysis of oxygen in fluorine gas mixtures at sample pressures from 10 psia to 60 psia. Optical or chromatographic methods do not provide reliable analytical results in this application.

A UTI quadrupole mass spectrometer and Atmospheric Pressure Sampling Module were controlled by a Hewlett-Packard data system under IEEE-488 protocol. All sampling and pump-out valves were controlled with this data system via a HP 3488A Switch/Control Unit so that gas sampling, analysis, and data reduction were fully automated. Calibration curves for oxygen content were generated with oxygen/argon mixtures that were standardized by gas chromatography and magnetic sector mass spectrometry. The oxygen content in the fluorine sample mixtures was determined by comparison of the instrument response to oxygen against the working calibration curve.

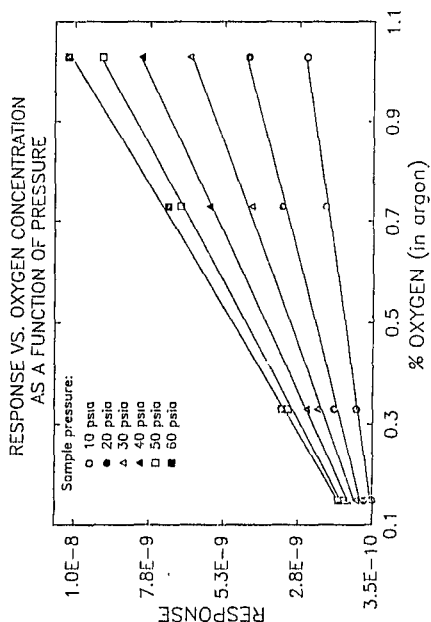
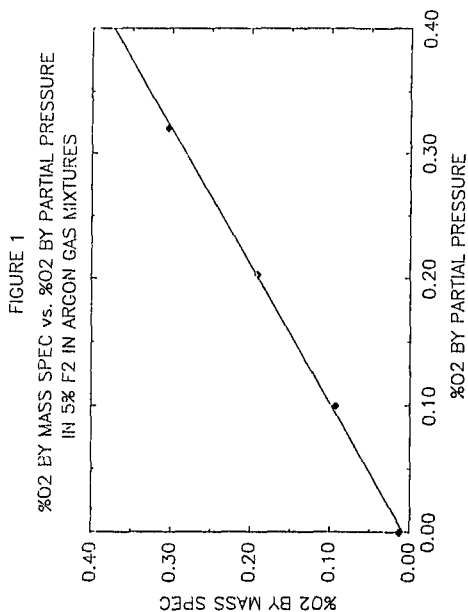
The validity of the non-matrix matched standards ($O_2/Ar/no F_2$) was evaluated by preparing four fluorine gas mixtures. Each mixture contained 5% F_2 . One cylinder had no added oxygen, the remaining cylinders contained 0.1%, 0.2%, and 0.32% added oxygen. The exact final O_2 concentration in these mixes is not known because the initial amount of O_2 present as an impurity in the fluorine is not known. However, the percentage of oxygen added to each cylinder is known by measurement of partial pressures. If the use of fluorine-free standards is valid, a plot of % O_2 by mass spectrometry versus % O_2 by partial pressures should yield a regression line with near unity slope and a non-zero intercept (as a result of the residual oxygen in the cylinder with no added oxygen). This is shown in Figure 1.

In most cases, calibrations were made at the same sample pressure as the analysis. Instrument response was corrected for pressure when this was not possible. However, the response was not a fixed function of pressure. At low sample pressures (10 psia - 30 psia), the response could be corrected by linear ratio of standard pressure to sample pressure. At higher pressures (30 psia - 70 psia) the instrument response followed a 0.7th root dependence on the pressure ratio (Figure 2). This results from gas flow into the ion source changing from molecular to viscous regimes as sample pressure increases. The results of multiple independent analyses are given in Table 1. In general the precision of the method is ± 5 rsd for oxygen levels greater than 0.1%. The precision degrades as fluorine concentration increases. Trace levels of oxygen can be quantified to less than 100 parts-per-million.

TABLE 1. OXYGEN ASSAY OF FLUORINE/ARGON MIXTURES

(Results reported as %O₂)

0.2% added O ₂ /5% F ₂	No added O ₂ /5% F ₂	No added O ₂ /20% F ₂
0.194	0.010	0.024
0.193	0.008	0.023
0.193	0.007	0.021
0.195	0.007	0.021
0.201	0.007	0.016
0.192		0.018
0.191		0.018
0.204		0.020
0.178		0.020
0.189		
$\bar{x}$ = 0.193	$\bar{x}$ = 0.008	$\bar{x}$ = 0.020
s = 0.007	s = 0.001	s = 0.002
RSD = 3.6%	RSD = 17%	RSD = 12%



MATRIX EFFECTS ON THE FAST ATOM BOMBARDMENT MASS SPECTRA OF PALLADIUM COMPLEXES

L. M. Mallis¹ and W. J. Scott

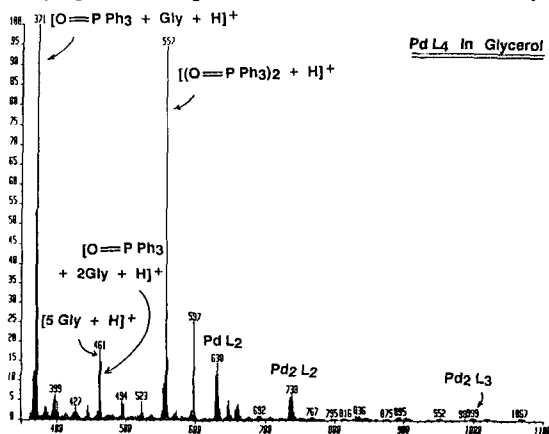
¹High Resolution Mass Spectrometry Facility
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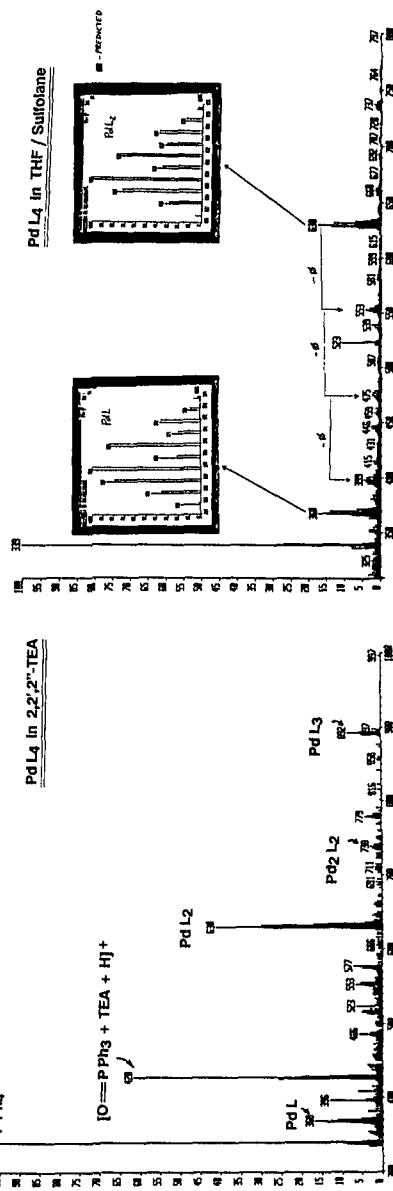
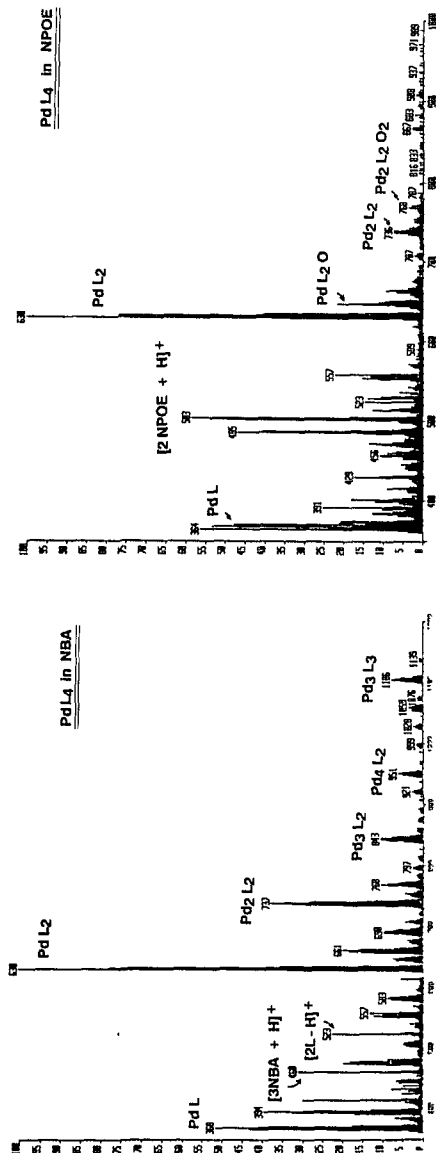
The use of palladium to catalyze the coupling of nucleophiles with electrophiles has become an increasingly important method for the formation of carbon-carbon bonds. The reaction is believed to proceed by an oxidative addition/ transmetalation/ reductive elimination scheme. While each step of the proposed catalytic cycle has precedent for the Group 10 metals, only the product of the oxidative addition step has been demonstrated to be formed under coupling conditions.

Fast atom bombardment mass spectrometry (FAB-MS) has been used to identify transient species in organic reactions. We are developing an understanding of the fragmentation characteristics of typical palladium complexes under FAB-MS conditions in order to apply this to a study of transient species which might be detected during coupling reactions.

In this paper, a study to determine the effects of matrix selection on the observed FAB spectrum is presented. These studies were performed using a VG ZAB-HF mass spectrometer equipped with an ION TECH saddle field atom gun, and an 11 250-J data system. It is important that the FAB matrix provide for an excellent ion yield while remaining inert to the coupling reaction being studied. It is interesting to note that use of a protic matrix causes the degradation of the palladium catalyst to form $Pd_xL_y^+$ ($x = 2-4$, $y = 0-3$) clusters which represents the degradation of the palladium catalyst. For example when PdL_4 is analyzed using glycerol, nitrobenzyl alcohol (NBA) or nitrophenyloctylether (NPOE), ions corresponding to $Pd_xL_yO_z^+$ ($x = 2-4$; $y = 1-3$; $z = 0-2$) are observed (see Figures 1, 2, and 3, respectively). In addition, clusters of the oxidized ligands (and complexed to the FAB matrix; see Glycerol spectrum) are observed suggesting that these more protic solvents are not reasonable choices for analyzing the catalytic process.

A spectrum of PdL_4 in triethanolamine is shown in Figure 4. Although an ion corresponding to the oxidized ligand is clearly observed, the fragment ions arising from the palladium catalyst are relatively clean of oxidized products. Recently, sulfolane has been used as a matrix for the analysis of organometallic compounds. A spectrum of PdL_4 in sulfolane is shown in Figure 5. It is interesting to note that the fragment ions present correspond unambiguously to the structure of the palladium catalyst. Investigation into the intermediates involved in the palladium catalyzed coupling reaction using sulfolane as the FAB matrix are currently underway.





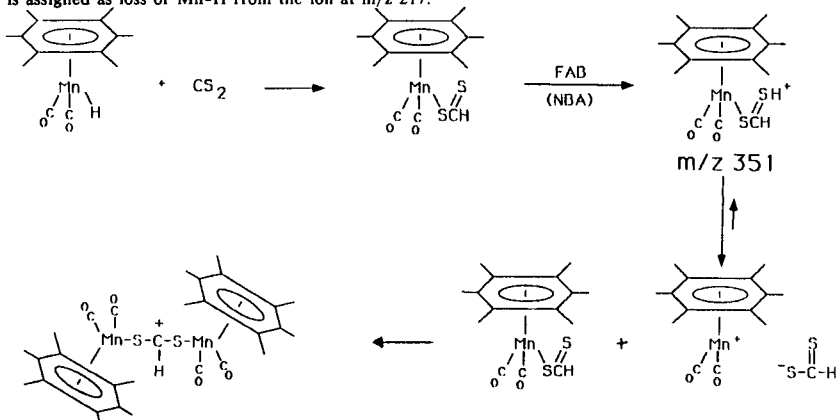
FAST ATOM BOMBARDMENT ANALYSIS OF SPECIES FORMED BY
THE INSERTION OF CARBON DISULFIDE INTO Mn-H BONDS

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Carbon disulfide is known to insert into metal hydride bonds.^{2,3} This paper reports the use of fast atom bombardment mass spectrometry (FAB-MS) to monitor the decomposition of an organomanganese compound which can be formed by two synthetic routes. $\eta^6\text{-(C}_6\text{Me}_6\text{)Mn(CO)}_2\text{H}$ will react with CS_2 in THF solvent to produce $\eta^6\text{-(C}_6\text{Me}_6\text{)Mn(CO)}_2\text{S}_2\text{CH}$ (**1**) by insertion of CS_2 into the Mn-H bond.⁴ In addition, the dithiormate may be synthesized by reacting $\eta^6\text{-(C}_6\text{Me}_6\text{)Mn(CO)}_3^+ \text{PF}_6^-$ with KS_2CH and Me_3NO in THF.⁵

In the FAB mass spectrum of **1**, an ion corresponding to the protonated molecular ions is observed at m/z 351 (see Figure A). Several structurally significant fragment ions are also observed in this spectrum. The base peak in this spectrum at m/z 294 is assigned as loss of 2 CO groups from the neutral molecule ($[\text{M} - 2 \text{CO}]^+$). The ion at m/z 273 corresponds to loss of the dithiormate group (S_2CH) while the ion at m/z 217 is assigned as loss of both CO groups and the dithiormate group (hexamethylbenzene manganese). It is interesting to note that the ion at m/z 161 is assigned as loss of Mn-H from the ion at m/z 217.



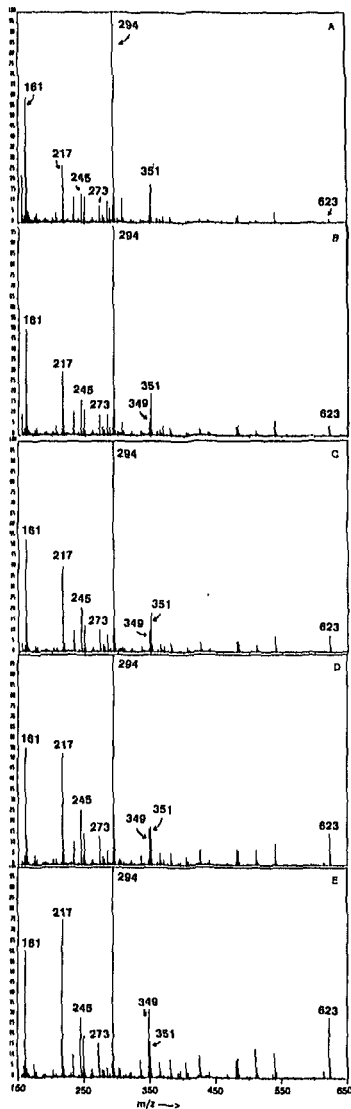
An aged sample of **1** also produces ions as described above. In addition, an ion assigned as a bimetallic species derived from **1** is observed. Repurification of **1**, followed by immediate FAB, analysis, showed a marked decrease in the ion corresponding to the bimetallic species and a corresponding increase in the protonated molecular ion. After one hour, FAB analysis established that the ion arising from the bimetallic species had again increased, demonstrating that the bimetallic species is being formed by a reaction of **1**, in the solid phase. This observation is enhanced when the bimetallic species is dissolved in THF (or the FAB matrix; i.e., nitrobenzyl alcohol) and heated for an extended period of time (30 - 60 minutes) under FAB ionization conditions. Figures A through E contain the FAB mass spectra for **1** dissolved in THF/NBA and bombarded for up to 60 minutes. By plotting the percent total ion current against the time of spectral acquisition, the relationship between the ions at m/z 623 and m/z 349 have been established. A proposed mechanism for the formation of m/z 623 and the fragment at m/z 349 are presented in Figures 1 and 3, respectively. Further studies to elucidate the exact structure of the ion at m/z 623, using FT-IR and MS/MS experiments, are currently underway.

2) Butler, I. S. and Fenster, A. E. *J. Organometal. Chem.* 1974, 66, 161.

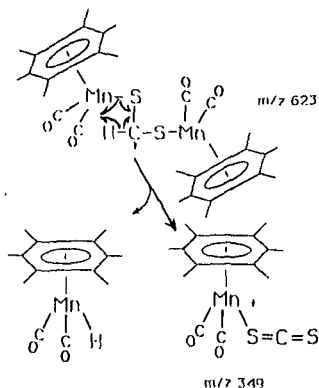
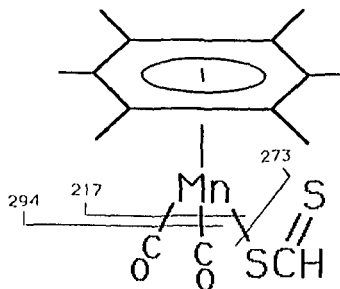
3) Yaneff, P. V. *Coord. Chem. Rev.* 1977, 23, 183.

4) Bernhardt, R. J., Doctoral Thesis, 1984, Department of Chemistry, University of Iowa, p. 129.

5) Wilmoth, M. A., Doctoral Thesis, 1985, Department of Chemistry, University of Iowa, p. 200.

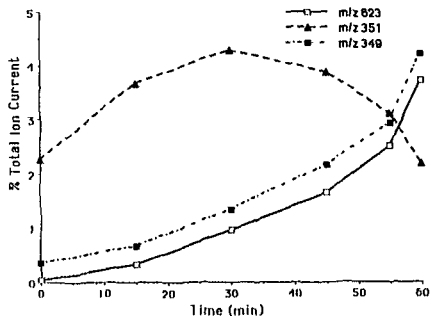


Figures A through E were acquired using the same sample loading for the solid sample mixed directly with the FAB matrix. The times associated with the acquisition of these spectra are as follows: A-15 min; B-30 min; C-45 min; D-55 min; E-60 min. Note the increase in production of the m/z 623 metallic dimer and the corresponding fragment ion at m/z 349.



Reaction for formation of m/z 349 fragment ion via a proposed four-electron homolytic bond cleavage reaction from the dimer observed at m/z 623.

Plot of % TIC vs. Time of Thermal Mixing Under FAB Conditions for a Solid Sample



EVALUATION OF A DOUBLE-QUADRUPOLE SPECTROMETER SYSTEM
FOR GLOW DISCHARGE MASS SPECTROMETRY

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As the technique of glow discharge mass spectrometry evolves into a more widely applied and accepted method of direct solids elemental analysis, two fundamental obstacles remain for the practitioner: 1) the requirement that the sample be conductive in nature and 2) the presence of isobaric interferences, specifically those related to molecular species. While nonconducting powders may be mixed intimately with a metal powder and a suitable sample disk press-formed, not all insulators occur in powder form. This problem has been addressed in our laboratory by the development of a radio frequency (rf) powered glow discharge device which allows the direct analysis of nonconductors without matrix modification. The desire to remove molecular isobars would suggest a couple of remedial measures. Double-sector instruments are useful in resolving atomic isobars, but many molecular interferences require resolving powers of $>10,000$. The second apparent option is the use of collision-induced dissociation (CID) schemes to remove molecular species. We describe here the use of a relatively simple double-quadrupole spectrometer for the removal of molecular species from rf-glow discharge mass spectra through collision-induced dissociation.

EXPERIMENTAL

Figure 1 is a diagrammatic representation of the double-quadrupole instrument employed in this laboratory. Ions exiting the ~ 0.3 torr ion source region pass through a differential pumping stage and into the analyzer region. These ions are focussed into the collision cell by a three-element lens system. The collision cell and mass analyzer are equipped with Extrel 3/8" quadrupole assemblies powered by 1.2 MHz generators. Argon is employed as the collision gas with its pressure monitored through the analyzer region ion gauge. Ions traversing the rf-only quadrupole are directed into the analyzer by a single-element lens. All of the spectrometer scan functions, ion lens/pole offset potentials, and ion detection were controlled by an Extrel C-50 mainframe. Due to the variations of mass throughput characteristics of the double-quadrupole arrangement, which are a function of the relative quadrupole d.c. offsets, spectra were taken at fixed collision cell offset potentials with the cell evacuated and then pressurized to assess the extent of molecular species removal. A rf-powered glow discharge source was employed in these studies with typical operating pressures of ~ 0.3 torr and powers of ~ 10 watts.

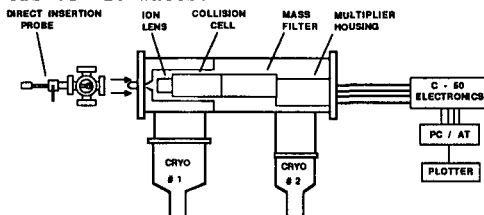
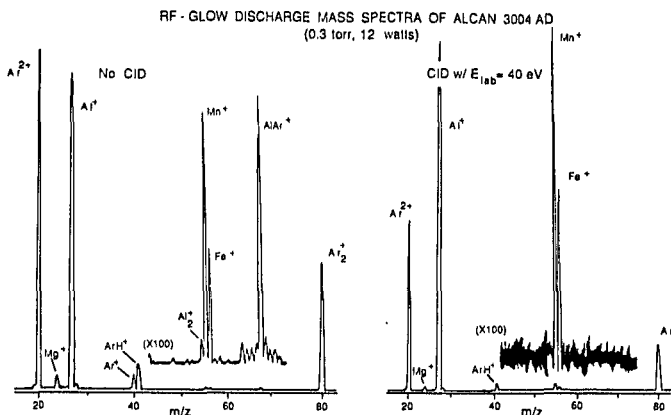


Figure 1. Double-quadrupole glow discharge mass spectrometer system.

RESULTS AND DISCUSSION

The first parameter investigated in the double-quadrupole CID-GDMS studies was the effect of collision cell pressure on the degree of dissociation of molecular species produced when sputtering a copper cathode. It was found that cell pressures corresponding to 4×10^{-4} in the analyzer chamber were the best compromise between dissociation and losses of analyte due to scattering. Studies were performed at collision energies (E_{lab}) of 20 and 40 eV. These values correspond to center-of-mass energies (based on a copper dimer species) of 4.8 and 9.6 eV. While both of these values should be sufficient to dissociate most of the molecular species, the latter proved to be more efficient. At this energy, each of the studied interferents (Ar^{2+} , $CuAr^+$, and Cu_2^+) were reduced to levels indistinguishable from background noise.

Shown below are a pair of mass spectra obtained from an Alcan 3004 AD aluminum alloy. In the "no CID" spectrum, the dimer of Al is seen at mass 54 as an isobar to the minor Fe isotope, with the aluminum argide ($AlAr^+$) present at mass 67. Employment of collision-induced dissociation to this sample eliminates both the dimer and argide molecular species. It should be pointed out that, while the CID does simplify the observed spectrum, the loss of the Al matrix ion signal is on the order of 80%. The actual noise level of the detector does not change when the cell is pressurized, the primary noise source seems to be from photons emitted from the plasma.



Studies performed on this system indicate that the relatively simple, double-quadrupole spectrometer geometry is a viable means of reducing the contribution of molecular species to glow discharge mass spectra. Future studies in CID-GDMS will focus on the reduction of the photon noise so that a dynamic range suitable for routine analyses can be achieved. Modifications of the interquadrupole lens will be made such that more uniform mass transport characteristics can be achieved. This is necessary in order to study the degree of molecular species dissociation as a function of collision energy.

The Relative Role of Penning Ionization in Glow Discharge Devices

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The use of glow discharges as ion sources for elemental mass spectrometry is a rapidly expanding field (1). In order to develop adequately glow discharge mass spectrometric methodologies, the fundamental mechanisms responsible for the creation of analytical ions is of interest. Two major mechanisms are believed to be responsible for the ionization of sputtered atoms, electron impact ionization and Penning ionization. Electron impact results from an energy transfer from an energetic electron, while Penning ionization results from a potential energy transfer from an excited metastable state of the discharge gas.

Electron impact ionization: $M^0 + e^- \rightarrow M^+ + 2e^-$

Penning ionization: $Ar^* + M^0 \rightarrow M^+ + Ar^0 + e^-$

In order to elucidate the ionization mechanisms present in the glow discharge, experiments were designed to quench the metastable levels of the rare gas and measure the resulting decrease in ion emission from a sputtered copper population (2). A glow discharge was operated in a 2.75 inch six-way cross at pressures of 0.3 - 1.2 torr, 0 - 4 mA, and 500 - 3500 volts. The cathode consisted of a 1.5 mm diameter brass pin with 5.0 mm exposed to the discharge. The optical system employed for atomic absorption and emission experiments consisted of an Instruments SA 0.32 m monochromator with a Hamamatsu 752 photomultiplier tube. A Pacific 124 photometer provided high voltage to the PMT and served as the readout device for atomic emission. For atomic absorption measurements, output from a pulsed hollow cathode lamp traversed the negative glow region of the discharge and the signal was monitored by a EGG PAR 5209 lock-in amplifier.

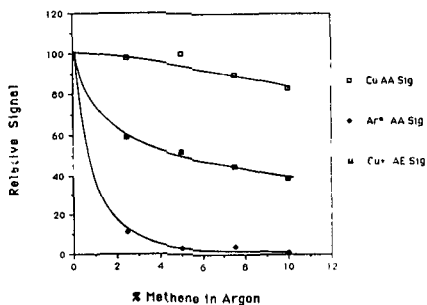


Figure 1

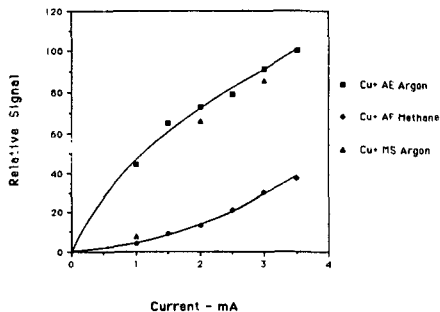
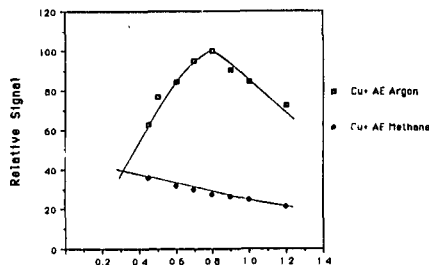


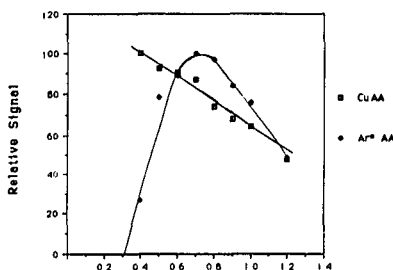
Figure 2

Methane was employed as the metastable quenching agent. As illustrated in Figure 1, low levels of methane added to an argon glow discharge will result in the removal of the Ar^* states without significantly impacting the ground state copper population. Under these conditions, the Penning ionization process should be removed, allowing ionization only through electron impact. Determination of the reduction of ion signal with methane relative to the signal present with pure argon should provide a measure of the relative role of Penning ionization in the glow discharge.

Figure 2 illustrates the behavior of copper ion signal as a function of discharge current for both pure argon and a discharge operating with 10% methane in argon mixture. The signal generated with the addition of methane shows the decrease due to the quenching of the argon metastable states. When the signals are corrected for the lower ground state copper population present with methane, a relative value of Penning ionization vs. current can be calculated. Penning ionization is observed to decrease linearly with increasing current, falling from 78% at 0.5 mA to 42% at 3.5 mA. Figure 3 exhibits the effect of pressure on the copper ion signal for both pure argon and the methane/argon mixture. The role of Penning ionization is clear in this figure, with the ion signal profile in argon peaking at 0.8 torr, matching the behavior of the Ar^* population, illustrated in Figure 4. With methane present in the discharge, the ion signal is reduced, and the profile matches that of the copper ground state, Figure 4. Again the relative role of Penning ionization vs. pressure may be calculated and it is found to rise from 0 at 0.3 torr to 60% at 0.8 torr, then level off up to 1.2 torr, the maximum pressure investigated.



Pressure - Torr
Figure 3



Pressure - Torr
Figure 4

References:

1. Bentz, B.L.; Harrison, W.W. *Prog. Anal. Spectrosc.* 1988, 11, 53.
2. Smith, R.L.; Serxner, D.; Hess, K.R. *Anal. Chem.*, in press.

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SEPARATION OF ATOMIZATION AND IONIZATION PROCESSES IN GDMS: C.M. Barshick and W.W. Harrison, University of Florida, Gainesville, FL. 32611. USA.

INTRODUCTION

Glow discharge mass spectrometry relies on a population of positive ions representative of the elemental composition of the sample cathode. While the intrinsic two-step sputter/plasma process offers the advantage of needing no secondary source of atomization or ionization, studying one process separate from the other is difficult, if not impossible. Limited success has been achieved in prior studies by focusing on correlations between discharge parameters (pressure, voltage, current, etc.) and ion signal. An alternative approach is to use one technique to generate a source of primary atoms in conjunction with a complementary glow discharge acting as the ionization source, incident energy and source pressures approximating the conditions present in the glow discharge.

EXPERIMENTAL

As the atom generator we have investigated two sources: (1) an excimer pumped dye laser and (2) a fast-atom source. Figure 1 illustrates the quadrupole mass analyzer and the position of the sample target relative to the laser and FAB beams. The dye laser output consisted of 10 nsec pulses in the 0.1 - 1.0 mJ range at 100 Hz. By focusing the beam to spot diameters of 100 micrometers, power levels of $10^8 - 10^9$ W/cm² were obtainable. Rhodamine 590 laser dye was used in all the studies at the wavelength of maximum power (583.0 nm). At these power levels there is essentially no wavelength dependence on the ablation process. An Ion Tech saddle field gun was used as the fast-atom source. The beam consisted of ca. 4 - 8 keV fast argon atoms at a current density of 1 - 10 μ A/cm². Machined conical pins (5.0 mm at the base and cut at a 45 degree angle)

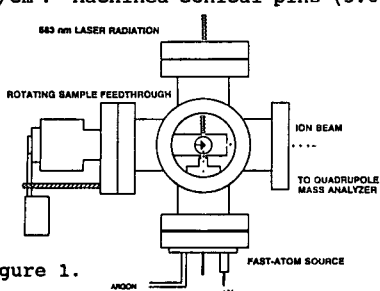


Figure 1.

and pressed disks (5.0 mm in diameter) were position on the end of a rotating sample holder 5 mm from the ion exit orifice. The samples were rotated at a constant 2 rpm. The laser and fast-atom beams were directed from a region of high vacuum (ca. $1 \times 10^{-7} - 1 \times 10^{-4}$ Torr) to a region of low vacuum (ca. 0.1 - 1.0 Torr) through a 0.5 mm aperture. In order for the fast-atom beam to impact the target it must traverse a distance of 1 cm through the high pressure region. The mean-free path is 0.5 cm through 1.0 Torr of rare gas at 8 keV. An auxiliary discharge was formed about a 1.0 mm diameter tungsten electrode positioned 180 degrees off the auxiliary atomizer axis, directly behind, and slightly above the target. The ions formed at the surface of the target and/or in the discharge plasma were extracted through a 1.0 mm orifice and focused into a quadrupole mass spectrometer.

RESULTS AND DISCUSSION

High vacuum sputter/ablation. The initial series of studies was performed under best vacuum conditions to determine the effect of laser pulse energy and FAB beam energy on typical metal/alloy materials; collision and redeposition processes are minimized in this way. Vacuum sputter/ablation studies were conducted at 2×10^{-7} Torr as measured in the analyzer chamber. ⁶³Cu ion signal was measured as a function of laser pulse energy and FAB beam energy. Laser pulse energy was varied from 0.05 - 1.0 mJ/pulse at a constant repetition rate of 100 Hz, and FAB beam energy from 4 - 8 keV. Ion signals showed linear increases as both pulse energy and beam energy were increased. Present orientation of the sample probe with the exit orifice limits sensitivity somewhat. Absolute copper ion signal ranged from ca. 2000 - 12000 counts/sec for laser ablation and ca. 50 - 500 counts/sec for fast-atom bombardment. These studies showed us that our experimental conditions permitted work in a linear response region of the sputter/ablation process.

Buffer gas. Introduction of a buffer gas into the sputter/ablation chamber produces collisions with the sputter-ablated atoms causing a fraction to be redeposited, analogous to the redeposition effects found in GDMS. With the controlled sputter/ablation studies performed here, the effect of increasing pressure is more clearly seen than in the glow discharge, where changing pressure also changes ion energy. Ion signal was monitored for the pressure range 0.0 - 1.0 Torr at (1) a constant laser pulse energy of 1.0 mJ/pulse at 100 Hz, and (2) a constant FAB beam energy of 8 keV. Figure 2A and 2B are plots of ^{63}Cu ion signal vs. pressure for the laser and fast-atom sources, respectively. As the pressure is initially increased, the ambient gas acts to contain the sputter/ablated atoms longer and to increase the probability for ionizing collisions. At ca. 0.4 Torr, the competing processes of containment redeposition, and ion neutralization through recombination create a maximum ion signal. As the pressure is increased above 0.4 Torr redeposition and recombination quickly dominate, as is evidenced by the sharp drop in ion signal.

LASER ABLATED ION SIGNAL VS. PRESSURE

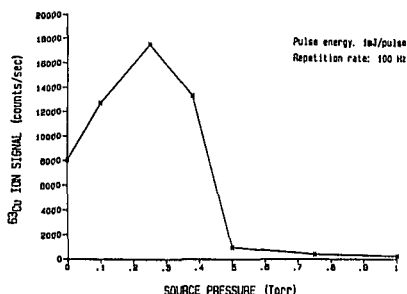


Figure 2A.

FAST-ATOM ABLATED ION SIGNAL VS. PRESSURE

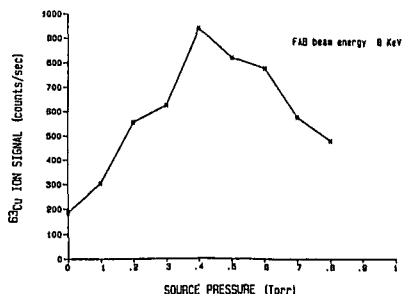


Figure 2B.

Sputter/ablation into a secondary discharge. The use of a secondary source of atomization in combination with an auxiliary glow discharge affords the opportunity to study two problems inherent to the discharge: (1) the inability to separate the sputter-atomization process from the ionization process, and (2) the inability to analyze nonconducting samples directly, since cathodic-samples must be conducting. The efficiency of a glow discharge in ionizing the ablated atoms should be dependent on the discharge current. Changes in the current at constant pressure should only affect the discharge and not the auxiliary atomization process. A plot of ion signal vs. discharge current for the laser ablated/secondary discharge system shows a sharp increase in ion signal up to ca. 4.0 mA, and then a steady decrease above this value. The changes in the observed ion signals are attributed to differences in the ionization efficiency. Higher currents and the corresponding higher electron densities would be expected to increase the ionization rate of the laser generated material. This is possibly the effect observed as the current is increased up to 4 mA. Above 4 mA there appears to be some competing processes that reduce the collection and/or ionization efficiencies. However, the discharge plasma changes in configuration with increasing current, and this could affect the extraction efficiencies also. Alteration in argon metastable population may also be created affecting ionization.

Nonconducting sample analysis. With the atomization/auxiliary discharge employed in these studies, a nonconducting sample can be analyzed directly without the need for a conducting matrix. This eliminates problems in finding a suitable contaminant free matrix. Preliminary results show a 10 fold increase in ion signal between the laser/auxiliary discharge system and the laser alone, indicating a potential for the direct analysis of nonconducting samples. The present electrode configurations are not optimized for maximum ion yield, so further increases in enhancement are anticipated.

STUDIES OF THIN FILMS IN GLOW DISCHARGE MASS SPECTROMETRY

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INTRODUCTION

The study of thin films includes the analysis of semi-conductors, micro-electronics, and other specialized systems. While some X-ray and electron spectroscopic techniques offer the advantage of non-destructive analysis, the sample is determined indirectly by detection of secondary electrons or X-rays generated from the sample. A second method used is that in which ions coming directly from the surface of the sample are detected. These methods use techniques in which atoms are sputtered from the surface of the sample, ionized and then detected. Falling in this class is glow discharge mass spectrometry (GDMS), which is the method of choice for these experiments. The disadvantage of this technique is that the sample is destroyed in the course of the analysis. An advantage is that actual atoms coming off from the surface are detected directly.

EXPERIMENTAL

GDMS has found use primarily in the analysis of bulk metal samples in the form of machined pins or pressed disks. A problem in looking at thin metallic films was the unavailability of a suitable sample holder. The plasma will sputter any portion of the sample exposed to the impinging gas atoms. When analyzing thin films it is desirable to sputter only the top of the sample because sputtering the edges will produce an ion signal from the lower layers in the sample. To solve this problem a sample holder was designed that would

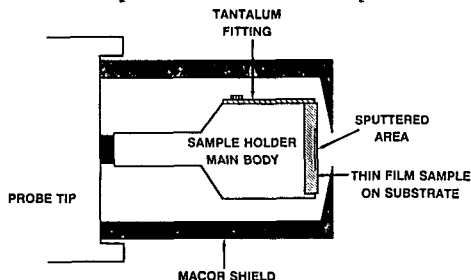


Figure 1.

shield the edges of the sample in the dark space of the plasma, not allowing bombardment by the gas discharge atoms. The holder was designed to attach the sample and its substrate onto the end of a direct insertion probe. Samples of approximately 5 mm square were held in place by a flexible tantalum fitting. The sample and holder were covered with a cylindrical Macor shield with a 4 mm hole allowing the discharge to sputter a circular area on top of the sample. A cross-sectional illustration of the sample holder with sample in place is shown in Figure 1.

The films analyzed were deposited on a variety of substrates including: silicon, sapphire, Kapton (a polyimide film), and gallium arsenide. Samples were in the thickness range of 500-10,000 angstroms, consisting of single element/single layer, multi-element/single layer, single element/multi-layer, and the matrix film. The metals analyzed were aluminum, copper, gold, and the alloy Cu₃Au. The analyses were carried out by monitoring the sputter profiles acquired as the metal was sputtered away from the surface. When looking at layers of 1000 angstroms or less the samples were studied using both argon and helium to compare the speed and results of the two different methods.

HELIUM DISCHARGE EXPERIMENTS

A second problem in GDMS is the fast sputter rate in an argon glow discharge. This problem became significant when looking at layers less than 1000 angstroms. The sputter times for layers of this thickness were on the order of 30 seconds or less. With large sputter rates, sputter profiles of very thin layers may provide poor results. If a layer is sputtered away in a short time period, any differences in the time from study to study will have a large overall error. This problem was solved by the use of an alternative discharge gas. The sputter rates of discharge gases vary due to a variety of parameters. The most predominant is the size of the gas atoms compared to the

sample atoms. Thus a larger gas atom will sputter a sample faster than a smaller gas atom. Helium was chosen because of its low cost, high purity, and availability. When using a helium discharge, the sputter rate is on the order of seven to eight times slower, thus the sputter profile will be spread out and the sputter time of a layer may be obtained more precisely. The plasma shape is unusual using helium because a sharp beam-like plasma was observed coming through the Macor hole. The size of the sampled area is the same, but the discharge seems to be concentrated at the center indicated by a deeper crater. A 1,000 angstrom Au/GaAs sample was analyzed using both argon and helium. Figure 2 shows the comparative sputter profiles for gold in both gases.

ANALYSIS OF MATRIX MATERIALS

Experiments to determine matrix composition were done for two reasons: (1) to see if there was anything present that might interfere with the analysis of the films and (2) to examine an organic polymer. Since sputter ablation of most films occurred rapidly, a complete mass spectrum of the elemental layers was not obtained. Any matrix interferences present could alter the analysis of the films since only one peak was monitored. If an interferant was present at a mass under investigation, the ion signal observed would not reflect the true value. The Kapton was considered non-conducting, so no discharge was expected to form about this material. When the discharge was formed with the samples on Kapton, it was thought that the metal was conducting enough current to cause formation of the plasma. After the metal was sputtered away the discharge remained, so redeposited metal may have caused the plasma to be maintained. In a later experiment the pure polymer was examined and found to sputter on its own.

RESULTS AND DISCUSSION

The sputter rate of the alloy Cu_3Au was found to be in the range of 35-40 angstroms per second monitoring the component metals, while the sputter rates of the pure metals are higher, copper being approximately 42 angstroms/second and gold about 63 angstroms/second. Comparable samples of aluminum had a rate of approximately 9.8 angstroms/second. Since the hole size in the Macor shield gets subsequently larger with each run the data were normalized by calculating the total volume sputtered in a given time. The calculated sputter rates for all of the thin films under investigation are shown in Table 1.

CONCLUSIONS:

1) A technique was developed for thin film analysis of a relatively small area of 10 mm^2 or less. 2) Small portions of a sample under investigation will be destroyed for each analysis. 3) Sputter rates on the order of 50-100 angstroms/second will not allow accurate analysis of samples less than 500-1,000 angstroms. 4) Alternative discharge gases such as helium may be used to slow the sputter rate for the study of thinner films.

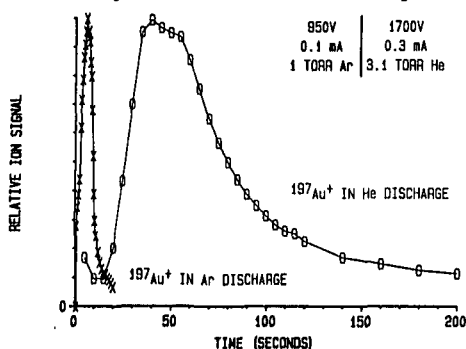


Figure 2.

TABLE 1: SPUTTER RATES FOR VARIOUS METALS

GAS	SAMPLE	ION	SPUTTER RATE (micrometer/second)
Argon	Al/Si	Al ⁺	8.7×10^{-1}
Argon	Au/GaAs	Au ⁺	7.0×10^{-4}
Argon	Au/Cu/Au/Si	Au ⁺	6.0×10^{-4}
Argon	Au/Cu/Au/Si	Cu ⁺	4.0×10^{-4}
Argon	Cu_3Au /Kapton	Au ⁺	3.8×10^{-4}
Argon	Cu_3Au /Kapton	Cu ⁺	3.5×10^{-4}
Helium	Au/GaAs	Au ⁺	9.3×10^{-3}
Helium	Cu_3Au /Kapton	Au ⁺	5.4×10^{-3}

ANALYSIS OF POWDERED METAL OXIDES
USING RADIO FREQUENCY GLOW DISCHARGE MASS SPECTROMETRY

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INTRODUCTION

Conventional glow discharge mass spectrometry (GDMS) allows a sample to be sputtered by applying a direct current (DC) to the sample while it is submerged in a noble gas. This limits the sample material to conductors such as metals or non-conductors mixed into a conducting matrix. An alternative method is to apply an alternating potential in the radio frequency (RF) range to the sample.

When a potential is applied to a nonconducting sample it will act like a capacitor. If the applied RF voltage drives the sample negative, positive ions begin to bombard the surface. Before the sample potential can reach zero, however, the applied RF voltage swings to a positive value and electrons begin to strike the surface. Since electrons are more mobile than ions, the surface potential quickly reduces to zero. As the process repeats itself, the difference in mobilities builds up a negative bias on the sample. This allows the sample to be almost continuously bombarded by positive ions.

EXPERIMENTAL

The RF system used in this study utilizes a wave-form generator to control the output of a RF amplifier, which in turn feeds the high voltage signal into an impedance matching box. A pi-network is used to match the impedance of the RF signal to the discharge impedance. The operating frequency of the RF source (here 6.2 MHz) is determined by the resonance frequency of the matching system.

To control the plasma and protect the holder from being sputtered the shield and grounding configuration in Figure 1 was used.

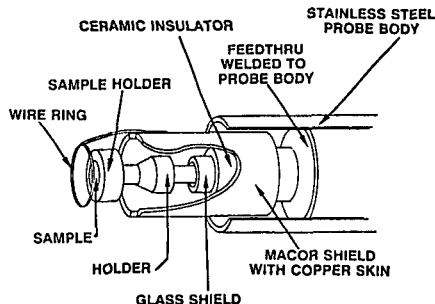


Figure 1.

RESULTS AND DISCUSSION

A comparison of DC and RF discharges using a copper disk shows that although the DC and RF spectra are similar, the RF spectrum shows a smaller sputtered species signal and enhancement of the water and gas peaks. This effect may be due to increased ionization of the contaminate gases in the RF discharge or reduced sputtering efficiency due to lower energy argon ions striking the surface. A weight loss study revealed that during sputter intervals of 30 minutes, the RF discharge demonstrated a weight loss of 1 microgram per second, slightly higher than the 0.7 microgram per second for the DC discharge. This indicates that the differences in DC and RF spectra originate in the steps beyond atomization.

Metal oxides are an example of a nonconducting material that can be directly sputtered with RF glow discharges. One concern in analyzing metal oxides is the effect of different plasma parameters on the amount of dissociation of the metal-oxygen bond. To study both RF plasma characteristics and their effect on oxides, this study focused on lanthanum oxide (because of its high bonding energy to oxygen), uranium oxide (which produces both monoxide and dioxide species when sputtered) and stannous oxide (which has a more "typical" bonding energy).

Powdered metal oxides have a large amount of water absorbed on their surfaces. Even after extended drying in an oven, samples made from compressed metal oxide powders can release water into the glow discharge for several hours. The amount of water present during an analysis will influence the dissociation of metal oxides. One possible explanation is that while the water is present

in large amounts, it is the primary current carrier. Water may not sputter the sample as efficiently as argon ions and the presence of water may quench metastable atoms capable of breaking the metal-oxygen bond.

The signal intensities of all the oxides in this study appear to follow the same pattern in relation to the distance from the sample to the exit orifice. By maintaining a constant pressure of 0.4 torr argon and 10 watts of power, the dark space for these samples was approximately 0.5 cm in length. The maximum signal appears to be within 1.5 mm of the dark space. As the distance to the exit orifice is increased, the oxide signals begin to go down at a faster rate than the metal signals. This effect may be due to the monoxide and dioxide species being broken up or scattered.

Increasing the applied power results in different responses for the oxide species depending on the current plasma environment. For stannous oxide, increased power yields an improved metal signal. Uranium oxide also shows an improved signal but the strongly bonded dioxide and monoxide dominate. Lanthanum oxide species lose signal strength with increasing power. Water may play an important role in each of these responses. In both the uranium oxide and stannous oxide, water is the most outstanding feature, whereas in lanthanum oxide the water signal is steadily decreasing.

Increasing the pressure of the glow discharge results in an increase in both metal and metal oxide signals. This is followed by a pressure range where the metal oxide peaks tend to lose intensity faster than the dissociated metal species. Uranium dioxide in particular loses signal intensity more rapidly than the monoxide species, an effect probably due to this molecule's larger size. The point where the analyte signal begins to decrease varies among the oxides studied. Factors influencing this inflection point include dissociation energy, collisional cross section and the amount of water in the discharge.

Many parameters of the RF glow discharge and their effect on the analysis of powders still remain undefined. In particular, clarification of the plasma conditions which can control the effects of water and other gas contaminants is to be investigated. Future experiments include the use of gettering agents in the discharge, the effects of changing the frequency of the discharge and the use of a second glow discharge to influence the ionization of the RF glow region. The extent of dissociation as the metal oxide is sputtered compared to recombination in the plasma will also be investigated.

CONCLUSION

This paper reports the initial results of sputtering powdered metal oxide samples using a small sample holder and non-conventional radio frequencies. Water and other contaminants appear to be strongly ionized in the RF system and introduce continually changing conditions that further complicate oxide analysis. Although the RF glow discharge appears to offer a convenient method to study non-conducting samples without a conducting matrix, the mechanisms which control the degree of dissociation of the metal-oxygen bond must still be investigated.

GETTERS AS PLASMA REAGENTS IN GLOW DISCHARGE MASS SPECTROMETRY (GDMS)
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INTRODUCTION: The use of compacted samples in glow discharge mass spectrometry (GDMS) for elemental analysis has extended the application of the technique. Non-conducting samples such as geologicals can be analyzed in compacted disc form by mixing them with certain metal powders. The presence of water, caused by its inevitable compression into the sample disc during the sample preparation process, is inherent to the method and quenches the signal to a considerable degree. In order to solve this problem, gettering materials have been chosen as the host matrices for their high performance in reducing gaseous impurities.

EXPERIMENTAL: Three metals with useful gettering properties were chosen to be sample host matrices: Ti, Ta and W. A mixture consisting of 10% La_2O_3 was prepared in a getter matrix. The mixture was shaken on a Wig-L-Bug for 3-5 minutes. A 4mm diameter disc was pressed at a pressure of 800-1000 psi and then mounted into a copper cup fixed on a probe tip. The probe was inserted into the ion source of a quadrupole mass spectrometer. An argon glow discharge was formed about 2-3 mm of the sample. Discharge parameters used were: 900 volts, 2.7 milliamps, 1.0 torr.

RESULTS AND DISCUSSION: Lanthanum oxide (La_2O_3) has been used as an analyte to evaluate the quality of several metals' "gettering" abilities. When sputtered into the discharge, La_2O_3 is detected by the mass spectrometer in two forms: La^+ and LaO^+ . The chemical components of the plasma, especially oxygen-bearing species such as water, have direct influence on the distribution of these two forms. Ratio R is defined as the atomic fraction: $R = \text{La}/(\text{La} + \text{LaO})$. Larger R, which is desirable for higher sensitivity of elemental analysis, can be achieved by the use of getters.

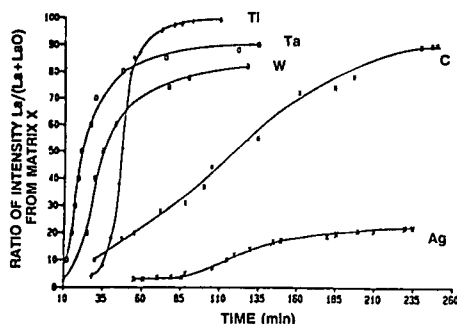


Figure 1

Metals of different gettering ability (Ag, C, W, Ta and Ti) have been mixed with 10% (weight percent) La_2O_3 ; matrix-dependent and time-dependent studies have been carried out. The "gettering" ability has shown an increase in the following order: $\text{Ag} < \text{C} < \text{W} < \text{Ta} < \text{Ti}$. Data are shown in Figure 1.

The mechanisms that getters undergo in the glow discharge have been studied with Ti. It is believed that sputtered Ti particles react with OH and H_2O as well as other "OH" involved clusters and remove them from the plasma. TiO , $\text{Ti}(\text{OH})_2$, $\text{Ti}(\text{OH})_3$, and $\text{Ti}(\text{OH})_3(\text{H}_2\text{O})$ molecules have been detected at early stages of sputtering. The decrease of these species along with sputtering time is accompanied by a simultaneous increase of R. This relation is shown in Figure 2 with TiO as an example. A chemistry process of La_2O_3 in the glow discharge is proposed to explain this phenomena in the following section.

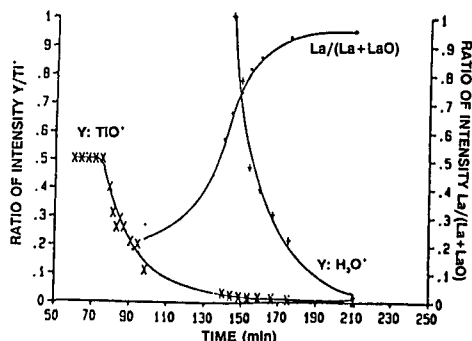


Figure 2

At early stages of sputtering when a large amount of the current carriers is water-dissociated H_n^+ ions, both sputtering and ionization are insufficient. Fewer Ti atoms are introduced into the plasma at this time. La atoms, if sputtered off the target surface, are recombined with oxygen atoms when going through the oxygen-rich plasma to the exit orifice. These oxygen atoms come from two sources: water dissociation and preferential sputtering from the sample. Gaseous impurities decrease as gettering reactions of Ti continue. More

Ar^+ atoms become the majority of the current carriers resulting in higher sputtering rate. The atomization of Ti is enhanced, which, in turn, speeds up the gettering process. As the point in time is reached when the plasma is composed of more Ti atoms than oxygen-bearing species, the recombination of La and O becomes less competitive resulting in a fast increase of R. Since the water contained in the ion source can be treated as two parts: in the plasma and on the sample surface, experiments were also carried out to study which part was more crucial to the formation of LaO . As shown in Figure 3, a Ti disc with 50% La_2O_3 was sputtered at a relatively low power ($V=520v$, $I=0.7mA$, $P=1.0torr$). Signal ratios of H_3O^+/Ti and LaO^+/La decreased to 10 and 1.0 respectively after 250 minutes of sputtering. Discharge power was manually raised up to 900v, 3.0mA 50 minutes later. This action enhanced the absolute signals of H_3O^+ , Ti^+ and LaO^+ resulting in an increase in both H_3O^+/Ti and LaO^+/La intensity ratios simultaneously. Both ratios dropped to less than 0.05 after another 200 minutes. It suggested that being a major oxygen carrier, water affected the formation of LaO more directly when present in the plasma than on the surface.

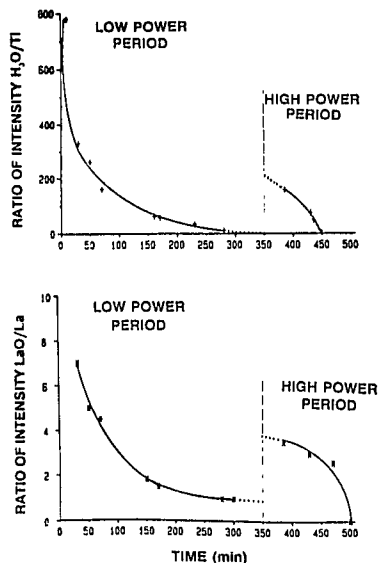


Figure 3

The ability of getters to eliminate gaseous interferences in the glow discharge offers potential in the analysis of compacted materials. Metal oxides with high bonding energies, when mixed with gettering reagents, can be analyzed in atomic form. Detection limits are thus increased. With getters generating a plasma which is relatively free of gaseous impurities, a better understanding of the chemical process that metal oxides undergo in the glow discharge is realized.

Future investigation will emphasize on:

- (1) Ionization of La from La_2O_3 by introducing argon metastable quenchers into the plasma after the "clean" point is reached.
- (2) Time-resolved studies on gettering process in GDMS.
- (3) Further quantitative analysis of non-conductive material by using getters.

KINETICS OF ETHYLIDYNE FORMATION USING LITD/FTMS

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Introduction

Our primary research goal is to probe the kinetics of chemical reactions on well-characterized surfaces and to identify the reaction intermediates which are present on these surfaces. We have approached this problem by coupling the techniques of laser-induced thermal desorption and Fourier transform mass spectrometry. The LITD/FTMS instrument uses an excimer laser to desorb molecular species from the surface and then ionizes and detects them with FTMS.¹ We present kinetic data on the conversion of ethylene to ethylidyne on Pt(111) which demonstrate that LITD/FTMS is well suited to the study of surface reaction kinetics.

The chemistry of ethylene on the Pt(111) surface is well characterized.² At low temperature (200K), ethylene molecularly adsorbs on Pt(111) with the C-C bond parallel to the surface. When the crystal is warmed to about 240K, the stable intermediate ethylidyne (CCH_3) is observed. At saturation coverages, thermal desorption of ethylene is observed with a peak maximum near 285K. The ethylidyne intermediate is stable on the surface up to temperatures near 450K, where it begins to lose hydrogen. Eventually complete dehydrogenation occurs, leaving only carbon on the surface.

We have measured the rate constant for the conversion of ethylene to ethylidyne on the Pt(111) surface at several surface temperatures (236K-274K) by using LITD/FTMS to monitor the change in the amount of ethylene on the surface as a function of time.

Experimental

The surface analysis LITD/FTMS instrument used for our experiments is a UHV chamber consisting of a bell jar and a side arm extension. The base pressure of the instrument is typically $< 7 \times 10^{-11}$ Torr. The bell jar is equipped with standard surface analysis equipment. The FTMS analyzer cell is located in the side arm extension, which is situated between the polecaps of a 0.6T electromagnet.

An experiment is begun by cleaning the Pt crystal and then cooling it to 210K. A near saturation coverage of ethylene is dosed onto the cold crystal, and it is moved 56cm down the side arm extension and positioned at a hole in one of the FTMS analyzer cell plates. A focused excimer laser beam (248nm) passes through another hole in the opposite cell plate and strikes the crystal. Low laser power is used ($\sim 2\text{mJ}$, focused to roughly $20\text{MW}/\text{cm}^2$) so that only neutral molecules desorb from the surface. The desorbed molecules are ionized by an electron beam, trapped in the analyzer cell by the magnetic field, and subsequently mass analyzed and detected by FTMS.¹ A complete mass spectrum from m/z 10 to 250 is acquired for each laser shot. The laser optics are adjusted so that the laser beam will hit a new spot on the crystal, and another LITD/FTMS spectrum is acquired. Several mass spectra are taken at 210K, then the crystal temperature is ramped (3-5 K/s) to the desired reaction temperature (between 236K and 274K) and is stabilized at that temperature while mass spectra are acquired from different spots as a function of time. As many as twenty non-overlapping spots can be probed during a given experiment. At the end of an experiment, the crystal is retracted into the bell jar and subjected to the cleaning and ethylene dosing procedures in preparation for another experiment.

Results

The rate of conversion of ethylene to ethylidyne was monitored by following the decrease in the laser-desorbed ethylene signal as a

function of time. Figure 1 shows the natural log of the absolute peak height for $C_2H_4^+$ as a function of time at five different isothermal reaction temperatures. The lines through the points are linear least squares fits to the data. Eight reaction temperatures were studied over a range from 236K to 274K. In each case, the ethylene was adsorbed at 210K, and the time $t = 0$ corresponds to the beginning of the crystal temperature ramp. Only data points accumulated after the crystal had reached the desired isothermal reaction temperature are plotted and fit. The decrease in the ethylene signal as a function of time is due to the conversion to ethylidyne. The reaction is expected to be first order with respect to ethylene coverage, and this is supported by the linearity of the kinetic data in Figure 1.

All the rate constant data were used to generate the Arrhenius plot in Figure 2. This plot gives an activation energy of 14.9 ± 1.4 kcal/mol and a preexponential factor of $4 \times 10^{10 \pm 1.2}$ /sec for the conversion of ethylene to ethylidyne on Pt(111). Recently T-NEXAFS³ and FTIR⁴ methods have been used successfully to probe this conversion reaction by directly monitoring the growth of the ethylidyne intermediate. Our results are in good agreement with both of these methods. ($E_a = 15.0 \pm 1$ kcal/mol and $\nu = 3.6 \times 10^{10}$ /sec by T-NEXAFS;³ $E_a = 14 \pm 1$ kcal/mol and $\nu = 10^{10 \pm 1}$ /sec by FTIR⁴).

Conclusion

We have presented the first rate constant measurements for a surface reaction using the technique of LITD/FTMS. The kinetic data show that FTMS is a sensitive and quantitative detection method and the combination of laser desorption with the FTMS detection provides a very powerful tool for studying surface reaction kinetics. LITD/FTMS is capable of following surface reactions on a short timescale, and because a complete, high resolution mass spectrum is obtained for each laser shot, the instrument can provide new insights on surface reactions which involve complex molecules or several surface species.

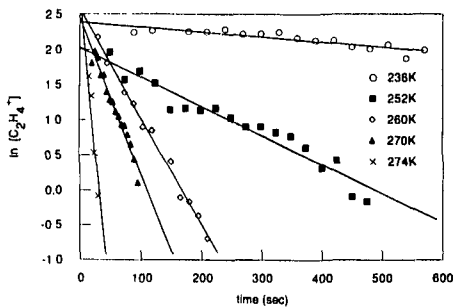


FIGURE 1

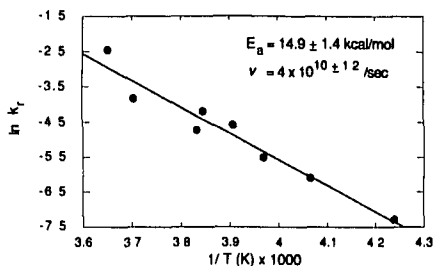


FIGURE 2

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Laser Desorption on Pt Surfaces: Experiment and Theory

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Department of Chemistry

A computer model has been developed to calculate the temperature jump on a metal surface resulting from a pulsed laser irradiation. Using this model, one can calculate the temperature rise as a function of time and position. From the calculated temperature rise, the probability of desorption of surface species can be obtained. By comparing the calculated desorption probabilities as a function of the absorbed power density to experimentally obtained values (using LITD/FTMS spectrometer), we could obtain the activation energy and the pre-exponential factor for the desorption of ethylene and the decomposition of ethylidyne on a Pt (111) surface.

Many surface chemical studies are performed by heating a surface with an adsorbed overlayer to induce surface reactions and/or desorption of the adsorbates. The probability that a species desorbs or reacts is determined by the instantaneous relative rate for these two processes. We consider here only first order desorption and reactions (eg. dehydrogenation). The rates can then be represented by the Arrhenius rate law $k = \nu \exp(-E_a/RT)$ where T is the temperature of the surface and E_a and ν are the activation energy and the pre-exponential factor, respectively. For specific processes, values for E_a and ν are available either from literature or can be approximated. Values for T can be calculated from the differential equation of heat flow. The basic assumptions for laser heating are: 1) once laser radiation is absorbed by the substrate, and the energy is converted to heat instantaneously. 2) The energy is considered to be deposited on the surface, and the lateral diffusion of the heat is small compared to the size of the laser beam.

Based on these assumptions, the temperature jump induced by the laser on the Pt surface can be calculated. Figure 1 shows the calculated surface temperature, rate of desorption reaction and the desorption flux on a Pt crystal as a function of time and power density. It is clear that the maximum rate of reaction occurs at 20 nanosecond, i.e. at the end of the laser pulse, where the maximum temperature occurs. However, the maximum desorption flux does not always occur at the end of the laser pulse. At high laser power density, the surface temperature increases quickly enough so that the maximum desorption flux occurs before the end of the laser pulse, and most of the molecules on surface can be desorbed. In contrast, if the laser power is relatively low, the maximum desorption flux would occur where the temperature is the highest. This effect of the laser power density on the desorption flux is illustrated in Figure 1.

The spatial distribution of the laser beam also affects the spatial desorption probability. Figure 2 depicts the desorption probabilities as a function of power density for different spatial laser profiles: rectangular and Gaussian beam profiles. For the rectangular beam, the temperature rise is the same across the laser spot, but for Gaussian beam, the temperature has a Gaussian distribution across the laser spot. Therefore, with the same laser power density, the rectangular beam would have higher desorption flux than the Gaussian beam. This is seen in Figure 2, where the desorption probability increases more rapidly in the rectangular spatial profile. From this effect, one could determine the shape of the laser beam used in the laboratory by matching the theoretical results with the experimental data. This is advantageous for determining the spatial profile of a laser beam which is difficult to determine in the laboratory.

By extending this method, one finds that varying the activation energy and the pre-exponential factor of a desorption reaction also affects the shape of the desorption probability vs. the absorbed power density plot. One can match the calculated results to the experimentally obtained data, and the activation barrier and the pre-exponential factor can be obtained. Figure 3 shows this type of matching for ethylene desorption. The activation energy and the pre-exponential factor are found to be 13 kcal/mole and $5 \times 10^{12} \text{ s}^{-1}$, respectively. Similarly, the activation energy and the pre-exponential factor of the laser driven decomposition of ethylidyne are 25 kcal/mole and $5 \times 10^{12} \text{ s}^{-1}$. The estimated error for these values is approximated 15 % due to the uncertainty in the measurement of the absorbed power density in the laboratory.

Laser power density, spatial distribution of the laser beam, and the activation energy and pre-exponential factor of a reaction all affect the desorption probability. One can take advantage of these variables and match the calculations to the experimental data. As a result, one can obtain information about the spatial profile of the laser beam and the kinetic parameters of a reaction in a real experiment.

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Figure 1: Calculated values for a) surface temperature b) rate of the desorption reaction and c) the desorption flux on a Pt crystal as a function of time and power density. The surface temperature (T_0) before laser heating is 200 Kelvin.

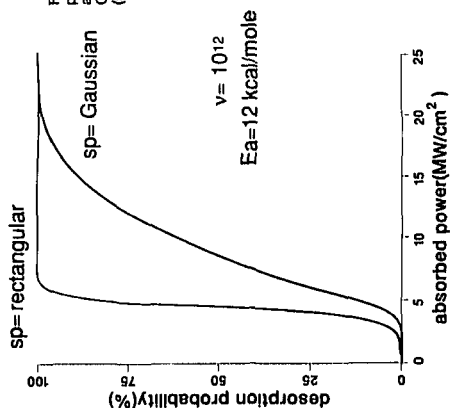
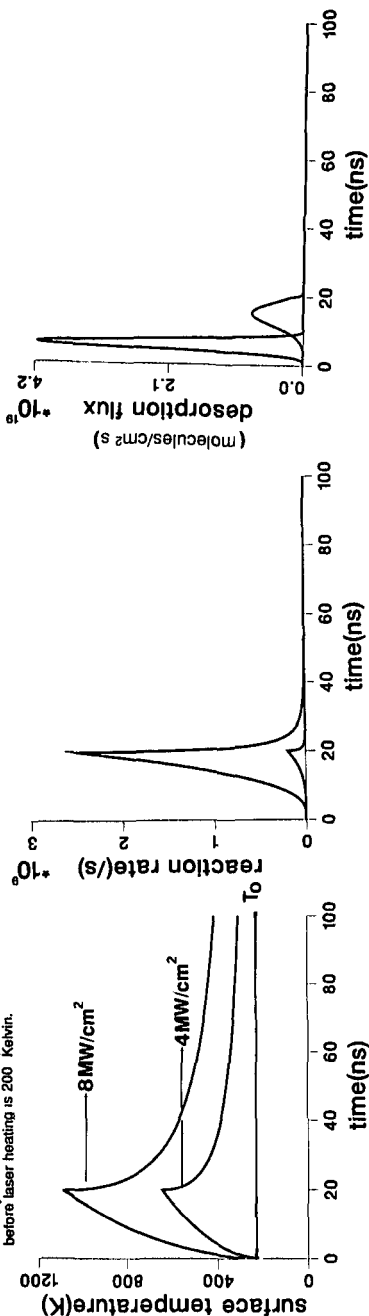


Figure 2: Calculated desorption probability in a Pt crystal as a function of absorbed power density for a (a) Gaussian and (b) rectangular beam (spatial profile).

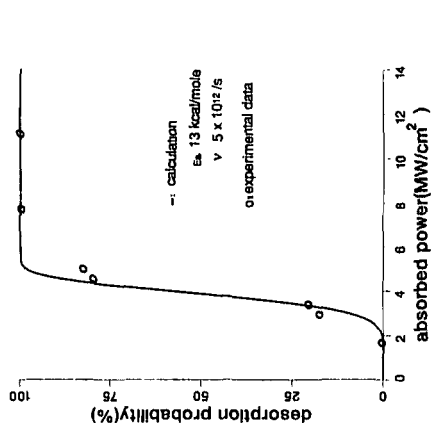


Figure 3: Comparison of theoretical results to experimentally obtained data for ethylene desorption. The activation barrier and the pre-exponential factor are determined to be 13 kcal/mole and 5×10^{12} s⁻¹, respectively.

TOF-SIMS of Polymer Materials

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TOF-SIMS has turned out to be a promising technique for the surface analysis and structure characterization of polymer materials[1,2,3]. Systematic investigations have been performed for standard polymer samples of polystyrene (PS), polymethylmethacrylate (PMMA), polyethyleneglycol (PEG) and krytox with average molecular weights of $M_n = 10^3 - 10^6$ u

High molecular fragments and oligomers up to 10,000 u are preferentially emitted from noble metal supported polymer films. Maximum yields of cationized secondary ions (SI) are obtained from etched silver substrates with a surface coverage of $1 \mu\text{g}/\text{cm}^2$.

For polymers with $M_n < 10,000$ u fragment ions are usually formed by a single chain scission of the polymer backbone. Fragment ion formation of polymers with $M_n = 10^4 - 10^6$ u can be explained by multiple chain scission events. The distribution of molecular ion peaks ($n \times \text{monomer} + \text{endgroups} + \text{Ag}$)⁺ represents the oligomer distribution of the sample.

Transformation probabilities of PS- and PMMA- oligomers, i.e. the probability of a certain oligomer to be emitted as an intact molecular ion, decrease by almost two orders of magnitude between 1000 and 7000 u. For PEG a corresponding decay is observed in the mass range $m = 1500 - 4500$ u.

Primary ion bombardment (Ar^+ , 12 keV) of a monolayer of polymer material results in an exponential decay of SI-intensities with time: $I = I_0 \exp(-\sigma j_p / e \cdot t)$. Damage cross sections σ of cationized oligomers increase proportionally to the molecular ion mass from $\sigma(1000 \text{ u}) = 3 \times 10^{-13} \text{ cm}^2$ to $\sigma(7000 \text{ u}) = 15 \times 10^{-13} \text{ cm}^2$. The same values of σ are observed for cationized fragments of corresponding chain length.

Stable, reproducible SI-spectra of insulating bulk polymers (foils, powders, liquids) are obtained by TOF-SIMS with a self-adjusting charge compensation system [4]. Damage effects by the compensating electrons (20 eV) can be neglected in all cases.

SI-emission from polymer surfaces is usually restricted to the low mass range $m < 300$ u (PS, PMMA). A different behaviour is, however, observed for some F-containing polymers. For the krytox samples a C-O-bond cleavage with charge separation results in the emission of intense positive and negative fragment ion series with m between 200 u and 6000 u (see figure 1).

PI-bombardment of the krytox oils in the dose regime of $10^{12} - 10^{13}$ PI/cm² has no influence on absolute and relative peak intensities. A regeneration of the polymer surface by diffusion may be responsible for this effect.

An exponential decay of fragment ion yields with increasing PI-dose density is found for PMMA. Corresponding damage cross sections are in the range of $\sigma(31 \text{ u}) = 0.3 \times 10^{-13} \text{ cm}^2$ to $\sigma(185 \text{ u}) = 1.3 \times 10^{-13} \text{ cm}^2$.

TOF-SIMS spectra of bulk polymers with typical dose densities of 10^{11} PI/cm² can generally be attributed to the undestroyed polymer surface.

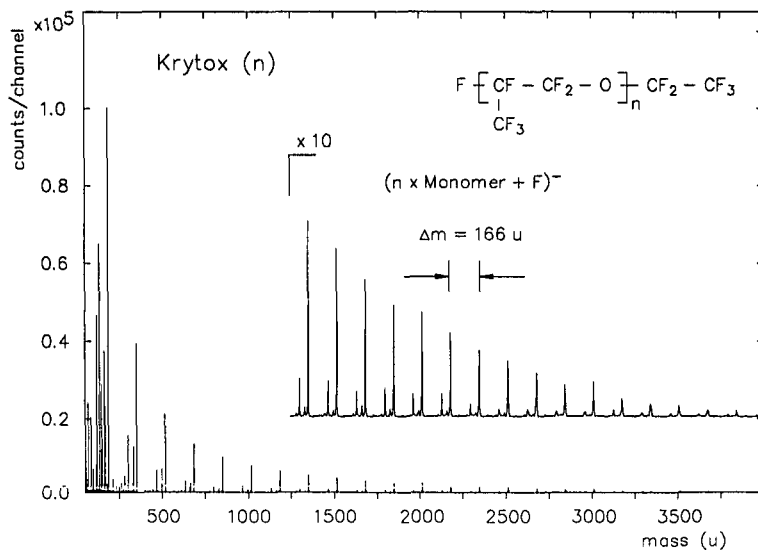


Figure 1: Neg. TOF-SIMS spectrum of a thick film of krytox ($M_n = 3900 \text{ u}$)

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The Effects of Polyatomic Primary Ions on the Yield and Extent of Fragmentation
of Secondary Ions in Molecular Secondary Ion Mass Spectrometry

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The translational energy, mass, and polyatomicity of the bombarding ion affect the yield and extent of fragmentation of secondary ions in molecular secondary ion mass spectrometry (SIMS). These effects are examined using Ar^+ , N_2^+ , CO_2^+ , CF_3^+ and SF_5^+ primary ions with analytes which included ammonium, arsonium, and phosphonium salts. As the energy of the bombarding ion is increased from 0.5 to 5.0 keV, the observed abundance of secondary ions increases, but the extent of fragmentation of the desorbed ions decreases. The SIMS spectra of $(\text{C}_6\text{H}_5)_4\text{PBr}$ at 0.5 and 5.0 keV CF_3^+ primary ion energies are shown in Figure 1. The intact cation (C^+) at m/z 339 and a $\text{C}_{12}\text{H}_9\text{P}^+$ fragment ion at m/z 183 are observed. As the CF_3^+ ion energy is increased from 0.5 to 5.0 keV, the observed secondary ion signal shows a forty-fold increase. The extent of fragmentation (339⁺/183⁺ ratio) decreases slightly as the CF_3^+ energy is increased. The SIMS spectra of $(\text{C}_6\text{H}_5)_4\text{PBr}$ at 0.5 and 5.0 keV Ar^+ primary ion energies are shown in Figure 2. As the Ar^+ energy is increased from 0.5 to 5.0 keV two dramatic effects are observed: (i) the observed secondary ion signal increases by a factor of ten and (ii) the extent of fragmentation decreases dramatically.

Figure 3 shows the observed ion abundance of the intact cation (C^+) of $(\text{C}_6\text{H}_5)_4\text{PBr}$ at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 keV Ar^+ , N_2^+ and CF_3^+ ion energies. CF_3^+ is more efficient than either Ar^+ or N_2^+ at desorbing this ion. The efficiency of secondary ion production follows the general order: $\text{SF}_5^+ > \text{CF}_3^+ > \text{CO}_2^+ > \text{Ar}^+ > \text{N}_2^+$, which is the order of masses of these ions: 127 > 69 > 44 > 40 > 28. This suggests that the efficiency of secondary ion production is controlled by the primary ion momentum and not the number of atoms in the bombarding ion. Diatomic N_2^+ ions are less efficient at secondary ion production than monoatomic Ar^+ ions.

The extent of fragmentation increases with decreasing bombarding ion energy as a result of increased internal energy deposition at lower bombarding ion energy [1]. Figure 4 shows the ratio of the intact cation at m/z 339 to the fragment ion at m/z 183 from $(\text{C}_6\text{H}_5)_4\text{PBr}$ at 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 keV Ar^+ , N_2^+ and CF_3^+ ion energies. When Ar^+ or N_2^+ ions are employed, the extent of fragmentation decreases significantly as the energy is increased, but when CF_3^+ is employed, it decreases only slightly (as shown in Figure 1). At a given primary ion energy, the extent of fragmentation follows the general order: $\text{Ar}^+ < \text{N}_2^+ < \text{CO}_2^+ < \text{CF}_3^+ < \text{SF}_5^+$, which does not follow the order of increasing masses of the primary ions. Ar^+ is associated with the least amount of fragmentation even though N_2^+ has a lower mass. This effect is attributed to the polyatomicity of the primary ions. At a given energy, the extent of fragmentation is directly related to the number of atoms contained in the primary ion. For example, diatomic N_2^+ ions induce more fragmentation than monoatomic Ar^+ ions. This effect is attributed to the independent actions of the constituent atoms in the ion. For example, a 5.0 keV N_2^+ ion acts like two 2.5 keV N^+ ions during energy deposition into the analyte molecules at the surface, thus promoting more fragmentation of the secondary ions. This effect is even more pronounced when primary ions composed of more atoms, such as SF_5^+ and CF_3^+ are employed.

The decrease in the extent of fragmentation is directly proportional to the velocity (v), but is inversely proportional to the mass (m) and the number of atoms (n) in the primary ion. Figure 5 shows plots of the intact cation/fragment ion ratios of $(\text{C}_6\text{H}_5)_4\text{PBr}$, $(\text{C}_6\text{H}_5)_4\text{AsCl}$ and $(\text{C}_4\text{H}_9)_4\text{NBr}$ with respect to the factor v/mn . This factor accounts for the effects of kinetic energy, mass and polyatomicity of the primary ion. In each case, the extent of fragmentation decreases approximately linearly as v/mn increases.

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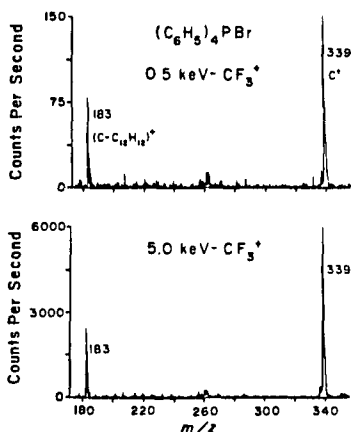


Figure 1 - SIMS spectra of $(C_6H_5)_4PBr$ at 0.5 and 5.0 keV CF_3^+ ion energies.

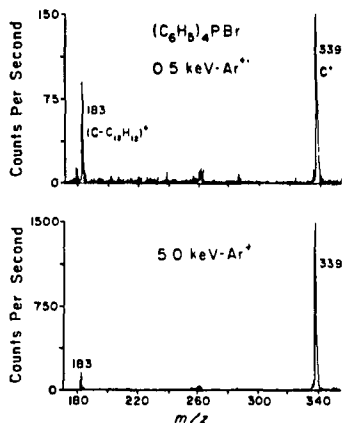


Figure 2 - SIMS spectra of $(C_6H_5)_4PBr$ at 0.5 and 5.0 keV Ar^+ ion energies.

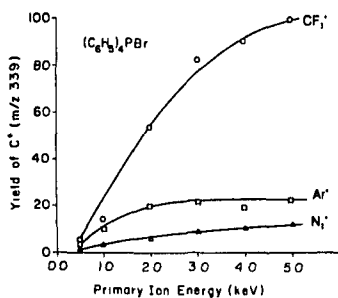


Figure 3 - Normalized abundance of C^+ of $(C_6H_5)_4PBr$ with respect to primary ion energy.

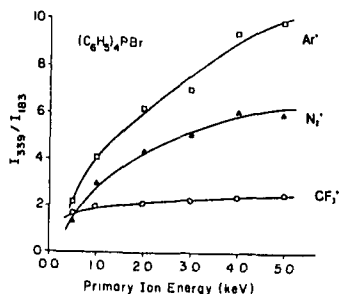


Figure 4 - $339^+/183^+$ ion ratio of $(C_6H_5)_4PBr$ with respect to primary ion energy.

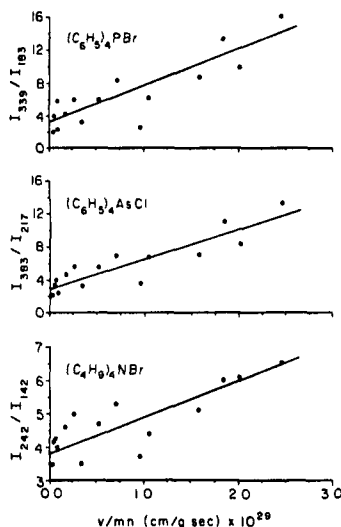


Figure 5 - Intact cation/fragment ion ratios plotted as a function of the factor v/mn .

ORGANIC SURFACE ANALYSIS WITH A TIME-OF-FLIGHT SECONDARY ION MICROSCOPE

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The analysis of organic surfaces by secondary ion mass spectrometry requires low primary ion exposures of the surface in order to avoid irreversible sample damage. Mass spectral analysis and measurement of ion distributions on biological samples is especially critical in this respect since the primary ion beam can cause topographical, as well as mass spectral changes of the substrate.

We have used a time-of-flight (TOF) secondary ion microscope for ion imaging and mass spectral analysis of such surfaces. The instrument is capable of transporting a magnified stigmatic image of the secondary ions produced at the sample surface to an imaging ion detector during the acquisition of the mass spectra. Secondary ion images and mass spectra can be acquired at primary ion doses of less than 10^{12}cm^{-2} , i.e. less than 0.1% of the sample surface is affected by the primary ion beam. The TOF ion microscope can be operated with imaged sample areas of $200 \mu\text{m}$ (magnification $m=60\times$) or $60 \mu\text{m}$ ($m=270\times$) diameter with lateral resolutions of up to $1 \mu\text{m}$. The mass resolution of the instrument is presently 3100 at mass 28. A detailed description of the experimental conditions is given by the authors in these proceedings.

This TOF-SIMS instrument can produce both elemental and molecular secondary ion images. Figures 1 through 3 illustrate the organic microanalytical capability of this instrument. Figure 1 is a positive ion TOF-SIMS mass spectrum of a residue of acetylcholine chloride (ACh-Cl) dispersed onto a silicon substrate. This spectrum contains the peaks at m/z 146, 129, 104 and 87 which are characteristic fragment ions produced in static SIMS analyses of ACh-Cl residues. The most intense peak in this group of fragments is the $(\text{M}-\text{Cl})^+$ ion at m/z 146. Figure 2 is a section of a positive secondary ion mass spectrum of thin sections of rat brain tissue. The sample was prepared by shock freezing and cryomicrotoming thin sections ($\sim 5 \mu\text{m}$ thick) which were subsequently freeze dried. The mass spectrum in Figure 2 was produced from the caudate putamen region which had been doped with approximately 2×10^{-7} moles/ cm^2 of acetylcholine Cl. The mass spectrum in Figure 2 also shows mass signals at m/z 104 and 146 which are characteristic for acetylcholine. Figure 3 shows three secondary ion images from the same region. These images were acquired using a specially modified resistive anode encoder (RAE) which provides a pulse counting, ion imaging detection capability. The intensities in the images shown are displayed in a gray scale. High secondary ion intensities correspond to darker regions of the images, whereas the white regions indicate that no ion was detected at this position.

The three positive ion images of Figure 3 include the total ion image along with the m/z 104 and the 146 cations produced from the ACh-Cl residue in the tissue. These images were acquired over a $200 \mu\text{m}$ diameter analysis area. If one assumes that the acetylcholine chloride doping of this tissue dispersed uniformly over the sample, these ion images represent sampling approximately 10^{-11} moles or less of the compound. The intensity distributions in the m/z 104 and 146 images correlate very well indicating that these ions are produced from the same regions on the sample surface. The lateral resolution in these images is on the order of $2 \mu\text{m}$ suggesting that molecular ion images of ACh-Cl could be produced at the cellular level. The signal of m/z 104 is, however not necessarily produced by a fragmentation of acetylcholine chloride since it could also be found in undoped regions of the rat brain section, as well as in other biological thin sections. The ion signal due to m/z 146 is not observed in the analysis of undoped caudate putamen regions of the rat brain (or other samples investigated) and is believed to be specific for ACh-Cl. We are currently exploring the use of the imaging TOF-SIMS technique for the localization of various catecholamines (e.g., dopamine, ACh-Cl, epinephrine and norepinephrine) within cellular regions of cancerous adrenal tissues (pheochromocytoma).

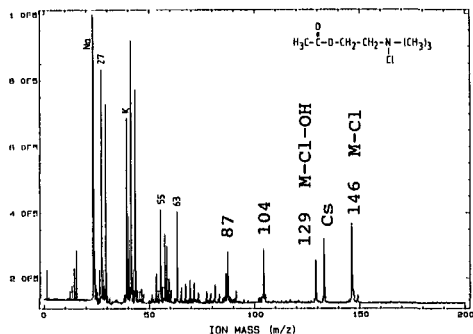


Figure 1: Positive secondary ion mass spectrum of acetylcholine chloride dispersed onto a silicon wafer.

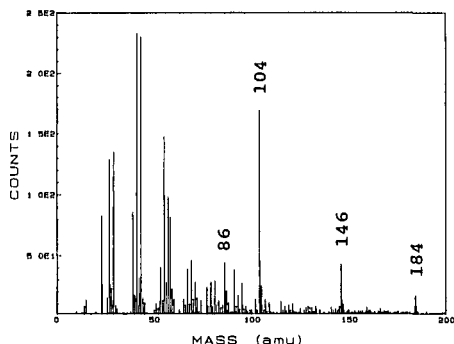


Figure 2: Section of a positive secondary ion mass spectrum from a thin section of rat brain tissue. The analyzed brain section had been doped with approx. 2×10^{-7} moles/cm² of acetylcholine chloride.

3a: Total pos. ion image 3b: m/z=104⁺ ion image 3c: m/z=146⁺ ion image



Figure 3: Three positive secondary ion images taken from the same spot of a rat brain tissue thin section doped with 2×10^{-7} moles/cm² acetylcholine chloride. The imaged sample areas are 200 μ m in diameter. Darker regions correspond to higher ion intensities.

Acknowledgments: The assistance of Dr. Kym Faull and Ms. Joan Hunter of the Stanford University Medical Center for the preparation of the tissue specimens is gratefully acknowledged.

EVIDENCE FOR ION NEUTRALIZATION BY ELECTRON TUNNELING
IN THE DESORPTION OF SPECIES FROM POLYMER SURFACES

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The desorption-ionization process in ^{252}Cf plasma desorption mass spectrometry (PDMS) has been found to be extremely surface sensitive(1). A molecule can give a small to nonexistent signal on one surface but may yield a significant peak when deposited on another surface. Little is known about these surface effects and how they influence the desorption-ionization process of different species. Molecules may desorb as neutral particles before being ionized or they may be desorbed as ions. Since molecules have several fundamental properties, there is likely to be a variety of desorption-ionization mechanisms based on a molecule's size, shape, composition, polarity etc. In this study the desorption-ionization process of I^- and CF_3SO_3^- from polyethylene terephthalate (PET), nitrocellulose (NC) and poly-3-methylthiophene (PMeT) and their modified surfaces have been investigated.

Samples were prepared by electrospraying(2) $10\text{nmol}/\text{cm}^2$ of salt onto the surface of interest. Surfaces were either the unmodified polymers or these polymers sputter-coated with 0.5 to 10nm of gold.

I^- exhibits very unusual behavior when deposited on PET and 0.5nm gold-coated PET. When CsI is deposited on PET the Cs $^+$ peak is easily seen while the I^- peak is very weak (Fig. 1a). If the PET surface is altered slightly by depositing 0.5nm of gold on it before depositing the salt, the I^- intensity increases by over 40 fold (Fig. 1b). The Cs $^+$ peak also increases but only by 8 times over that observed for the bare PET. If heavier coatings of gold are deposited onto the surface the intensities of I^- and Cs $^+$ start to decrease (Table 1).

The positioning of the gold layer is important. Experiments were performed in which CsI was electrosprayed onto 1nm gold-coated PET or in which 1nm gold was deposited onto the previously deposited salt. Both Cs $^+$ and I^- are attenuated when the gold layer is deposited over the salt but the Cs $^+$ peak was only attenuated by 13% while the I^- peak decreased by 83% (Table 2).

To see how other polymer surfaces effected this desorption process NC, oxidized PMeT, and reduced PMeT along with these surfaces coated with 0.5 or 10nm of gold were investigated. The important result obtained here is that all three surfaces showed an increase in I^- and Cs $^+$ intensity when going from their unmodified surface to their surface coated with 0.5nm of gold. There was no clear trend when CsI was deposited on 10nm gold-coated polymer but the ions of interest generally decreased on these surfaces.

LiSO_3CF_3 was also examined on PET, 0.5nm gold-coated PET and 10nm gold-coated PET. A similar but not as dramatic trend was observed as in the case of CsI. Although the initial CF_3SO_3^- peak was intense on the bare PET, the 0.5nm gold coating effected the desorption of CF_3SO_3^- as well as Li^+ enhancing each of their signals by 1.5 times that of the uncoated PET surface. When LiSO_3CF_3 was electrosprayed onto 10nm gold-coated PET the Li^+ and CF_3SO_3^- intensities were smaller than those of the salt deposited on uncoated PET. This decrease in ion intensity with an increase in gold coverage could be due to the fact that with heavier coatings of gold, gold can now act as a dissipator of energy and hinder the desorption process.

The above results can be explained in terms of electron tunneling through one of the proposed mechanisms. In the first case neutral species are desorbed from the surface. If an iodine atom desorbs, it must extract an electron from the surface. Since the electrons in the polymer surface are tightly bound it is difficult for the iodine atom to remove an electron. When gold is present, electrons are available

near the surface which can tunnel from the gold to the iodine atom forming a detectable species. On the other hand, Cs^+ has to lose an electron to the polymer surface and does this rather easily as seen by its intense peak on the PET surface; however, if gold is present Cs^+ can donate its electron more readily to gold's conduction band and is enhanced by this process. Thus in this case, gold can donate or receive electrons and enhance ion formation.

A second possible mechanism is that ions are desorbed from the surface and that the presence of gold prevents their neutralization. If I^- is desorbed a positive charge is left behind and there is the possibility for the electron in I^- to tunnel back into the surface. If gold is present, gold is able to donate its electrons to the positively charged site decreasing the probability of an electron tunneling from I^- . When Cs^+ departs the surface a negative charge remains. For this situation, an electron from the surface can tunnel to Cs^+ neutralizing it but when gold is present the excess electrons on the surface can be accommodated by gold and the neutralization of Cs^+ can be avoided. In this case, gold acts as a donor or recipient of electrons at the surface and prevents the neutralization of ions.

Electron tunneling is one possible mechanism for ion activation/deactivation. This study has shown that of the polymer surfaces examined, electron tunneling can occur and is influenced by the presence of small amounts of gold.

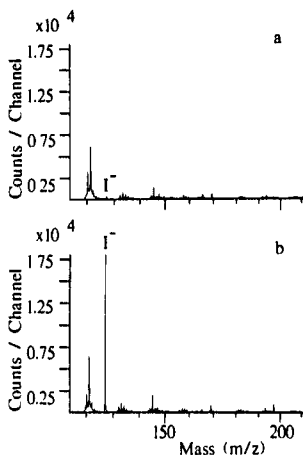


Figure 1. CsI electrospayed on (a) PET and (b) 0.5nm gold-coated PET. The presence of the gold enhances the I^- peak by more than 40 times.

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Table 1
 I^- and Cs^+ ion intensities for CsI electrospayed onto various thicknesses of gold-coated PET.

Au(nm)	I^-	Cs^+
0	1200	78300
0.5	47500	623900
1	37900	369800
2	32700	325500
5	18200	73100
10	6400	30300

Table 2
 I^- and Cs^+ ion intensities for CsI electrospayed on 1nm Au-coated PET and 1nm Au coating of previously deposited CsI.

	I^-	Cs^+
CsI on 1nm Au	37900	369800
1nm Au on CsI	6400	322800

Enhancement of Static FAB Spectra
Data of Azo Sulfonated Dyes

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A study was initiated to find a matrix or procedure that would improve FAB spectral quality of polysulfonate and/or polysulfonic acid compounds, especially those of azo dyes. A commercially formulated sample, "Pergasol Red Mix76" exhibits a good FAB spectrum. The commercial sample is an aqueous solution of a disulfonated azo dye containing a small amount of triethanolamine. Intense ions relative to the glycerol matrix ion at m/z 551 were observed at m/z 526 and m/z 527 (figure 1). These ions represent the $(M-H)^-$ and $(M)^-$ ions respectively. Sodium adduct ions are also evident in the spectrum. Less intense ions relative to the glycerol ion were observed when the neat sample was analyzed (figure 2). The analyte of each sample was also found to have a significantly different surface activity in glycerol. When a glycerol matrix containing an azo dye is modified, its FAB spectrum may be enhanced. Figure 2A is an example showing the Pergasol Red neat material in a glycerol/DMF matrix. Its FAB spectrum and surface activity is better than that of the neat material in glycerol alone.

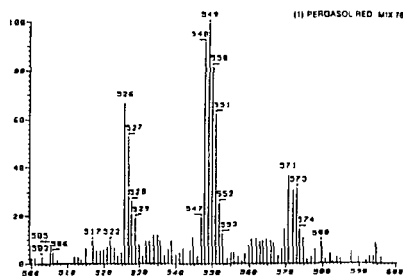


Fig. 1. Negative FAB spectrum of Pergasol Red "Mix76" in glycerol
(Surface Activity = 10.6 dyne/cm).

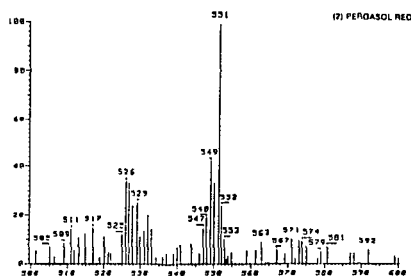


Fig. 2. Negative FAB spectrum of Pergasol Red neat material in glycerol
(Surface Activity = 2.1 dyne/cm).

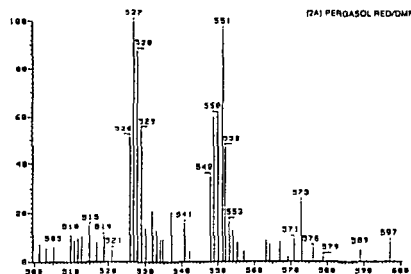


Fig. 2A Negative FAB spectrum of Pergasol Red in glycerol/DMF
(Surface Activity = 2.2 dyne/cm).

Modifying the glycerol matrix appears to influence the surface activity of Pergasol Red and in turn its FAB spectral ion intensity.

Initially the commercially formulated sample labeled Pergasol Red "Mix76" revealed significant surface activity along with intense FAB spectral ions. After a prolonged period of time the surface activity and spectral ion intensity diminished. A laboratory formulation paralleling the commercial sample was prepared but did not duplicate the results of the original material.

An unknown highly surface active but volatile component is suspected to have been present in the original sample which has evaporated on standing. Work is planned to investigate the change in sample behavior with time and its possible connection to the presence of a volatile component.

A dramatic enhancement in spectral quality was achieved when an aqueous solution of the polysulfonic acid or sulfonate azo dye was passed through a cationic exchange resin column (1) prior to FAB mass spectral analysis (figures 3 and 4). Cibacron Red, which is a trisulfonated azo dye (empirical formula $C_{31}H_{24}ClN_7O_{10}S_3$) exhibits only low intensity high mass ion clusters in its FAB spectrum relative to the glycerol matrix peak at m/z 183. The material analyzed after column treatment exhibits a pronounced peak at m/z 784 corresponding to the $(M-H)^-$ ion of the free acid azo dye.

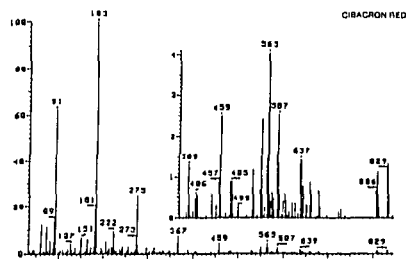


Fig. 3. The negative FAB spectrum of Cibacron Red before column treatment

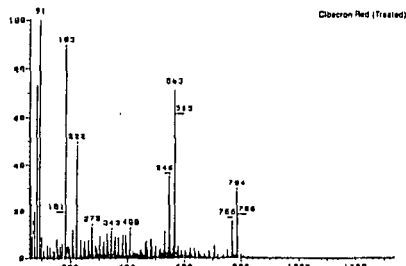


Fig. 4. The negative FAB spectrum of Cibacron Red after column treatment

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ON THE DYNAMICS OF LOW AND MEDIUM ENERGY COLLISION-INDUCED
DISSOCIATION OF IONS

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Collision-induced dissociation (CID) of ions has greatly enhanced the role of mass spectrometry in analytical and structural chemistry over the past two decades. It has been the subject of numerous studies characterizing the process and the effect of various parameters, viz. collision energy, collision gas and gas pressure, internal energy, and angular scattering. With the advent of new ionization methods and high mass range mass spectrometers, CID has been extended to study large biological molecules. Our understanding of the CID process, though sufficient to apply it successfully for analytical applications, is incomplete except for diatomic and probably triatomic ions. It is essential to understand the dynamics of the collisional activation and dissociation process if it is to be applied universally to large polyatomic ions. In this paper we present some results on the dynamics of the CID process for acetone molecular ion in the medium and low energy range.

Experiments were performed on a tandem hybrid mass spectrometer described earlier [1]. Briefly, a high energy (3keV) ion beam from VG7070E double focusing mass spectrometer generated by 70 eV electron impact ionization is decelerated to the desired final energy and focused at the collision region where it collides with a supersonic neutral beam at right angles. Fragment ions are energy and mass analyzed at a series of scattering angles using a hemispherical energy analyzer and a quadrupole mass filter. Data are presented in the form of center-of-mass (CM) velocity contour maps.

Figures 1 and 2 show velocity contour maps for the dissociation of acetone molecular ion to acetyl ion at 40.9 and 10.3 eV collision energy respectively. The intensity maxima in both the figures lie in the forward scattering region at non-zero angles. The energy transfer into acetone ions (>4 eV), derived from P(T) curves, is many times more than the thermochemical threshold (0.65eV) even though the ions were generated by 70 eV electron impact. These results confirm an earlier observation [2] from CID of 5 keV acetone ion that the dissociation proceeds via low impact parameter collisions with extensive momentum transfer.

As the collision energy is lowered, the intensity maximum shifts from forward scattering to the backward scattering region and at energies close to thermochemical threshold almost all the ions are backscattered. Figure 3 shows such an example at 0.65 eV collision energy. This is the first observation of backward scattering in a CID process suggesting that very low impact parameters (nearly head-on collisions) can be accessed in low energy collisions. The consequence of such a phenomenon on the collection efficiency in CID would depend strongly upon the experimental conditions. Clearly the role of this new parameter in CID needs to be examined in detail. Experiments are currently in progress in our laboratory to explore whether this kind of mechanism is generally important in low energy CID.

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Figure 1.

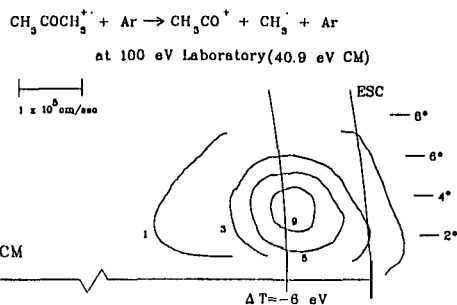


Figure 2.

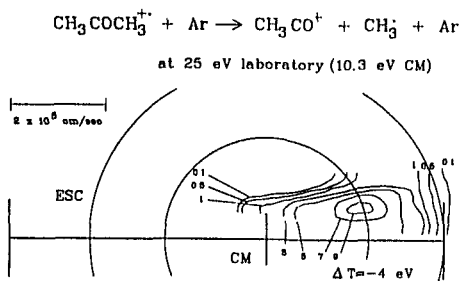
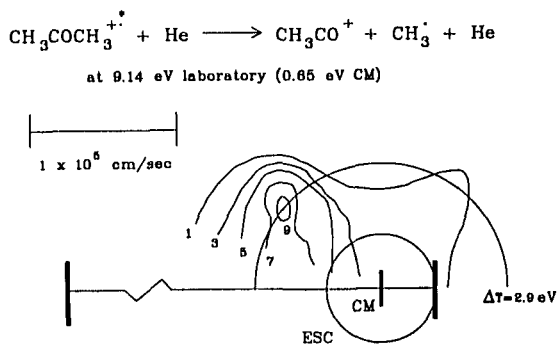


Figure 3.



Center-of-mass velocity contour diagrams for the dissociation of acetone ion to acetyl ion at (1) 40.9 eV, (2) 10.3 eV and (3) 0.65 eV collision energy.

DETECTION OF LONG-LIVED EXCITED STATES OF POLYATOMIC IONS IN MOLECULAR
BEAM STUDIES OF COLLISION-INDUCED DISSOCIATION PROCESSES

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The energy distributions of product ions in ion-molecule reactions provide a direct assessment of energy available in the primary ions and utilized to drive these reactions. If the reactant ions is initially electronically or vibrationally excited, less translational energy is required to overcome the endoergicity of a reaction than in the case of ground state ions. The presence of such excited states can therefore be identified by measuring the relative translational energy differences between reactant and product ions. We have used this method to discern the role of internal excitation in collision-induced dissociation (CID) of acetone and methane molecular ions.

CID of acetone and methane molecular ions was studied by two different instruments. Acetone ion CID was performed on a recently developed hybrid tandem beam mass spectrometer[1] where a VG 7070E double focussing mass spectrometer generates a high energy (3 KeV) primary beam of acetone molecular ions. The ion beam was decelerated to the desired energy at the collision center by a series of cylindrical and rectangular tube lenses and then collided with a vertically moving supersonic beam of He neutrals. The fragment ions were energy and mass analyzed by a hemispherical energy analyzer and a quadrupole mass filter at different laboratory scattering angles. Methane ion CID was studied using a different crossed-beam apparatus[2]. The high-pressure ion source customarily used in this apparatus was replaced by a low pressure source to generate a methane ion beam with LAB energy 750 eV. The ion beam is decelerated to its final collision energy by an exponential lens and crossed at 90° with a supersonic neutral beam of Ar seeded with He. Energy and mass analysis of fragment ions were done by a 90° cylindrical energy analyzer coupled with a quadrupole mass filter.

Figs. 1 and 2 show the center of mass (CM) velocity contour maps for the dissociation of acetone ion into acetyl ion at CM collision energies of 0.65 and 0.45 eV respectively which are equal to and slightly below the thermochemical threshold of the reaction. If contributions of internal energy of acetone ion to the CID reaction are taken as negligible, the CID products will be observed only at the CM in the 0.65 eV experiment (with small reaction cross section) and no products will be observed in the 0.45 eV experiment. The fact that acetyl ions were observed in both experiments indicates that internal energy of acetone ion plays a key role in the CID process. The important and surprising observation from these contour maps is that acetyl ions are predominantly backward scattered with intensity maxima lying well outside the elastic scattering circle (ESC). This can only occur as a result of nearly head-on collisions with conversion of excess internal energy into kinetic energy. Analysis of the recoil kinetic energy of the CID product ions indicates that the conversion energy (internal to translational) is about 2.9 eV, which matches the internal energy content of acetone ions formed in the first electronically excited A-state. This observation strongly suggests the possibility of potential curve crossing which cause the de-excitation of acetone ions (A → X transition) with excess energy being released as kinetic energy. The superelastic backward scattered peak totally disappeared when the electron energy to generate acetone ions was lowered from 70 eV to 12.5 eV as shown in Fig. 3. Most of the observed CID products fell within the ESC and near the CM, confirming the excited state mechanism we propose.

Methane ion CID, on the other hand, shows three distinct maxima in the contour map (Fig. 4). The most important observation here is the peak beyond the ESC in the direction of the ion beam. This could be explained only by the existence of a long-lived excited state (2E (D_{2d})) of CH_4^+

which dissociated to the ground states of CH_2^+ and H_2 with excess energy released as the relative translational energy[3].

The above two cases demonstrate that the excited states of primary ions can play an unique role in CID processes which have not been realized before. More studies are required before a generalized explanation can be proposed.

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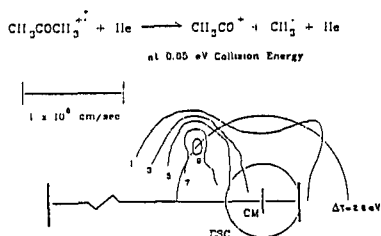


Fig. 1

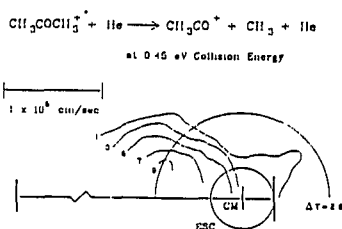


Fig. 2

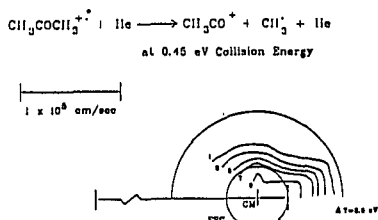


Fig. 3

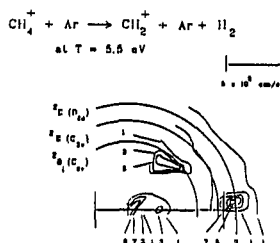


Fig. 4

Fig. 1-3 Scattering contour maps for the CID of acetone molecular ion to acetyl ion on collision with He at labelled CM collision energy. Primary acetone ions in Figs. 1 and 2 were generated by 70 eV electrons; Fig. 3 utilized 12.5 eV electrons. Elastic scattering indicating no energy conversion between translational and internal modes is represented by the circle marked ESC. The circle labelled $\Delta T = 2.9$ eV indicates the location of velocity vectors for CH_3CO^+ products with internal-to-translational energy conversion ($\Delta E = 2.9$ eV) corresponding to the energy difference between the A-state and the ground electronic state assuming that the products are formed in their ground states. Fig. 4 Scattering contour map for the CID of methane molecular ion to methylene ion by collision with Ar at 5.5 eV CM collision energy. The circles indicate the locations of velocity vectors for CH_2^+ products with internal-to-translational energy conversion corresponding to the indicated initial state of CH_4^+ dissociating to ground state CH_2^+ and H_2 .

ANGLE AND ENERGY RESOLVED COLLISION-INDUCED DISSOCIATION STUDIES
OF THE PROPANE MOLECULAR ION.

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The CID of propane was studied at high (keV) and low energies (<100 eV) using a triple sector tandem mass spectrometer and a hybrid crossed beam tandem mass spectrometer, respectively. The hybrid instrument uses a rotatable energy analyzer and quadrupole mass filter that characterize the CID daughter ions by energy, angle, and mass. The angular and energy distribution of each daughter ion can be observed and then mapped in center of mass (CM) coordinates.

Propane molecular ion is representative of polyatomic ions whose unimolecular decomposition is well described by the quasi-equilibrium theory (QET) of mass spectra. The collision-induced dissociation (CID) of the propane molecular ion is characterized by a quick vibrational excitation followed by a slow unimolecular decomposition. The results obtained are consistent with statistical (QET) predictions for ions with a significant amount of internal vibrational excitation. A modest amount of additional energy must be deposited by collision to induce dissociation. The daughter ions are forward scattered close to the elastic scattering circle (ESC). All daughter ions have maximum intensity at non-zero scattering angles. The amount of energy deposited in the collision process increases with the scattering angle. More endothermic daughter ions are scattered at larger angles and appear farther inside the ESC.

Figure 1 shows the distribution of $C_2H_5^+$ from the CID of propane ions with argon collision gas at 100 eV lab. The CM coordinate system is used and shows the ion intensity as a function of velocity about the CM. The CM collision energy is 47.5 eV and the radius of the ESC is 9,950 m/s. The maximum is at a CM scattering angle of 4 degrees and is 700 m/s off axis of the primary ion and is 250 m/s inside the ESC (2.4 eV). In studies at keV energies the energy deposited to produce mass $C_2H_5^+$ averaged about 1.7 eV corresponding to a velocity shift of 20 m/s inside the ESC and accompanied by a velocity spread of 300 m/s resulting from kinetic energy release.

Figure 2 displays the distribution of $C_2H_5^+$ ions resulting from collision of propane ions with helium at 100 eV lab. At this lab energy the CM collision energy is 8.3 eV. The ESC has a radius of 1740 m/s. The $C_2H_5^+$ peak maximum is at a CM scattering angle of 27 degrees and 290 m/s inside of the ESC (2.5 eV) and 700 m/s off axis of the primary ion beam. The distribution is similar to the one obtained from argon collision gas but the velocity spread parallel to the primary ion beam is broader while the velocity spread perpendicular to the primary ion beam is narrower. Distributions obtained at 13.5 eV lab with neon collision gas are also centered 850 m/s off axis of the primary ion velocity vector.

The distribution of $C_2H_4^+$ from collisions of propane with argon at 100 eV is shown in figure 3. The maximum intensity is shifted 340 m/s from the primary ion beam and is 170 m/s inside the ESC (1.6 eV). The maximum intensity is at a CM scattering angle of 2.0 degrees. At keV energies the propane ions are excited by 1.0 eV to produce $C_2H_4^+$ which is a shift in velocity of 12 m/s. At this collision energy $C_2H_4^+$ has a velocity spread of 270 m/s due to kinetic energy release. $C_2H_4^+$ is scattered through smaller angle and has less energy deposited than $C_2H_5^+$.

The angular distribution of the CID process results from the superposition of scattering from collisions and release of kinetic energy when a neutral fragment is ejected from the parent ion and the daughter ion is formed after several vibrational periods of the excited parent ion. It is difficult to separate the contribution from scattering from the contribution of energy release. From the diagrams mentioned above it is apparent that the velocity spread from energy release is less than the velocity spread caused by angular scattering for $C_2H_5^+$.

The result obtained at intermediate and high energies are consistent with studies performed at eV energies. No change in mechanism is discernable between eV and keV CID. The systems studied show significant off axis

scattering from collisions and kinetic energy release. The off axis velocity for $C_2H_5^+$ is remarkably insensitive to changes in CM collision energy. Our prediction is that any difference in CID spectra between beam and quadrupole methods are the result of different acceptance angle of these two methods. The angular and velocity distributions resulting from the CID process must be convoluted with instrumental angular and energy transmission functions to explain the difference in data collected by beam methods and by triple quadrupole instruments. Furthermore the relative abundances of the daughter ions observed should be sensitive to the acceptance angles and energy bandpass of the instrument used to observe the CID process.

Mass 28 from collisions with Argon at 100 eV

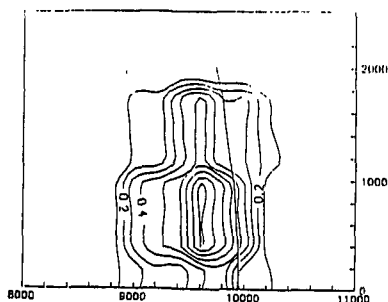
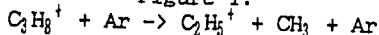


Figure 1.



Mass 28 from collisions with Helium at 100 eV

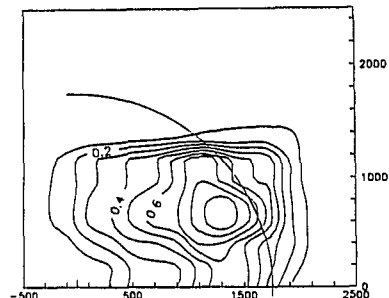
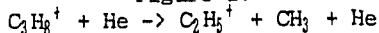


Figure 2.



Mass 28 from collisions with Argon at 100 eV

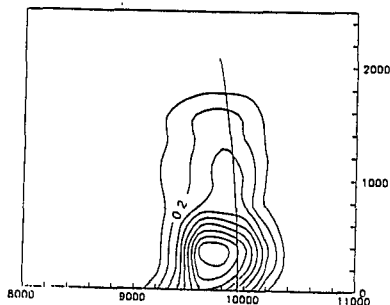
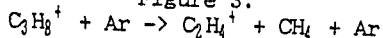


Figure 3.



ACCELERATION REGION PROCESSES

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The method of Acceleration Region Kinetics (ARK), has been applied to investigations of uni-molecular fragmentation of nitrous oxide molecular ions, n-butylbenzene and nitrobenzene-d5[1,2]. The ARK technique is similar to that of Field Ionization Kinetics (FIK) and has the advantage that it may be used with a standard Nier-type source.

In the reaction



unimolecular dissociation occurs when m_1^{z++} has fallen through a fraction b of the accelerating potential V_0 ; the daughter ion falls through the remaining fraction $(1-b)$. When $b = 0$, m_2^{y+} is formed in the source; when $b = 1$, m_2^{y+} is formed in the 1st FFR and the apparent mass/charge ratio is given by $m^* = m_2^2 x / m_1 y^2$. For decompositions throughout the acceleration region the apparent mass-to-charge ratio, m_b^*/e , is given by

$$\frac{m_b^*}{e} = \frac{m_2}{ye} \left[\frac{m_2 x}{m_1 y} b + (1-b) \right] \quad (2)$$

The kinetic energy of the fragment when it exits the acceleration region will be:

$$KE_{m_2} = \left[\frac{m_2}{m_1} x b + y(1-b) \right] eV_0 \quad (3)$$

and the ion will be passed at an ESA setting E_b such that

$$E_b = \left[\frac{m_2 x}{m_1 y} b + (1-b) \right] E_0 \quad (4)$$

The value of b , may be calculated from equation (5):

$$b = [(1 - E_b/E_0) / (1 - m_2 x / m_1 y)] \quad (5)$$

When the process is a charge stripping or charge exchange reaction, as opposed to a decomposition, then the relevant settings of the mass spectrometer are given by:

$$m_b^* = \frac{m_2}{y} (1-b) \quad (6)$$

$$E_b = y(1-b)E_0 \quad (7)$$

A plot of m^* vs. E is referred to as an ion incidence map. Such maps are employed to investigate processes which occur throughout the acceleration region such as:

- (1) half-lives of fast decay processes not seen in conventional mass spectra [1,2]
- (2) field ionization of Rydberg states *e.g.* $Ar^{z+} \rightarrow Ar^{z+}$ by the accelerating field of about $10^4 V cm^{-1}$: A linear pressure dependence suggested that Ar^{z+} ions may be created in this manner, but the results have not been conclusive.
- (3) estimation of ion residence time in the Nier-type source of a mass spectrometer: Comparison of ARK results with those of FIK are being carried out in the hope of determining the absolute age of the ions detected. Compounds employed so far include n-butylbenzene, methyl cyclopentane and cyclohexene.
- (4) an investigation of the production of F^- from SF_6^- : The same diagrams constructed for positive ions may be employed for the study of negative ions. SF_6 has been chosen for study because of its high cross-section and because it has been studied extensively with other techniques.

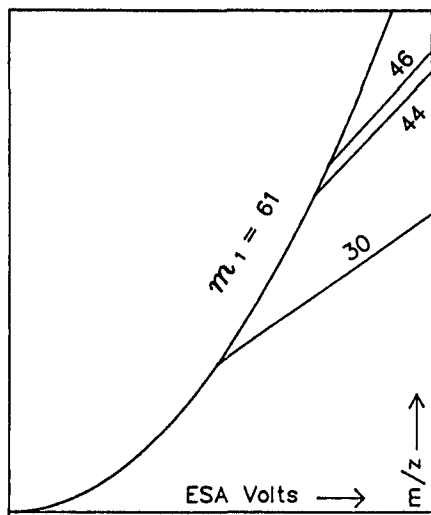
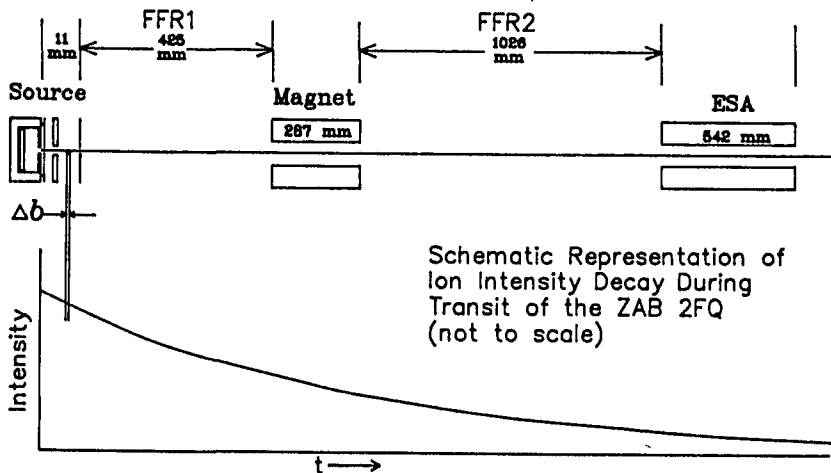


Figure 1. Ion incidence map for nitromethane. The parabola is the locus of all decompositions of the parent ion m/z 61. The line segments represent acceleration region processes for the production of the ion indicated. The points of intersection are located at the terminal point of the acceleration region process: that is, where $b = 1$ and the process occurs in the first field-free region (FFR1).

Signals encountered during Ion Kinetic Energy scans may be plotted on the map and thus the mass of the product ion can be determined.

Figure 2 (below) shows a schematic representation of the acceleration region kinetics (ARK) technique. The production of daughter ions, m_2^{v+} , is measured throughout a window, Δb , which is very narrow. Kinetic data collected in this region is related to a time in the life of an ion which is earlier than that of FFR1 processes. The window is of the order of 10^{-7} s wide and the resolution is of the order of 10^{-5} s.



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A COMPARISON OF INTERNAL ENERGIES OF IONS FORMED BY CHARGE INVERSION COLLISIONAL
ACTIVATION AND DIRECT EXCITATION BY COLLISIONAL ACTIVATION

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Collision induced dissociation has developed as a useful analytical technique; however, for high molecular weight species, it is desirable to deposit large amounts of energy into the ion. The amount of energy deposited can be increased by using multi-collision conditions (e.g., high collision-gas pressures or long collision cells), but such methods reduce sensitivity and could lead to unwanted complications of the data (e.g. thermal rearrangements reactions, etc.). Another approach that has not been extensively evaluated is charge-inversion collisional activation. An evaluation of the mechanism of charge-inversion collisional activation is performed with emphasis on the energetics of the charge-inversion process.

All experiments were performed on a Kratos MS-50TA. Positive ions were formed by electron impact, but the negative ions were formed by dissociative electron transfer. The charge-inversion spectra were obtained by mass selecting a negative ion, colliding the ion with a neutral target atom (keV collision energy). The mass-analysis of the positive ions formed by the high energy collisions is accomplished by reversing the polarity on the second ESA and energy analyzing the resulting positive ions.

The high amount of energy deposited in the charge-inversion reaction is observed from a comparison of the positive and negative ion collision induced dissociation with the charge inversion spectra. The products of the charge inversion reaction correspond to a high degree of fragmentation of the reactant ion, for example, the most abundant ions in the charge inversion spectra of $\text{Fe}(\text{CO})_4^-$ and $\text{Cr}(\text{CO})_6^-$ are $\text{M}(\text{CO})^+$ and M^+ ($\text{M} = \text{Fe}, \text{Cr}$); however, $\text{M}(\text{CO})^{+/-}$ and $\text{M}^{+/-}$ are less than 4% of the total ion current in the collisional induced dissociation spectra of $\text{Fe}(\text{CO})_4^+$, $\text{Cr}(\text{CO})_6^+$, $\text{Fe}(\text{CO})_4^-$ or $\text{Cr}(\text{CO})_6^-$.

Other evidence for the deposition of large amounts of energy in the charge inversion reaction can be estimated by energy loss measurements. The energy loss value is calculated using equation 1;

$$\Delta E = e(V/E_0)(m_f/m_p)(E_1 - E_2)$$

where V is the ion source accelerating potential, E_0 is the potential of the electrostatic analyzer and $E_1 - E_2$ is the energy shift in electrostatic units. Figure 1 shows a plot of the energy loss accompanying charge inversion for several systems the data is presented as a function of the ratio of fragment ion mass to parent ion mass. The energy loss value correlates with the energetics of the dissociation reaction. For example, the energy loss observed for Fe^+ is greater than that observed for $\text{Fe}(\text{CO})^+$. Conversely, collision induced dissociation is accompanied by very small energy loss, and, to a first approximation, is independent of the fragment ion mass.

The excess energy used to induce the charge inversion reaction arises by multiple collisions with the target gas. Pressure studies of the charge inversion signal were consistent with multiple collisions. For example, the relative intensities of the product ion in the charge inversion spectrum of $\text{Fe}(\text{CO})_4^-$ were monitored as a function of the percent attenuation of $\text{Fe}(\text{CO})_4^-$ (pressure). A significant increase in the low m/z ions is observed. Also, the relative abundance of $\text{Fe}(\text{CO})^+$ (normalized to $\text{Fe}(\text{CO})_4^-$) increases with the percent attenuation with both helium and argon collision gases (Figure 2). Note that the charge inversion signal is very small until the main beam is attenuated by more than 10%.

In conclusion, charge inversion collisional activation deposits large amounts of energy into the ion, but the mechanism for energy deposition is multiple collisions, which can reduce sensitivity and lead to unwanted complications of the data. To our knowledge the mechanism of charge inversion has not been determined, but two plausible mechanisms are; i) electron detachment followed by collisional ionization and fragmentation of the re-ionized species, or ii) electron detachment, collision induced dissociation of the neutral fragments followed by reionization of the neutral fragments. Note that both mechanisms are multi-collision processes; therefore, both proposed mechanisms are consistent with the results presented in this paper.

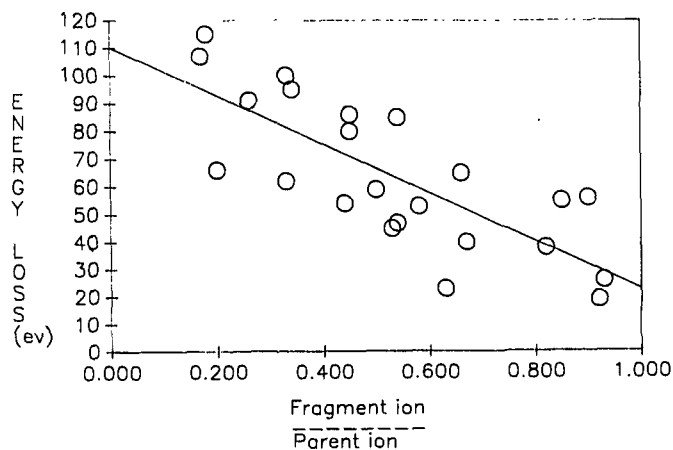


Figure 1. Energy loss accompanying charge inversion for several systems vs fragment ion to parent ion ratio.

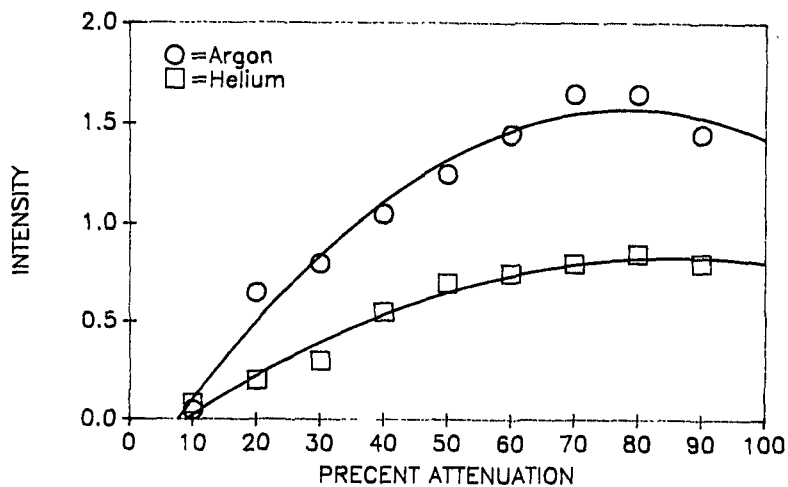


Figure 2. Relative abundance of $\text{Fe}(\text{CO})^+$ (normalized to $\text{Fe}(\text{CO})_4^-$) as a function of percent attenuation.

COLLISION INDUCED DISSOCIATION VERSUS TARGET GAS EXCITATION : A REINVESTIGATION OF KINETIC ENERGY LOSS ACCOMPANYING ACTIVATION OF CHLOROPHYLL-a IONS.*

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The dissociation of precursor ions collisionally activated by keV collisions with a neutral target gas is often characterized by a substantial translational energy loss (ΔE_F) of the projectile ion (1). The extent of this energy loss was recorded by Bricker and Russell (2) for the major fragmentation pathway of chlorophyll-a ($m/z:893 \rightarrow m/z:614$) using different collision gases. They found that the translational energy losses for this fragment (ΔE_F), recorded via a mass-analyzed ion kinetic energy (MIKE) scan, progressively increased as Kr, Ar, Ne and He were successively used as targets. When these results were used to calculate the corresponding translational energy loss undergone by the precursor ion [$\Delta E_F = \Delta E_F \times 893.5/614.2$] (1), values remarkably close to the ionization energies (I.E.) of the respective target gases were obtained. This was interpreted (2) as evidence that, in the keV collision energy regime, CA of the precursor ion and excitation of the collision gas via ionization are competitive processes.

There are several reasons why experimental results of this nature are at best ambiguous and should be treated with caution. Firstly each CID MIKE peak obtained previously (2) represents a composite of fragment ions formed by unimolecular dissociations occurring throughout the length of the field-free region, together with fragment ions formed via CID processes (3). Consequently the energy loss measured as a simple displacement of the MIKE peak when gas is added to the collision cell does not represent the true value of ΔE_F . In fact the observed displacement will depend on the relative lengths of the field free region and the collision cell, on the relative ion optical collection efficiencies from these two regions, and on the degree of precursor ion attenuation (3). If provision is made to electrically float the collision cell these two components can in principle be separated (3). Secondly the values for ΔE_F were obtained (2) from single scans of the appropriate region of the MIKE spectrum with inherently poor signal to noise ratios. Thirdly, the ΔE_F value for xenon as collision gas was not reported (2) and no data were presented for any diatomic gases. In order to clarify these ambiguities it was decided to repeat these experiments with and without electrical floating of the collision cell, with multi-channel accumulation (MCA) of successive MIKE scans, and with a wider range of collision gas types.

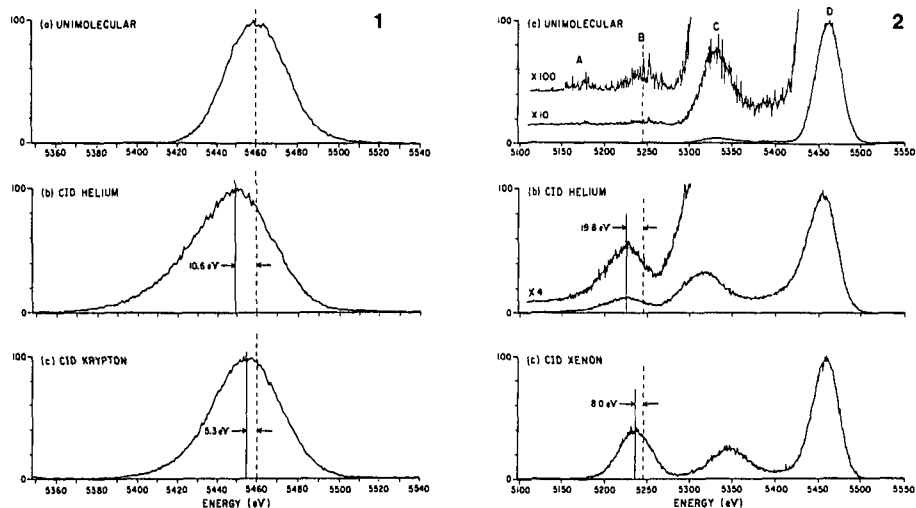
MIKE spectra were recorded using a VG Analytical ZAB-EQ tandem mass spectrometer under the following conditions: initial translational energy of the precursor 7936.0 eV; energy width of precursor ion beam, measured at double-focus point in the absence of collision gas, 5eV at half peak height; attenuation of precursor ion ~ 50%

Figure 1 illustrates typical results obtained with the collision cell at ground potential. On addition of collision gas the peak maximum is not only shifted to a lower energy, but the resultant MIKE peak is now asymmetric. As expected (3) the shift varies with the degree of attenuation of the precursor ion; for example with helium as target gas ΔE_F was found to vary from 6eV at 15% to 15eV at 90% attenuation. If the collision cell is floated at -700 V the MIKE peak shown in Figure 1(a) splits into a number of components (labelled B, C and D) as shown in Figure 2(a) [peak A corresponds to a different fragment mass than m/z 614]. Peak B was identified as corresponding to unimolecular dissociations occurring within the floated cell. Peak C, whose shift is also a function of the float potential but not in accordance with that expected for dissociations occurring within the cell (3), is possibly due to dissociations occurring in the fringe-field between the floated cell and ground. Figures 2(b) and 2(c) show the results obtained with He and Xe respectively as collision gases. The shift in the peak maximum of the true CID component (peak B) from its unimolecular value can now be used to evaluate ΔE_F . This shift was found to be invariant to increasing collision gas pressure. The calculated values for ΔE_F obtained with Ne, Ar, Kr and Xe as collision gases were the same within experimental error. The values obtained for H_2 , D_2 and He were also indistinguishable from one another, but were appreciably larger than that obtained for the heavier gases. The present results are displayed graphically as a function of I.E. in Figure 3 and unambiguously show that there is no correlation between the translational energy loss of the precursor ion (either M^+ or MH^+) and the I.E. of the target gas. In particular both H_2 and D_2 give practically identical shifts to that obtained for He, but have considerably lower ionization energies. Also the shift obtained with Ne is closer in value to that observed with the heavier targets yet its I.E. is only 3 eV lower than that of He.

* NRCC #30275

Compared to previous work (2) this study was conducted with several instrumental advantages; a floatable collision cell, an MCA facility and a tandem quadrupole for unambiguous mass analysis. While the complete interpretation of the results summarized in Figure 3 is open to debate, the earlier interpretation (2), in terms of target gas excitation as a necessary accompaniment to CID of chlorophyll-a ions, is now seriously in doubt.

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Figures 1 and 2: MIKE spectra of M^+ m/z 892.5 from FAB ionization of chlorophyll-a showing the m/z 614 fragment ion region. Figure 1: collision cell at ground; Figure 2: collision cell floated at a potential of -700 V.

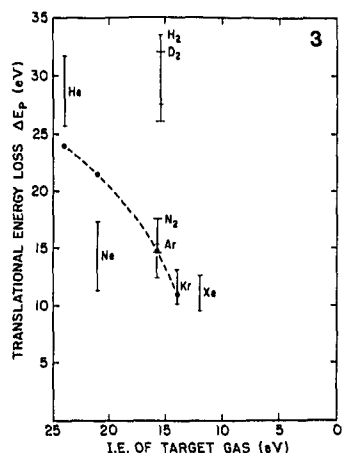


Figure 3. Plot of energy loss (ΔE_p) accompanying CID of the M^+ ion of chlorophyll-a as a function of the ionization energy (I.E.) of the target gas. The data points represented by solid circles and connected by a dashed line are estimated from the plot of the original results of Bricker and Russell (Figure 5 reference 2).

INTERNAL ENERGY UPTAKE OF SURVIVING IONS FROM KILOVOLT COLLISIONS*

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Collisional activation (CA) of ion beams in the kilovolt energy range with target gas gives rise to a wide variety of competing reactions. A proportion of the excited projectile ions will survive the collision without dissociating during the time frame of observation ($\approx 10^{-5}$ s after collision). These survivors have experienced at least one collision which could in turn result in a substantial loss of their translational energy (ΔE). Their energy distribution as observed in the MIKE spectrum is superimposed on those precursor ions unaltered by their journey in the flight tube. These mixed energy profiles account for the shoulder observed on the low energy side of the precursor peak [1-3].

In addition to their translational energy profiles, surviving ions differ from the unaltered precursors by their internal energy content [4]. An example of such a phenomenological observation is illustrated in Figure 1 for the formation of the A_4 and B_4 fragments from CA of Leu-enkephalin impinging on He. The upper part of Figure 1 shows the main beam profile obtained on an hybrid BEqQ tandem mass spectrometer with and without collision gas. The fragment ions A_4 and B_4 resulting from the unimolecular dissociation in q (rf only collision cell) of precursor ion having selected ΔE values are shown in the lower part of Figure 1. It is observed that as ΔE is progressively increased from 0 to 20 eV, the intensity ratio of 397/425 rises from a value of 0.3 to 1.1. Similar observations were made when the same experiment was repeated on a 4 sector tandem mass spectrometer of BEEB geometry. However in the latter situation the relative increase of the 397/425 ratio as a function of ΔE was less pronounced, probably reflecting the different time frame of observation for these two instruments.

The internal energy uptake of surviving ions of different ΔE values was estimated using the intensity ratio of fragment m/z : 91 and m/z : 92 from *n*-butyl benzene obtained on a 4 sector instrument. The relative increase of this ratio with ΔE was compared with the calibrated energy "thermometer" from PEPICO experiments [5]. Thus it was estimated that precursor ions of $\Delta E=12$ eV apparently had 0.6 eV of internal energy. One possible explanation for this lack of correspondence of ΔE with internal energy is that in the usual MIKES experiment no collimation is made in the non-focussing z direction. This lack of collimation would result in the transmission of ions having a broad range of scattering angles, which in turn translates into detection of ionic species having less internal energy uptake than that calculated directly from the value of ΔE estimated from the difference in sector plate potentials. In addition, of course, some of the inelasticity must end up as kinetic energy of the target.

This was investigated by analyzing the main beam energy profile of m/z :912 CsI cluster, and of m/z : 343 from perdeutero acetyl galactose, as a function of z direction using an angle resolving slit prior to the collector. Figure 2 illustrates the distribution of the precursor beam from m/z : 912 upon CA with He projected on the y - z plane. It can be observed that as the angle-resolving slit is progressively moved away from the central ion trajectory, the energy profile becomes broader. Furthermore as the slit position approaches a value of 3 mm the mixed ion profiles composing this peak tail are resolved into two components B and C, corresponding to the unscattered and scattered species. When the same experiment was repeated with Ne or Ar as target gas two changes were immediately obvious. First the energy profile of scattered ions lay much closer to the unscattered ion distribution for any z position. In order to achieve the resolution of B and C profiles with Ne collision gas, it was necessary to move the slit further out toward 4 mm, whereas using Ar collision gas no resolution could be achieved within the limit of the instrument (z direction). Secondly, the low energy tail of scattered ions was less intense compared to the situation when He was used as target gas.

The information accumulated thus far concerning the extent of internal energy uptake of survival ions in kilovolt collisions by MIKES indicates that measurement of a ΔE value, via sector potential measurements cannot be quantitatively transposed into excitation energy of the precursor ion. In the absence of z collimation this disparity is likely to be more severe when He is used as a target since a larger distribution of scattered ion species will be transmitted for a given offset of electric sector field (apparent ΔE).

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Figure 1: Top: Narrow MIKE spectrum of the protonated molecular ion region of Leu-enkephalin, MH^+ : 556. Broken lines represents parent ion profile no collision gas; solid lines, He collision gas (50% attenuation). Bottom: Narrow mass region of fragment formed unimolecularly in q but arising from precursor ions of different apparent translational energy losses, ΔE . (ΔE : 0 eV for centroid energy value of main beam, no gas)

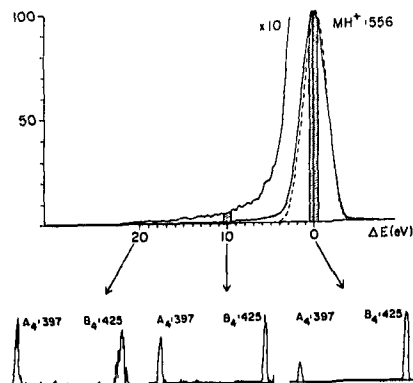
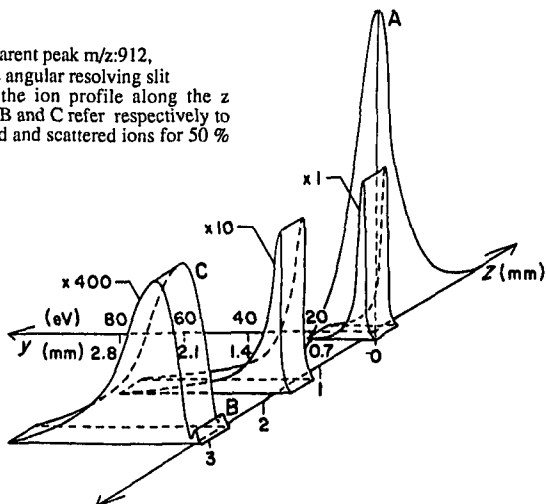


Figure 2: Translational energy distribution of parent peak m/z :912, $(CsI)_3Cs^+$ for different settings of the angular resolving slit (z direction). Peak A represents the ion profile along the z direction for ΔE : 0 eV no gas. Peak B and C refer respectively to the energy distribution of unscattered and scattered ions for 50 % attenuation with He collision gas.



REFLECTION OF POLYATOMIC IONS UPON COLLISIONS WITH SURFACE

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In 1985, a BQ hybrid mass spectrometer (Fig. 1) was developed for the study of polyatomic ion/surface interactions.¹ This instrument uses a solid surface in the second field free region to induce collisional activation (CA) of the incident mass-selected parent ion. A surface as a collision partner is superior to a collision gas in many respects. For example, surface collisions deposit a larger portion of the laboratory kinetic energy in the ions as internal energy with a narrower energy distribution than those of comparable experiments using gaseous targets for collisional activation.² Furthermore, the BQ instrument includes capabilities for linked scanning the two analyzers.³ This allows reflection of ions to be studied in a single mass scan and the present study utilizes this procedure to study polyatomic ion stability upon collision (typically at 35 eV) with a stainless steel surface. The information gained leads to a better understanding of the nature of ion/surface collisions including the competition between surface-induced dissociation and neutralization.

Figure 2 illustrates the four types of experiments performed. All are identical in the first step of mass analysis, i.e. ions produced in the EI source are mass analyzed by scanning the acceleration voltage at a fixed magnetic field strength. The ions are either allowed to collide with the surface (in reflection mode) or to avoid it (in deflection mode) by varying the extraction lens potential after the surface. Before reaching the detector, the ions traverse a quadrupole mass filter either linked to the first analyzer in a neutral loss of zero scan or operated in the rf-only mode. In the Reflection Spectrum (Fig. 2a), the two analyzers are linked and only those ions which survive collision upon the surface without a change in their mass-to-charge ratio or charge state are recorded. When the ions are steered past the surface without collision, the spectrum is referred to as linked analyzer Deflection Spectrum (Fig. 2b). In contrast, when the quadrupole is operated in its rf-only mode, all ions emerging from the surface reach the detector. If collision upon the surface is permitted, the spectrum is modified by the extent of neutralization (or charge changing, in general) of an incident ion, without contribution from fragmentation, and is referred to as the Reflection and Dissociated Ions Spectrum (Fig. 2c). Otherwise, the mass spectrum is obtained which is also labeled as single mass analyzer Deflection Spectrum (Fig. 2d).

Two examples are offered in Fig. 3 and 4. Comparison of the percent relative abundances of ions in the linked analyzer deflection and reflection spectra of phenetole (Fig. 3) reveal that m/z 63 reflects particularly well. Other particularly stable ions include $C_6H_5^+$ (m/z 50) and $C_6H_7^+$ (m/z 65). These ions are less susceptible to fragmentation or neutralization and thus reflect in greater yield than other ions in the spectra. Some ions which reflect well have an even number of electrons others do not. Molecular ions and saturated hydrocarbon ions are relatively unstable in the reflection spectrum. Comparison of the reflection spectrum of phenetole with the spectrum of reflected and dissociated ions reveals that charge changing (neutralization) is the major loss process except that m/z 79 and m/z 66 dissociate readily. Results obtained for limonene (Fig. 4) show that the even-electron ion $C_{10}H_{17}^+$ (m/z 67) and the odd-electron ion $C_{10}H_{15}^+$ (m/z 68) have nearly equal abundances in the deflection spectrum but only the former even electron ion reflects. On the other hand, $C_{10}H_{13}^+$ dissociates extensively while $C_{10}H_{11}^+$ does reflect well. From these data and others, it is clear that the ease of neutralization with the surface does not parallel that of gas phase charge exchange processes although the ease of dissociation can be predicted from a knowledge of gas phase collisional activation.

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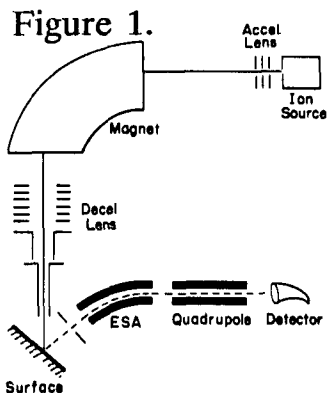


Figure 2.

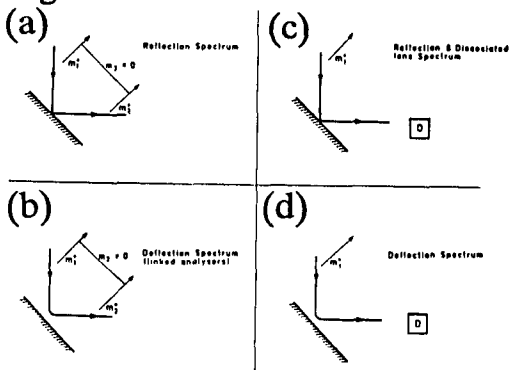


Figure 3.

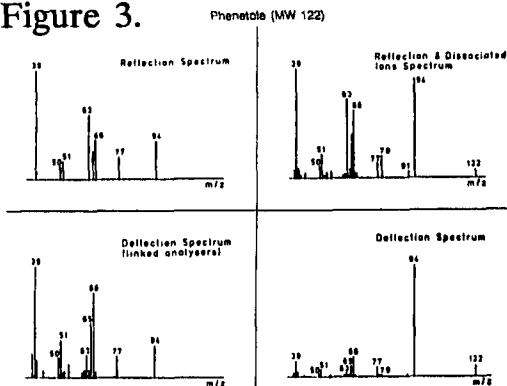
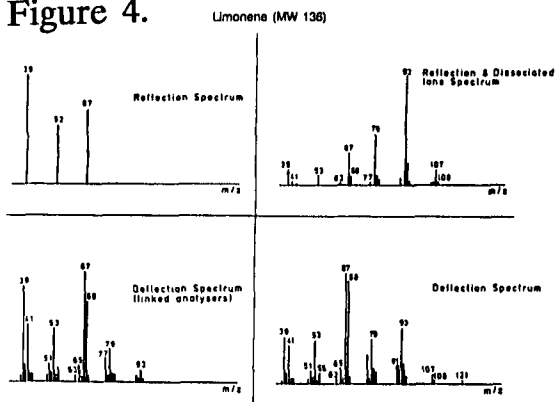


Figure 4.



EVALUATION OF PHOTON INDUCED DISSOCIATION IN A FOUR-SECTOR MASS SPECTROMETER.

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Since the installation of the tandem mass spectrometer at M.I.T. we have acquired the collision induced dissociation (CID) mass spectra of numerous samples from a variety of compound classes in the mass range from 100-3500 daltons. During these studies parameters such as collision gas and collision energy have been varied in an effort to define the limits of this process. A major limitation is the decreased efficiency of fragmentation with increasing mass, estimated to be only a few percent at m/z 2000. This is thought to be a consequence of the increased degrees of freedom available to dissipate the energy with increasing mass, fixed maximum kinetic energy of the precursor ions (10 kV in the JEOL HX110) and the limited amounts of collision gas which can be introduced into the high vacuum region. In an attempt to overcome the problems associated with the gas collision process, we have investigated the feasibility of photon induced dissociation (PID) at 193 nm in a magnetic tandem mass spectrometer.

There have been several reports of PID of underivatized peptides using excimer lasers in ion storage devices (1,2), however, the experimental parameters necessary to determine if it would be a useful technique for tandem magnetic deflection instruments have not been defined. Therefore, the efficiency of PID, the effect of laser power on the type and extent of fragmentation and a comparison with gas collisions at the same sampling frequency were investigated on a JEOL HX110/HX110 tandem mass spectrometer equipped with a multichannel array detector (3). A collision cell was constructed that can be electrically floated to 10 kV and permits either laser or gas induced fragmentation. Preliminary experiments were performed with a model R2440 excimer laser on a two week loan from Questek, Inc. Billerica, MA. This laser has a 15 nsec pulse width, 100 Hz repetition rate and 275 mJ maximum power at the wavelength employed in these experiments, 193 nm. The laser emits a beam profile of 25x6 mm which is collimated by a cylindrical lens to a 22x0.5 mm photon beam profile at the point of intersection with the ion beam. The laser beam enters the mass spectrometer through a 14 cm diameter fused silica window located directly above the collision cell. In the interaction region the laser beam is perpendicular to the plane of the ion beam and its long axis is parallel to the ion flight path. The 193 nm line of the excimer laser was chosen because peptides absorb strongly at this wavelength and it has sufficient energy to cleave a chemical bond.

Due to the low overall sampling frequency in our initial experiment, 0.01% compared to conventional gas collisions, we were limited to recording PID MS/MS spectra of samples which produced abundant secondary ion yields in MS-1. One such example, angiotensin III produced a PID MS/MS spectrum which exhibited all the fragments that are observed in a conventional gas collision experiment, i.e. a_n and d_n , including the d_4 ion for Ile (Figure 1b). Furthermore, the overall sensitivity of the PID spectrum was significantly better than that of a CID MS/MS spectrum acquired with the same low duty cycle (Figure 1c). A plot of the relative abundance of the precursor and product ions as a function of laser power (Figure 2) showed increasing immonium and decreasing high mass ion abundance with increasing laser power and vice versa. These data indicate that PID is a viable technique. Presently modifications are being made which will increase the effective PID duty cycle from 0.01 to 5-10%. These modifications will be combined with a pulsed Cs^+ gun which will produce precursor ions only when the laser is on. This will result in an effective laser/ion beam interaction frequency of unity and permit a detailed comparison of PID versus CID MS/MS.

Acknowledgement. This work was supported by a grant from NIH (RR00317) to K. Biemann.

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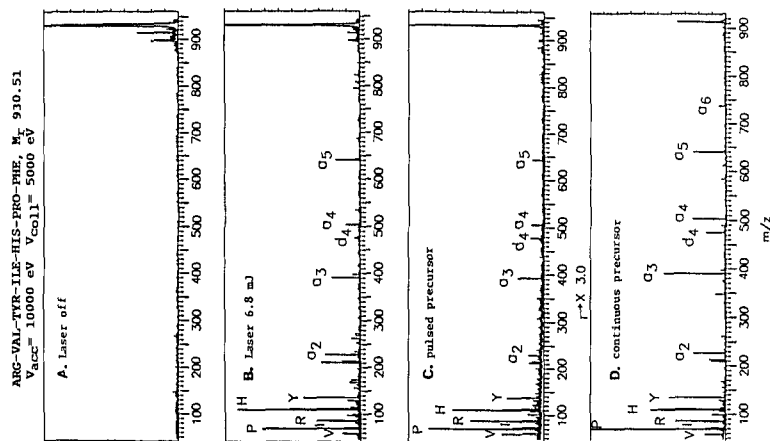


FIGURE 1. Comparison of the efficiency of PID versus CID MS/MS for Angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe, M_r 930). In A-C, the precursor ions are pulsed into the collision cell at 100 Hz with a pulse width=2.2 μ sec (T_2 , Figure 4). A) Laser off, B) laser on, 6.8 mJ/pulse and C) helium collision gas. D) Conventional helium collision spectrum. The same integration time was used for the acquisition of all four MS/MS spectra.

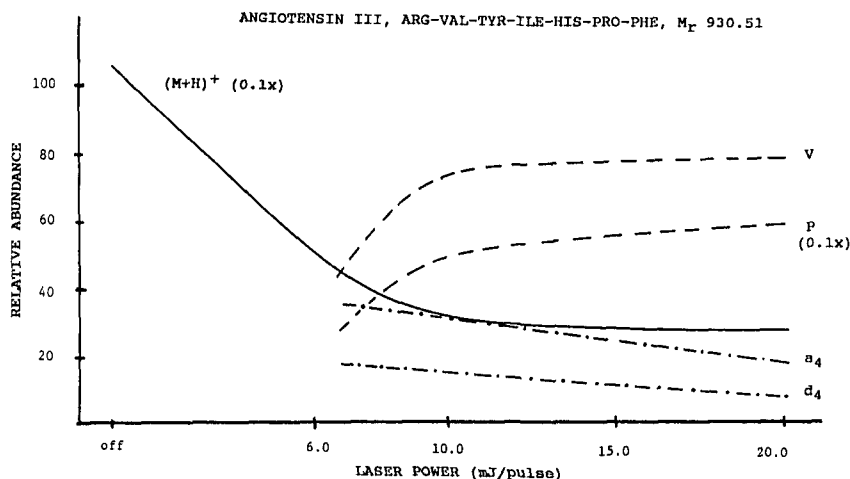


FIGURE 2. Plot of the change in relative abundance of the precursor ion of Angiotensin III $(M+H)^+$ and several of its' product ions, a_4 and d_4 , and the immonium ions for Val (V) and Pro (P), as a function of laser power (mJ/pulse) for PID MS/MS. As the laser power increases the yield of the immonium ions increase while the $(M+H)^+$ and higher mass fragment ions decrease in relative abundance.

MEASUREMENTS OF DAUGHTER ION MASS SPECTRA IN A REFLECTING TIME-OF-FLIGHT MASS SPECTROMETER

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Measurements of daughter ion mass spectra arising from decomposition of a given parent ion provide useful structural information about the parent. Such measurements can be carried out on a reflecting time-of-flight mass spectrometer [1] by determining the correlation between neutral and charged daughters from decomposition of parent ions in the first leg of the flight path. This gives information about the decay paths of the parent ions.

Efficiency of the Correlated Measurement (MS/MS)

For a given decay, the number of events when both neutral and charged fragments are detected is measured by the number of counts (N_{coin}) in that peak in the correlated spectrum. The total number of daughter ions detected from this decay (N_+) is measured by the number of counts in the corresponding peak in the overall reflected spectrum. $\epsilon_n = N_{\text{coin}}/N_+$ is therefore a direct measure of the efficiency of detecting a neutral fragment when the corresponding daughter ion has been detected. Fig. 1 shows the ratio N_{coin}/N_+ as a function of m^*/m for $[M+H]^+$ and $[M+Ag]^+$ decay in GGF (m^* and m are the masses of the daughter ion and parent ion, respectively). As might be expected the efficiency decreases as the energy of the neutral fragment becomes smaller.

The total number of neutrals detected from decay of a given parent ion N_n is given by the number of counts in that peak in the neutral spectrum. The ratio of $N_{\text{coin total}}$ to the total number of neutrals detected gives the average efficiency $\epsilon_+ = N_{\text{coin total}}/N_n$ for detecting charged daughters from the decay of a given parent when the corresponding neutral fragments have been observed. Fig. 2 shows the average efficiency for detecting charged daughters as a function of secondary ion acceleration voltage for $[M+H]^+$ and $[M+Na]^+$ decay in GGF.

Recently we reported our first measurement of the efficiency of such correlated measurements [2]. This has now been considerably increased by enlarging the front aperture of the ion reflector and increasing the acceleration voltage of the secondary ion to increase the transmission and the detection probability. If we assume the efficiencies for detecting the neutral and charged daughters are independent, we may estimate the overall efficiency for detecting both charged and neutral fragments from any decay as the product $\epsilon_n \epsilon_+$. From Fig. 1 and Fig. 2, with 10kV acceleration voltage, a 25% efficiency for detecting both charged and neutral daughters from a typical decay ($m^*=m/2$) was achieved, an improvement by an order of magnitude over our previous results.

The Resolution of Daughter Ions

When the ion mirror is optimized for the parent ions, it produces a considerable improvement in their resolution. Fig. 3 shows the mass spectra of $[M+H]^+$ ions of melittin, both with and without the mirror. The improvement is then less for daughter ions, since the daughters do not spend a long enough time in the mirror to provide full compensation for their velocity spread. However, the electric field in the mirror can also be optimized for a given daughter ion (instead of the parent); under this condition the parent will pass through the mirror. For a parent of mass ~ 1000 u, unit daughter mass resolution may be obtained for all daughters by recording the spectrum in several segments. For example, Fig. 4 shows the daughter ion doublet at masses 278/279 from the $[M+H]^+$ parent ion of Leu-enkephalin with corresponding adjustments in the mirror voltage. Clearly the resolution for the daughters is greatly improved when the voltage on the ion mirror is optimized for the daughters instead of parents. Thus this technique provides satisfactory daughter ion resolution coupled with high efficiency.

Applications of the Correlation Method to Larger Peptides

The use of the correlation method to produce daughter ion mass spectra has been applied to some larger peptides, e.g. proenkephalin, LHRH, tyrothricin (a mixture of cyclic decapeptides of masses ~ 1300 u) α -neoeendorphin, substance P, α -endorphin etc. Considerable structure and sequence information can be extracted from the daughter ion spectra from metastable decay. Fig. 5 shows the daughter ion spectra of α -endorphin.

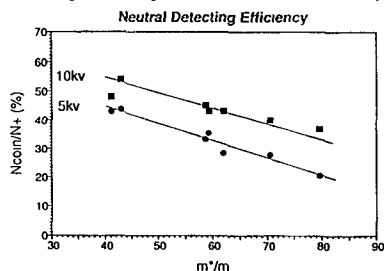


Figure 1

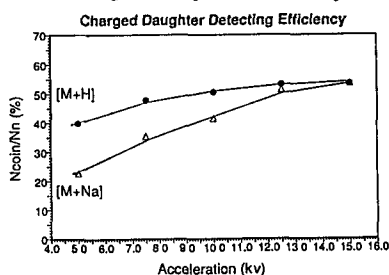


Figure 2

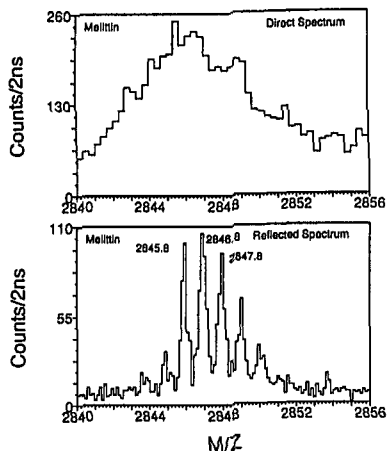


Fig. 3 Direct and reflected mass spectra of melittin.

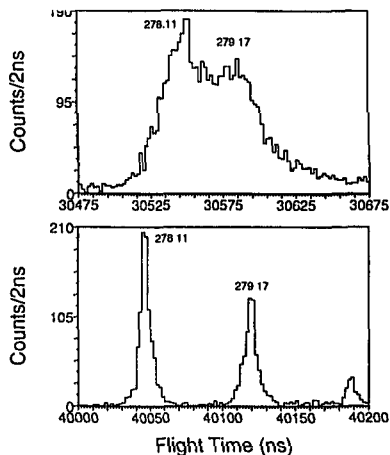


Fig. 4 Daughter ion doublet from the $[M+H]^+$ parent ion of Leu-enkephalin with the mirror optimized for the parent ion (top) and for the daughter (bottom).

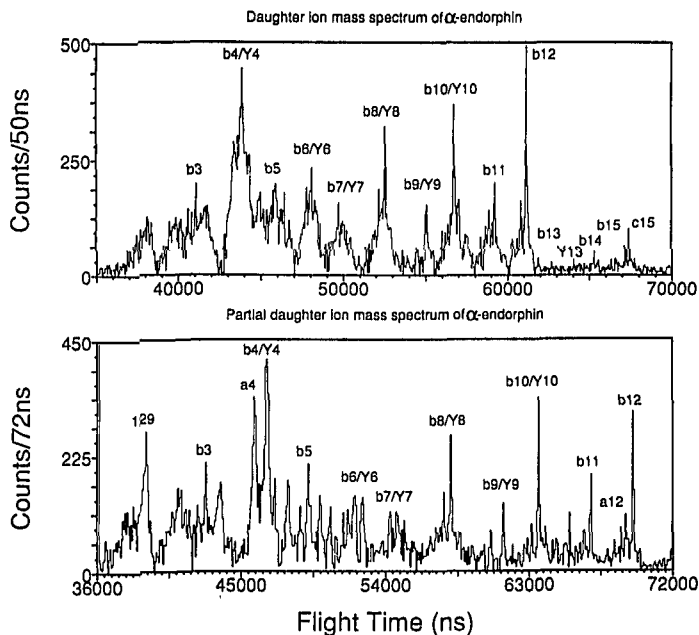


Fig. 5 Daughter ion mass spectra of α -endorphin with mirror optimized for the parent (top) and for the daughter b12 (bottom).

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CHARGE SEPARATION AND ELECTRON CAPTURE INDUCED DISSOCIATION OF DOUBLY CHARGED PORPHYRIN IONS

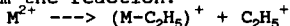
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Electron ionization of a porphyrin forms abundant doubly charged ions (Fig. 1). The gas-phase chemistry of doubly charged porphyrins has been little studied and mass spectrometric methods of porphyrin analysis that incorporate the propensity of porphyrins to form doubly charged ions have not been developed. Reported here are preliminary results from a study of the gas-phase decompositions of doubly charged free-base and metal containing alkylporphyrins. Doubly charged porphyrins were observed to undergo four types of decomposition reactions: a) metastable ion decomposition (Fig. 2a), b) collision induced dissociation (CID, Fig. 2b), c) charge separation (Fig. 2c), and d) electron capture induced dissociation (ECID, Fig. 2d). The charge separation and ECID results are discussed here.

All spectra were acquired using a BE instrument. Ion source voltage (4.5 keV) and magnetic field were fixed to allow doubly charged ions of interest to enter the reaction region between B and E and the electric sector voltage was scanned from 0-2E so as to detect ionic products formed from metastable ion decompositions, CID, charge separation, and ECID in this region.

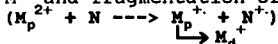
Charge Separation ($M_p^{2+} \rightarrow M_{d1}^+ + M_{d2}^+$). A large kinetic energy release is associated with charge separation of doubly charged porphyrins producing broad, dishd peaks. The kinetic energy release arises predominantly from the Coulombic repulsion of the fragment ions and can be used to calculate the charge separation in the transition state (1). Several different charge separation reactions can occur as witnessed by the multiple charge separation peaks observed (Fig. 2c).

Kinetic energies released were measured at FWHM of the charge separation peak, ($M-29$)⁺ arising from the reaction:



for several different porphyrins. The charge separation calculated from the kinetic energy release data is similar for all the porphyrins studied indicating that the metal has little effect on charge separation (Table 1). Since the diameter of the porphyrin macrocycle is approximately 8 Å, the charges on the transition state ion must be located on opposite sides of the molecule. Note that the charge on the stable doubly charged free-base porphyrin, as determined by molecular orbital calculations, is distributed between the meso carbons and nitrogens, a charge separation of approximately 2 Å.

ECID. ECID consists of two events: charge exchange of M^{2+} with the collision gas to form the M^+ and fragmentation of M^+ (2).



The rare gases did not charge exchange with the doubly charged porphyrins, but the collisions with the rare gases did promote, as do other target gases, collision-induced charge separation ($M^{2+} + N \rightarrow M_{d1}^+ + M_{d2}^+ + N$).

Both isobutane (IP = 11.4-11.7 eV) and ammonia (IP = 10.2 eV) charge exchanged with the doubly charged porphyrins. The charge exchange process was maximized using ammonia at a beam attenuation of 50% (Fig 2d). ECID occurs when the singly charged porphyrin is formed in an excited state (M_p^{+*}) and fragments. The only unambiguous ECID product observed is loss of CH_3 from M_p^+ .

2E Mass Spectra. M^+ formed from M^{2+} by charge exchange has a kinetic energy-to-charge ratio twice that of M^+ formed in the ion source. By using ammonia as the collision gas, setting the electric sector voltage at 2E, and scanning the magnetic sector, a 2E mass spectrum is obtained (Fig. 3). Only the doubly charged species in the mass spectrum that undergo the reaction $M^{2+} \rightarrow M^+$ are observed in the 2E mass spectrum. The 2E scan, therefore, may be of use when analyzing complex matrices for porphyrins.

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 May 21-26, 1989, Miami Beach, FL

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Fig. 1

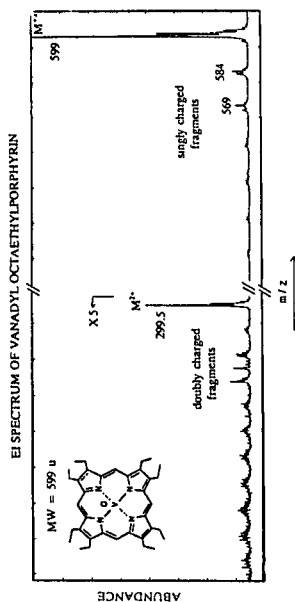


Table 1

PORPHYRIN	KINETIC ENERGY RELEASE (eV)	CHARGE SEPARATION (ANGSTROMS)
etioporphyrin-III	1.62	8.9
nickel etioporphyrin	1.62	9.0
octaethylporphyrin	1.62	9.0
vanadyl OEP	1.59	9.0

Fig. 3

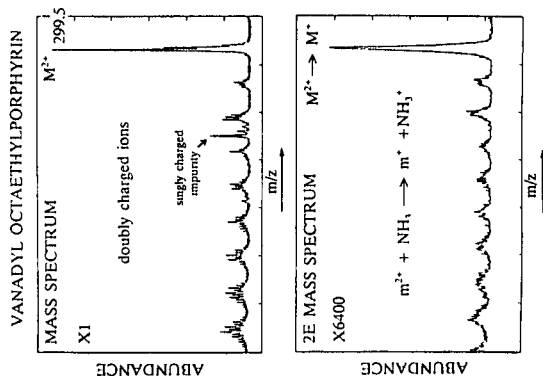
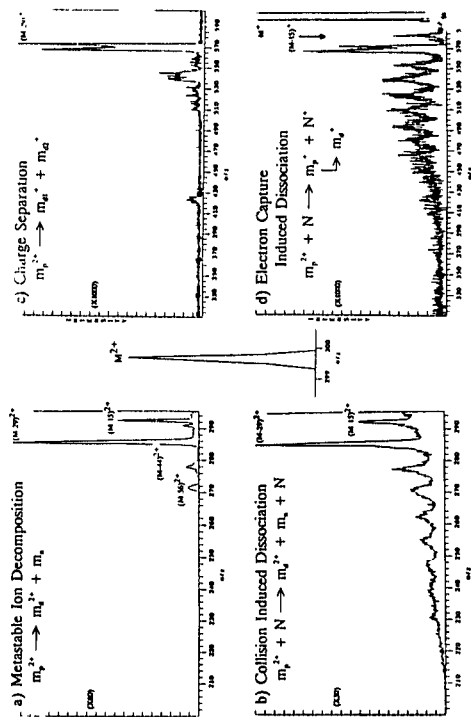


Fig. 2



DISSOCIATION OF LARGE IONS IN FTMS

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Characterization of large molecules by tandem mass spectrometry requires new methods of ion dissociation to yield structurally useful fragments. Dissociation techniques capable of high internal energy deposition, or non-ergodic dissociations should be of increasing value with increasing molecular size. Collisionally activated dissociation (CAD), photodissociation, and surface induced dissociation (SID) in a Fourier transform mass spectrometer (FTMS) are compared.

CAD is the most common technique used in tandem mass spectrometry, with dissociation efficiencies approaching 100% for low mass ions in FTMS. However, when the mass of the precursor ion (m_p) is much larger than that of the target, the center of mass collision energy (E_{CM}) becomes inversely proportional to m_p , thereby reducing the available energy for internal excitation of high mass ions. In addition, the maximum kinetic energy obtainable in FTMS is inversely proportional to m_p . For an ion at mass 4700 undergoing a collision with Ar, ~0.12 eV of internal energy will be deposited (~0.25 E_{CM}) which corresponds to absorption of a single IR photon from a CO₂ laser.

Ions formed by plasma desorption and laser desorption are trapped in an elongated cell of a prototype Nicolet FTMS-2000 (1). CAD of gramicidin D (MW 1881) using pulsed Ar collision gas results in a dissociation efficiency of only a few percent (Figure 1a). Photodissociation with 193 nm deposits 6.4 eV per photon absorbed. Extensive structurally-useful fragmentation is observed for a variety of peptides up to mass 2000 with dissociation efficiencies up to 100% (Figure 1b).

For SID, ions formed by FAB are accelerated into a QQ-FTMS (2) and undergo dissociations on the front screened trapping plate of the cell. Substance P (MW 1348) underwent extensive dissociation with 48 eV collision energies (Figure 2). Few fragments above m/z 600 were observed, even for peptides up to ~3100 MW (e.g., RSBP 3056 MW) indicating substantial internal deposition as reported by Cooks (3).

Using a pair of grounded screens inside the trapping plates of a FTMS cell can increase resolution for high mass ions by up to 4x, consistent with Marshall's observations (4).

Electrospray ionization (ESI) (5), capable of producing highly protonated species from peptides up to 130,000 MW has been coupled to a modified QQ-FTMS (2). The high resolution capabilities of FTMS will make possible analysis of peptide mixtures with unprecedented accuracy. For example, our computer simulations show that with 10⁵ resolution at m/z 1000, substitution of a valine for an alanine (+C₂H₅) in bovine albumin (Ave. MW ~66075) at the 10% level could readily be distinguished (Figure 5), making this a very promising technique for monitoring mutations in peptides. Preliminary results show abundant multiply charged ions for a variety of peptides up to ~17,000 MW. Photodissociation (193 nm) of ($M + 2H$)⁺⁺ of gramicidin S (MW 1142) formed by ESI produces abundant ($M - H$)⁺ and sequence fragments (Figure 4) indicating this will be a useful technique for obtaining structural information from large molecules.

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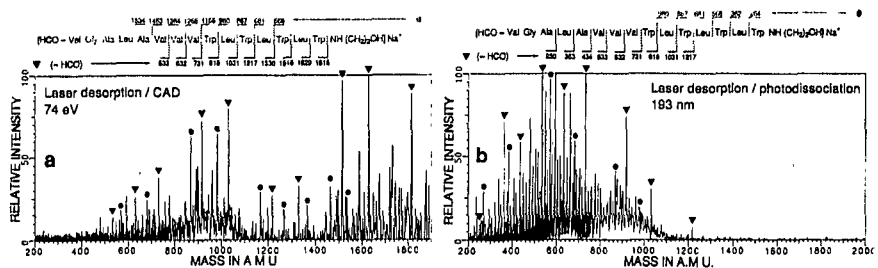


Figure 1: Dissociation of $(M + Na)^+$ of gramicidin D formed by laser desorption by a) CAD (25 scans) and b) photodissociation with 193nm (1 scan).

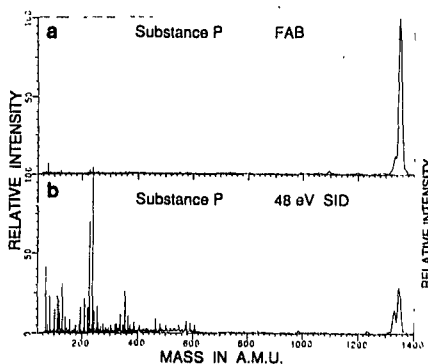


Figure 2: a) FAB and b) SID spectrum (48 eV collision energy) of substance P.

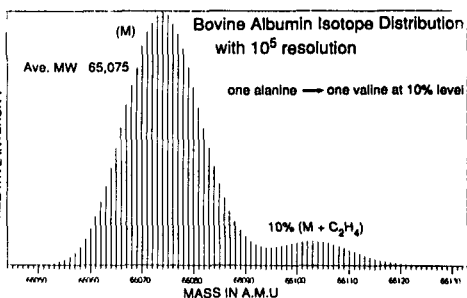


Figure 3: Simulated isotope distribution for bovine albumin with a valine substituted for an alanine at the 10% level.

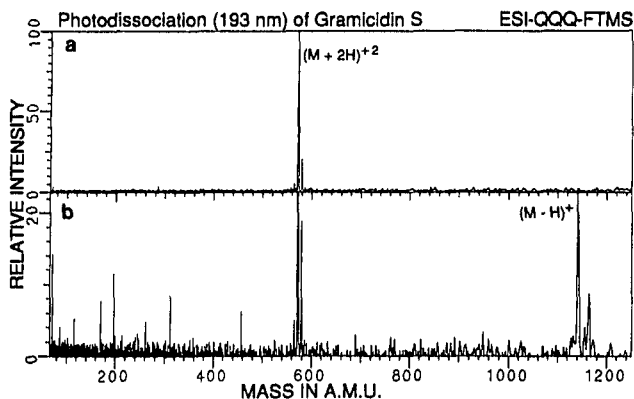


Figure 4: a) ESI-QQQ-FTMS mass spectrum of gramicidin S and b) photodissociation (193 nm) of $(M + 2H)^{++}$.

FURTHER INVESTIGATION OF THE ENERGETICS OF CHARGE REMOTE FRAGMENTATION

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INTRODUCTION

The internal energy requirements for charge-remote fragmentation have been estimated by employing several different methods. Estimates have ranged from less than 2eV^1 to greater than $10\text{eV}^{2,3}$. A theoretical treatment has yielded activation energies of 4 and 5eV for the 1,4 and 1,2 hydrogen elimination mechanisms, respectively⁴. Particularly troubling have been the difficulties in observing charge-remote fragmentation by using low kinetic energies and CA with massive targets. Because of these problems, an extended study of the charge-remote fragmentations from a model ionic species has been performed.

In all of the employed methods, the amount of kinetic energy deposited into the internal modes of the incident ions is inferred indirectly. However, the kinetic energy displacement of the daughter ionic species can be measured directly from which the kinetic energy displacement of the population of parent ionic species that fragmented to the selected daughter species can be directly calculated. From the kinetic energy loss, the internal energy uptake can be calculated assuming no internal energy uptake by the target gas and no significant scattering of the parent population⁵. This method is well suited to sector instruments and appears to be more direct than other methods. It was employed in this study.

EXPERIMENTAL

All experiments were performed on a Kratos MS-50TA (Triple Analyzer) of EBE configuration. The parent ionic species were selected by MS-I, the magnet and ESA₁. Collision gas was admitted to the second collision cell which is located between the magnet and ESA₂. Beam suppression in CA was typically 25% which results in over 85% of the collisions being single events. ESA₂ was controlled by a custom-built MID which generates sweeps of programmable width and which performs like a precision programmable voltage divider. Sweeps in energy space by ESA₂ was performed in peak-switch format with constant proportional energy widths. The final β slits were closed sufficiently to make the peak of the parent species be Gaussian in energy space. Data were collected by a Nicolet 1170 signal averager then transferred to the host Data General Nova 4X computer. The peaks were fit by Gaussians from the parameters of which were calculated the kinetic energy loss, ΔT , of the population of the parent ionic species that gave rise to the selected daughter ionic species, and the energy width and kinetic energy release, T_r , of the daughter species. From the kinetic energy loss was calculated the apparent internal energy gain, Q_a , of this specific population of parent ions by assuming no internal energy gain by the target gas and no scattering of the beam of parent ions by CA.

The model species used in this study was n-dodecyl sulfate anion (LS-) which was generated by Ar FAB of a solution of sodium n-dodecyl sulfate (SLS) in triethanolamine (TEA). This species was chosen because the spectrum of charge-remote fragmentations is cleaner than for n-alkyl carboxylate anions in that the first seven fragmentations give essentially single peaks and there is less interference from other fragmentations.

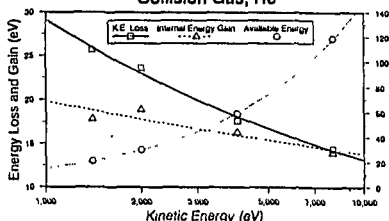
RESULTS AND DISCUSSION

Parameters relating to the energetics of fragmentation were determined for six of the first charge-remote fragmentations from n-dodecyl sulfate anion at kinetic energies of 8.0, 4.0, 2.0, and 1.4keV and with He and Ar as collision gasses. A summary of the results are presented in the graphs later in this section. There are several interesting trends in the data:

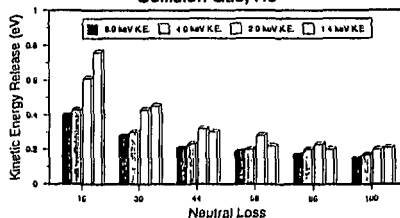
1. At a set kinetic energy of the parent ionic species and with a given collision gas, there are no large individual variations from the average of the data over the series of charge-remote losses except for methane.
2. The average values of kinetic energy loss and corresponding internal energy gain with He as collision gas are much greater than the corresponding values with Ar as collision gas.
3. With He as the collision gas, the average values of kinetic energy loss increase dramatically with decreasing kinetic energy. The corresponding values with Ar as the collision gas remain almost constant. In both cases the kinetic energy losses for methane loss start diverging from the average as the kinetic energy is lowered. In addition, the values for ethane and hexane losses decrease as kinetic energy is decreased with Ar CA.

4. At a set kinetic energy and with a given collision gas, kinetic energy release (KER) decreases as the mass of the neutral loss increases. With Ar as collision gas, the KER for a given product, except for methane loss, increases slightly with decreasing kinetic energy. But with He, the corresponding values of KER increase significantly as the kinetic energy is decreased and at kinetic energies of 2.0 and 1.4 keV, become such that energy widths of the losses are constant.

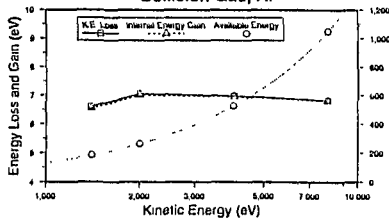
**KINETIC ENERGY CONVERSION
AS A FUNCTION OF KINETIC ENERGY
Collision Gas, He**



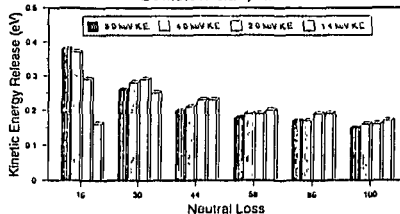
**KINETIC ENERGY RELEASE
AS A FUNCTION OF KINETIC ENERGY
Collision Gas, He**



**KINETIC ENERGY CONVERSION
AS A FUNCTION OF KINETIC ENERGY
Collision Gas, Ar**



**KINETIC ENERGY RELEASE
AS A FUNCTION OF KINETIC ENERGY
Collision Gas, Ar**



CONCLUSIONS

The divergent trends in kinetic energy loss and internal energy gain with respect to kinetic energy indicate some fundamental difference in the mechanisms of CA between He and Ar. The sense of the trend with respect to available energy in the case of He strongly indicates that excitation of He does not play a role. With He CA, the trend of KER's that ends in constant energy widths at 2.0 and 1.4 keV kinetic energies indicates that some other mechanism besides KER is dominating peak broadening. If the difference between He and Ar CA is scattering in the He case, rms values of the scattering angle, θ_s , are .21°, .34°, .54°, and .63° for kinetic energies of 8.0, 4.0, 2.0, and 1.4 keV, respectively.

The diverging values of kinetic energy loss and corresponding internal energy gain for methane loss over the average values as kinetic energy is decreased is consistent with a postulated difference in mechanism for this loss.

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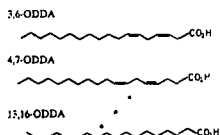
CHARGE-REMOTE FRAGMENTATION OF A SERIES OF HOMOCONJUGATED OCTADECADIENOIC ACIDS: A CA MS/MS STUDY

J.S. Crockett and M.L. Gross

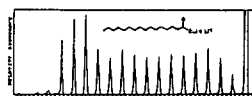
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A nearly complete series of Octadecadienoic Acids was utilized to investigate the effect of location of a 1,4-double bond moiety upon charge-remote fragmentation (CRF). The series also gave the opportunity to investigate the effectiveness of activating various ionic precursors; i.e., Lithium Cationized $[M - H + 2Li]^+$, Barium Cationized $[M - H + Ba]^+$, and Carboxylate Anion $[M - H]^-$.

OCTADECADIENOIC ACIDS



NORMAL CHARGE-REMOTE FRAGMENTATION MECHANISM



Charge-remote fragmentations have been studied in previous work involving a wide variety of compounds and different precursor ions.¹ This is the first time a continuous series has been used, allowing investigations of CRF as a function of ionic precursor and location of the double bonds.

CAD of an straight chain fatty acid gives a characteristic CRF pattern for which the 1,4-H₂ elimination mechanism has been proposed.² The methylene separated double bonds of the octadecadienoic acids interrupt the normal CRF pattern with a characteristic identifiable pattern.

EXPERIMENTAL

The acids were synthesized by William Christie and obtained from the Hormel Institute, Austin, Minn.³ Ions were desorbed from three different matrices by adding an acid (ca 1 ug) to a matrix on a copper probe tip. Matrices used were 3-NBA/LiI/GLY, Ba(OH)₂, and triethanolamine.

CAD spectra was acquired with a Kratos MS-50 triple analyzer tandem mass spectrometer (EB/E). It is equipped with a commercial Kratos FAB source, an Ion Tech saddle field FAB gun, and a Kratos DS-55 data system.

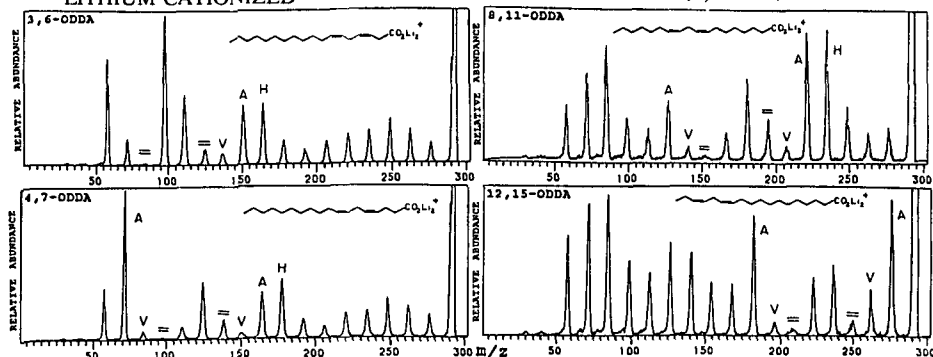
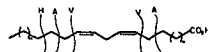
The FAB-desorbed precursor ions were produced by bombardment of sample/matrix with 7-8 keV Ar atoms followed by acceleration through an 8-kV potential. Mass-selection was achieved by using MS-I, CA with He in the collision cell followed, reducing the beam by 30%. Scanning MS-II resulted in structurally informative MIKES spectra.

RESULTS AND DISCUSSION

LITHIUM CATIONIZED

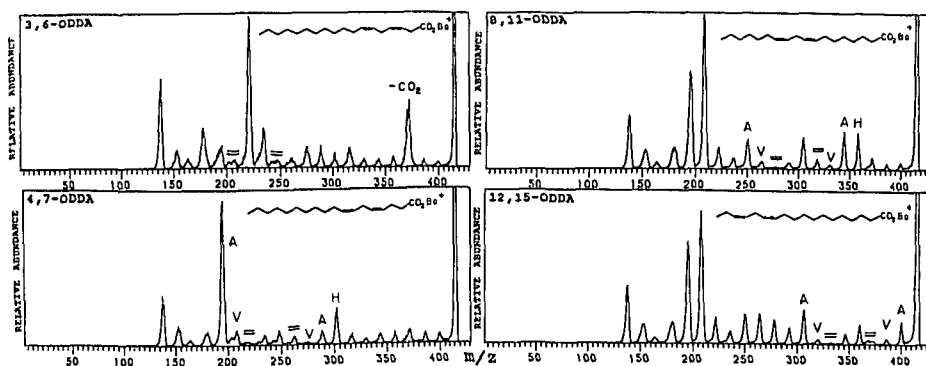
LEGEND

V ventic cleavage
A allylic cleavage
H homolytic cleavage



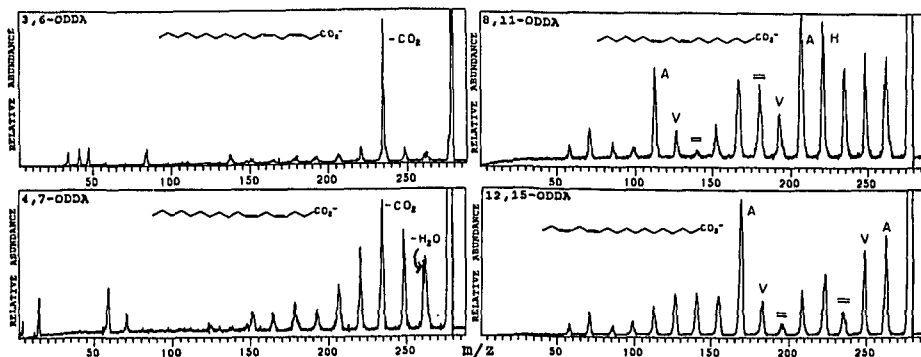
The lithiated species undergo facile CFR upon CA to give the most uniformly consistent spectra, containing principally CRF, resulting in reliable structural information with little dependence upon the location of the double bonds. Charge-driven neutral losses do not interfere with the CRF. Fragmentations due to competing charge-proximate, homolytic reactions occur with similar relative abundance as CRF, and are seen at the low mass end of the spectra. They do not interfere with structural identification. The lithiated precursor is the most generally applicable to structural determination.

BARIUM CATIONIZED



The bariated species undergoes facile CRF; however, the CRF appear suppressed by charge-proximate homolytic cleavages. Structural identification is still possible and the location of the double bonds has little effect upon the use of the bariated species for structural determination. In the case of the 3,6-acid, however, the CRF is complicated by the charge-driven CO_2 loss.

CARBOXYLATE ANION



CRF of the carboxylate anion is significantly affected by the location of the double bonds. Charge-driven neutral losses dominate the spectra when the double bonds are within a distance of five carbon atoms from the carboxyl group. Beyond this point, CRF is intense enough to be structurally informative. The 3,6- acid loses mainly CO_2 , the 4,7- acid loses both CO_2 and H_2O . Water loss also occurs when the double bonds are in the 5,8- and 6,9- positions interfering somewhat with CRF. As the functional group moves further down the alkyl chain, the intensity of CRF increases as does the structural information obtained from it.

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STRUCTURE INFORMATION DERIVED FROM KINETICS OF DISSOCIATION REACTIONS

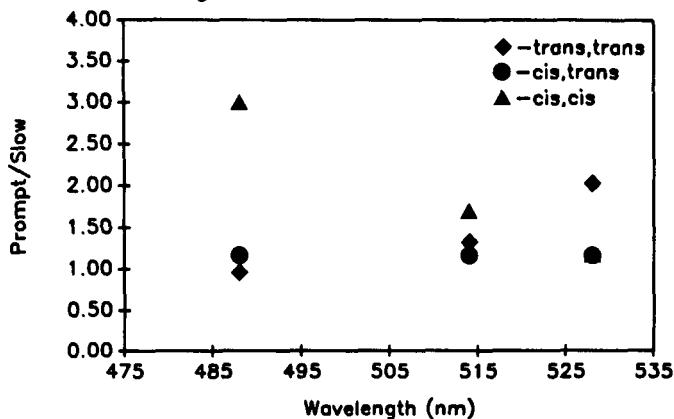
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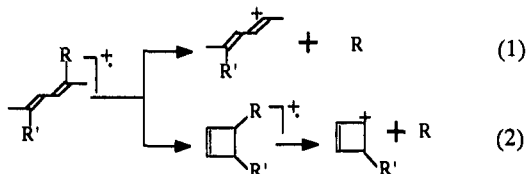
The unimolecular chemistry of activated diene ions has been studied by a variety of gas-phase ion chemistry techniques. One of the long standing issues is the occurrence/non-occurrence of diene radical cation to cyclobutene radical cation rearrangement reactions prior to dissociation. Our studies probed this question; the method used is laser-ion beam photodissociation and dynamical data on the photodissociation reaction is obtained by using a biased activation cell.¹

The laser-ion beam photodissociation experiments were performed using a Kratos MS-50 tandem mass spectrometer and a Coherent Cr-18 argon ion CW laser and components for performing phase sensitive detection.² The laser-ion beam photodissociation apparatus has been modified by the addition of a biased activation cell. The activation cell can be biased (± 0.8 kV) with respect to the adjacent lenses and act like a symmetrical einzel lens decelerating the ions as they enter the cell and then accelerating them as they exit the cell. We recently reported on the novel use of a biased activation cell to study "prompt" versus "slow" dissociation reactions of photoexcited ions. The biased activation cell provides a means of distinguishing ions which dissociate promptly (< 50 ns within the biased activation cell) from those which are longer lived (50 ns to several microseconds and dissociate outside the biased activation cell).¹ The separation of "prompt" and "slow" dissociations can be used to estimate the rates of photofragment ion formation. For example, photodissociation rates of $> 5.6 \times 10^6$ and $5.6 - 0.29 \times 10^6$ can be specified for a 500 amu ion photodissociating within the activation cell and between the exit of the activation cell and the entrance to the second electrostatic analyzer, respectively.

The major emphasis of this paper is on the unimolecular chemistry of diene radical cations, specifically, questions related to direct dissociation versus rearrangement prior to dissociation. As an approach to the question of rearrangement, it is interesting to compare the prompt/slow ratios for the photodissociation reactions of diene radical cations. For example, the *trans,trans*-2,4-hexadiene, *cis,trans*-2,4-hexadiene and *cis,cis*-2,4-hexadiene $C_6H_{10}^{+\bullet}$ ions photodissociate to yield $C_5H_7^{+\bullet}$ and $C_4H_6^{+\bullet}$.³ For the photofragment ion the prompt/slow ratio for *trans,trans*- $C_6H_{10}^{+\bullet}$ is ca. 50/50, for *cis,trans*- $C_6H_{10}^{+\bullet}$ is ca. 60/40, and for *cis,cis*- $C_6H_{10}^{+\bullet}$ is ca. 75/25. Whereas for the photofragment ion the prompt/slow ratio for *trans,trans*- $C_6H_{10}^{+\bullet}$ is ca. 80/20 and for both *cis,trans*- $C_6H_{10}^{+\bullet}$ and *cis,cis*- $C_6H_{10}^{+\bullet}$ is $> 95/< 5$. There is also a distinct wavelength dependence for prompt/slow ratios for the loss of CH_3 from the 2,4-hexadiene isomers as shown in Figure 1 below.



For example, the *cis,trans*-C₆H₁₀⁺ shows no significant wavelength dependence, whereas the *trans,trans*-C₆H₁₀⁺ isomer shows an increase in the prompt/slow ratio with decreasing energy and the *cis,cis*-C₆H₁₀⁺ isomer shows a decrease in the prompt/slow ratio with decreasing energy. In an earlier paper these isomers were differentiated on the basis of kinetic energy release data for the photofragment ions.³ The isomer dependent kinetic energy release data were interpreted in terms of the photoexcited *trans,trans*-C₆H₁₀⁺ ions undergoing a slow rearrangement reaction prior to dissociation. The prompt/slow photodissociation ratio data for the C₆H₁₀⁺ isomers is also consistent with the interpretation of the kinetic energy release data. On the basis of the photodissociation data we propose the following model for photodissociation of diene radical cations.



Reaction 2 corresponds to dissociation of diene radical cations by a rearrangement reaction, while reaction 1 corresponds to direct dissociation. For simplicity, we show the rearrangement reaction occurring through a cyclobutene intermediate; however, dissociation through some other structural intermediate cannot be ruled out.

In general, for the diene systems studied, it was found that ions having an excited state which lies near the energy of the dissociation threshold both prompt and slow dissociation was observed. However, photoexcited dienes formed by absorption of ultra-violet radiation access excited states well above the dissociation threshold and only prompt dissociation is observed. This is consistent with our proposed model as well as field ionization kinetics work by Gross et al.⁴ which shows that diene radical cations with energies near the dissociation threshold dissociate via a rearrangement reaction while ions with higher internal energies dissociate directly. This differs from the results found for transition metal systems which were studied; these systems showed only prompt dissociation products. Also for systems which are thought to undergo photodissociation from a repulsive state, such as the propargyl, chloride and bromide,⁵ only prompt dissociation was observed.

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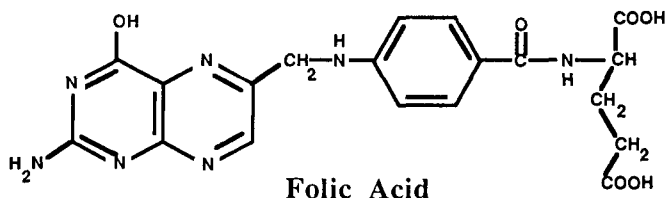
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Influence of Cationizing Metal Ions on Fragmentation in Folic Acid Derivatives

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Folate is an essential nutrient for humans that varies in substitution and oxidation state on its pteridine ring and in the number of γ -glutamyl residues in its peptide chain. Differences in the bioavailability of the various folates which occur in foods have been attributed in part to these structural variations, and provide an impetus for the development of mass spectrometric methods for characterization of folates in foods and determination of bioavailability using stable isotope techniques. Experiments in our laboratories previously demonstrated the potential of FAB ionization and parent ion MS/MS scanning for the analysis of the varied forms of intact folates [1].



Fast atom bombardment mass spectra of folates extracted from biological samples are frequently complicated by cationization by alkali metal cations such as Na^+ and K^+ . We have observed that this effect becomes more pronounced as the length of the polyglutamate chain increases. This effect has the potential to interfere with the identification of naturally occurring folates if alkali metal cations are not sufficiently removed from samples before analysis.

In order to establish fragmentation characteristics of metal ion cationized folates, daughter ion spectra of $[\text{M}+\text{H}]^+$, $[\text{M}+\text{Li}]^+$, $[\text{M}+\text{Na}]^+$, and $[\text{M}+\text{K}]^+$ ions of various folate derivatives were generated using constant B/E and MIKE scans. Daughter ion spectra of $[\text{M}+\text{H}]^+$ ions generated from most folate derivatives yields simple spectra characterized by: (1) loss of the polyglutamate chain, yielding various pteroyl cations; and (2) fragmentation at or adjacent to the pteridine ring. Cationization by Li^+ yields a similar set of fragments, with the metal ion retained in both daughter ions. However, as the size of the alkali metal ion is increased, numerous new kinds of

-
1. A. J. Clifford, A. D. Jones, and S. R. Missler, presented at the 34th Annual Conference on Mass Spectrometry and Allied Topics, Cincinnati, OH, June 1986, pp. 312-313.

daughter ions are observed which arise from various fragmentation pathways. Most of these new fragmentation pathways involve either the loss of small neutral fragments from the polyglutamate chain or the migration of the metal ion from the pteridine ring to the aminobenzoylglutamate portion of the molecule. The larger metal ions (Na^+ , K^+) yielded only minor amounts of either the pteroyl cation or its metallated analog.

Collisional activation experiments (CAD/MIKES) suggest that the decomposition pathways peculiar to the larger cations are facile processes that occur readily without collisional activation. The most apparent effect of the addition of collision gas (He ; $E_{\text{lab}} = 8 \text{ keV}$; 50% attenuation) was an increase in the abundance of the alkali metal cation as a daughter ion.

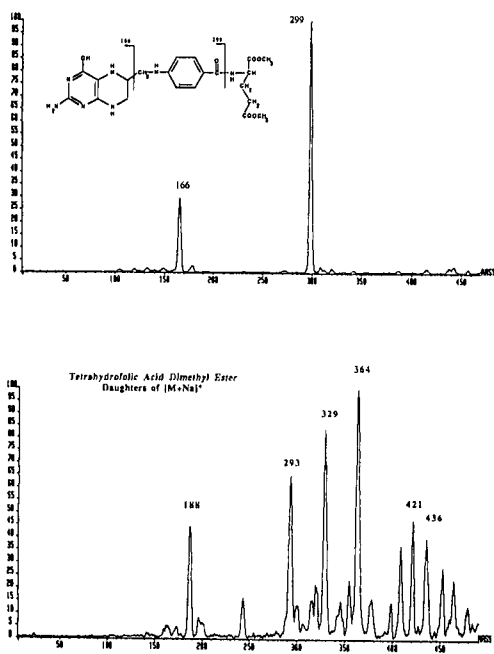


Figure 1. CAD/MIKE Daughter Spectra of $[\text{M}+\text{H}]^+$ ions (upper spectrum) and $[\text{M}+\text{Na}]^+$ ions (lower spectrum) obtained from tetrahydrofolic acid dimethyl ester.

This work has been supported by NIH grant 1R01 DK38637

CONTROLLED ENHANCEMENT OF STEREOCHEMICAL EFFECTS BY MS/MS/MS: ACCESS TO ENDOTHERMICALLY FORMED PRECURSOR IONS WITH UNIQUE DECOMPOSITIONAL PATHWAYS

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A tetraquadrupole MS/MS/MS (Nermag R30-10 modified principally by T. Blasco and S. LeMeillour) with consecutive surface-induced dissociation/ collisionally-activated decomposition (SID/CAD) capability¹ has been used to investigate decompositional behavior of bifunctional terpenes. Stereoisomeric distinction between exo (1) and endo (2) hydroxyl forms of tetracyclic terpenes (Fig. 1, MW = 334 amu) further containing a neighboring ketal group, was established using either CAD or SID. Positive CI-NH₃ MS/MS differentiation was based upon prominent loss of (NH₃ + C₂H₄O) by consecutive decomposition from (M+NH₄)⁺, or very weak C₂H₄O loss from MH⁺ (Fig. 1A). Both processes are stereospecific for the exo isomer (1), and m/z 291 is formed in each case.

To explain this stereochemical effect and the apparent contradiction (i.e. stronger decomposition to m/z 291 from the stabilized ammonium adduct), different protonated forms of 1H⁺ are postulated to exist. In the ion source, (1+NH₄)⁺ (having NH₄⁺ solvated by the ketal group (1a+NH₄)⁺) dissociate readily to form 1aH⁺, where the ketal group is protonated. Upon consecutive decompositions these 1aH⁺ ions do not yield m/z 291. On the other hand, (1c+NH₄)⁺ having NH₄⁺ solvated by the exo hydroxyl group, have much slower rates of source decomposition to 1cH⁺ (protonated hydroxyl, endothermic process), so other competitive processes such as nucleophilic substitution² preferentially take place at this site. 1cH⁺ protonated at the hydroxyl group thus represents a higher energy variety of MH⁺. Stereospecific formation of m/z 291 for the exo isomer (1) is believed to proceed via decomposition of this latter form of MH⁺. 1cH⁺ ions constitute a small fraction of the total 1H⁺, because although the daughter ion at m/z 291 is certainly significant, it still represents a very minor source process.

For the endo hydroxyl isomer (2), NH₄⁺ molecules can be solvated simultaneously by ketal and hydroxyl groups. Here, the 2aH⁺ form is likewise stabilized by this chelation process. The kinetics of decomposition of the cyclobutanol moiety from 2aH⁺ (or other forms) is apparently very sluggish compared to decomposition of the ketal group, hence, m/z 291 is not formed. M/z 291 is thus diagnostic of the exo-hydroxyl structure (1) in CAD or SID spectra of MH⁺, even though present in very low abundance. By selecting (1+NH₄)⁺ with the first quadrupole, then forming 1H⁺ via SID, production of molecules protonated at the hydroxyl group (1cH⁺ form, disfavored in the source) can be heavily promoted. Subsequent CAD of these 1cH⁺ yields m/z 291 as the base peak (Fig. 2A). This ion never appears in the corresponding MS/MS/MS spectrum of the endo hydroxyl isomer 2 (Fig. 2B).

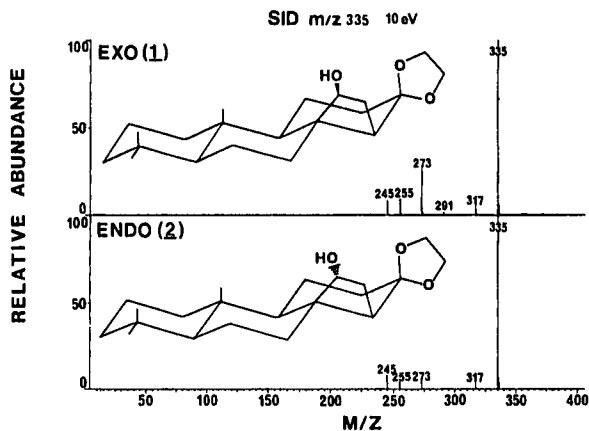
Such MS/MS/MS experiments are thus shown to offer distinct advantages over MS/MS for accentuating stereochemical effects. Enhancement of diagnostic signals is achieved by promoting the formation of a specific structure of precursor ions. If these precursors are formed endothermically in the ion source, their source yields will be minor. Formation of the precursors may be promoted via the use of collisions to induce a selected initial structure to give the disfavored precursor. In this manner, diagnostic ions which were minor ions in the MS/MS spectrum, may become the dominating ions in the MS/MS/MS spectrum.

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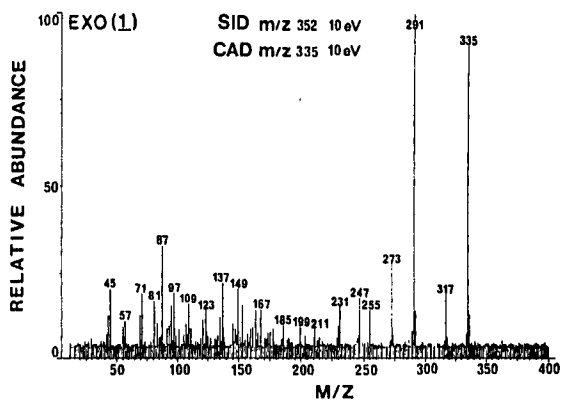
FIGURES

1a

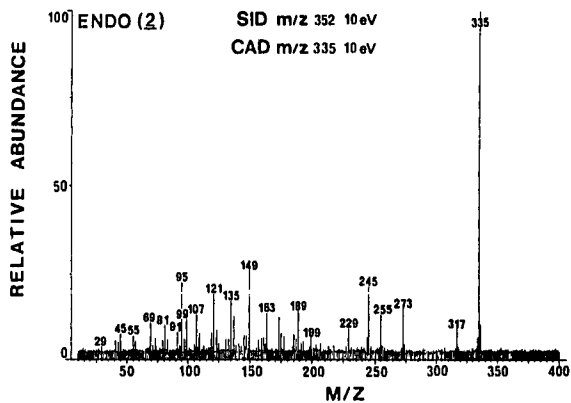


1b

2a



2b



Collisionally Activated Dissociation of Laser Desorbed N-Alkylpyridinium Cations

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Appearance energies (AE) have been estimated from collisionally activated dissociation (CAD) results for laser desorbed N-substituted pyridinium cations. These AE were compared with the energy differences,

$$\Delta\Delta H_i = \Delta H(\text{Py}) + \Delta H_i(\text{R}^+) - \Delta H_i(\text{PyR}^+) \quad (1)$$

determined by AM1 calculations.

Ions formed by laser desorption were trapped in the analyzer cell of a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. The trapped ions were excited to high translational energies by application of an RF pulse and allowed to collide with argon atoms. By varying the RF amplitude the relative kinetic energy per collision and thus the degree of CAD could be changed. The maximum amount of translational energy that can be converted into internal energy is given by Equation 2,

$$\text{KE}(\text{relative}) = \mu_r g^2 / 2 \quad (2)$$

where μ_r is the reduced mass and g is the difference in the velocity of the ion and neutral. Assuming that the fragment ions are in the ground state, the relative kinetic energy represents the maximum amount of energy available for fragmentation. By plotting the percentage of fragmentation versus the relative kinetic energy and extrapolating to zero dissociation, estimates of the fragment ion appearance energies were made.

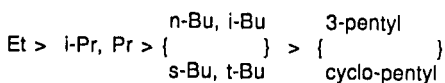
Samples were dissolved in MeOH or EtOH and deposited on a solids probe. A pulsed CO₂ laser was used to desorb the ions from the probe. An argon background pressure of 1×10^{-6} torr was maintained to serve as a collision gas. A delay of 1s before the CAD pulse allowed time for the desorbed neutrals to be removed and for collisional and radiative thermalization of the trapped ions. Calculations were performed by the AM1(1) method using the MOPAC program with complete geometry optimization. Standard bond lengths and bond angles were taken as the starting points. Planarity of the phenyl and pyridine rings, and C_{2v} symmetry of the pyridine rings were assumed, but conformations of the substituents were optimized.

Two distinct pathways for the CAD of these ions have been observed. Direct bond cleavage of the alkyl substituent produces pyridine and a carbonium ion as represented by pathway 1 in Figure 1. Many ions which by beta-elimination can yield a strain-free olefin dissociate by pathway 2 producing a pyridinium cation and an olefin.

For all N-alkylpyridinium cations observed to dissociate via pathway 1, plots of the percentage of dissociation versus the relative kinetic energy were made. Typical plots are shown in Figure 2. Determination of the x-intercept provides an upper limit for the activation barrier for dissociation. Assuming small activation barriers for simple bond cleavage, these values are estimates of the bond energies.

The $\Delta\Delta H_i$ (Equation 1, quantities calculated by AM1) values are plotted against the CAD appearance energies in Figure 3 for selected ions. Energies for R⁺ and possible tropyli and spiro isomers are shown since it is possible that the carbocation, R⁺, undergoes rearrangement during the dissociation.

Nine compounds dissociated by both pathways 1 and 2. For simple alkyl groups both the AE for olefin formation and the calculated height of the transition state (TS), $\Delta H^\ddagger$ decrease with the number of carbon atoms contained in the N-alkyl group:



AE = 92.9	72.4-66.8	61.5-51.9	52.9-43.3 kcal/mol
$\Delta H^\circ = 71.1$	55.3-47.3	45.1-38.4	33.9-29.4 kcal/mol

The alternative dissociation into $R^\bullet$ or olefin involves distinct transition states. The energy of the transition state for $R^\bullet$ formation is dominated by the possibility of reorganization into a more stable isomer. By contrast the TS for olefin formation is dominated by the size of R, and much less influenced by the primary, secondary, or tertiary nature of the substituent.

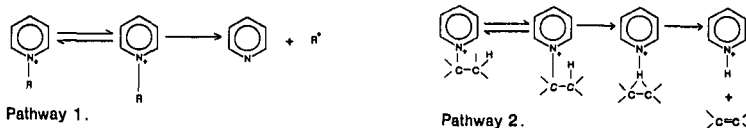


Figure 1.

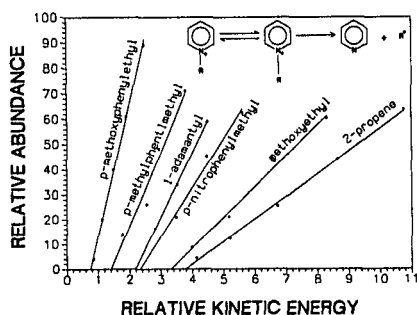


Figure 2.

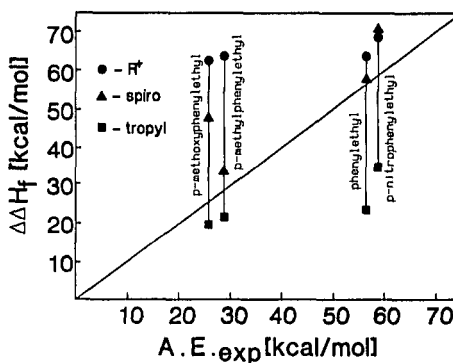


Figure 3.

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Two-Laser Infrared Multiphoton Dissociation of Gaseous Ions Studied by FTICR

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Ions trapped in a Fourier transform ion cyclotron resonance mass spectrometer (FTICR) experienced enhanced infrared multiphoton dissociation (IRMPD) when irradiated sequentially by two carbon dioxide lasers in a unique probe/pump scheme. A pulsed laser tuned to a resonance infrared absorption band promotes ions to vibrationally excited states. Subsequent irradiation with a fixed-frequency continuous wave (cw) laser produces fragmentation. The extent of IRMPD for the dual-laser experiment was greater than that observed for either single-laser experiment.

A probe/pump experimental sequence employing both a pulsed and a cw laser is presented. The pulsed laser was tuned from 10.8 to 9.1 μm (925 to 1100 cm^{-1}) to probe the ions' resonant infrared absorption bands. The power of this probe laser was low enough such that little or no dissociation occurred. Ions with resonance absorption bands were promoted to vibrationally excited states, presumably into the vibrational quasi-continuum. A fixed-frequency cw laser pumped sufficient energy into the ions that the dissociation threshold was exceeded and dissociation occurred.

Experiments were carried out in a FTICR mass spectrometer using a 1.935 tesla superconducting magnet. Ions were made using a standard EI source. Illustrated in Figure 1 is the pump/probe experiment. The pulsed laser was reflected by a gold plated mirror into the cell through a hole in an excitation plate. The cw laser entered the cell through a hole in the trapping plate that was covered with a stainless steel mesh of ca. 90% transparency. The wavelength and power of the cw laser were kept constant at 10.61 μm and 50 W, respectively.

The vibrational cooling rate of protonated diglyme molecules experiences was measured. The amount of fragmentation was recorded as a function of the delay time between the two laser events. The amount of fragmentation is enhanced at short delay times and decreases with increasing delay time. At a pressure of 5×10^{-8} torr the initial energy from the pulsed laser had dissipated after about 400 ms, and no enhancement was observed at longer delays.

The amount of "enhanced" IRMPD was measured as a function of the probe laser output energy. These experiments examined the feasibility of using other types of probe lasers that have modest to low energy output for photodissociation studies. For most samples studied to date, enhanced IRMPD was observed until the probe laser's pulse energy was reduced to about 50 mJ.

Previous single laser(1) studies have shown that the IRMPD spectrum of C_3F_6^+ correlates with that of the neutral IR absorption spectrum. Similar results were obtained using the 2-laser probe/pump approach. Shown in Figure 2 is the 2-laser IRMPD spectrum obtained by tuning the probe laser at a constant output energy of 250 mJ/pulse. However, when the output was reduced to 100 mJ/pulse no IRMPD was observed for C_3F_6^+ (see Figure 3) unless the pump laser was present.

A strong correlation was also observed between the neutral IR absorption spectrum and IRMPD spectrum of chloroethane. These two spectra are shown in Figure 10. The width of the P and R branches appears narrow in the IRMPD spectrum, this results from the limited number of lines available from the CO_2 laser.

Tunable wavelength sources that by themselves cannot produce photodissociation can be used in combination with a more powerful pump source to provide useful information. Further studies are planned that will exploit irradiation of ions in other regions of the infrared spectrum.

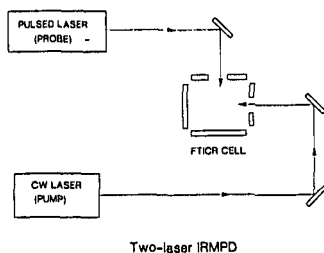


Figure 1.

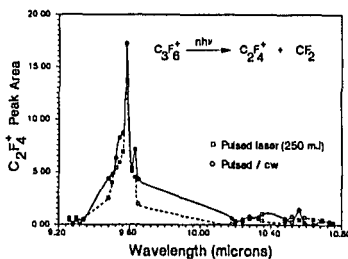


Figure 2.

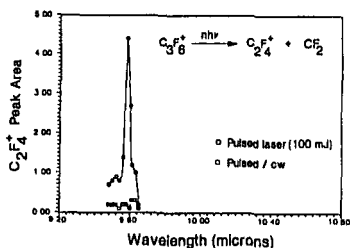


Figure 3.

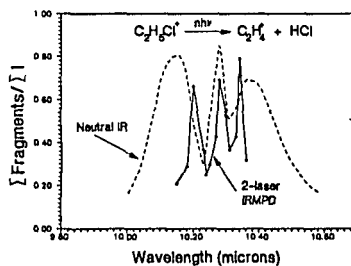


Figure 4.

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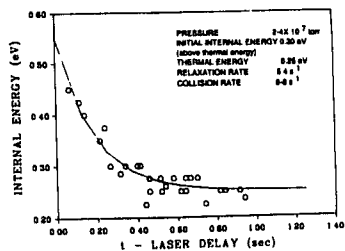
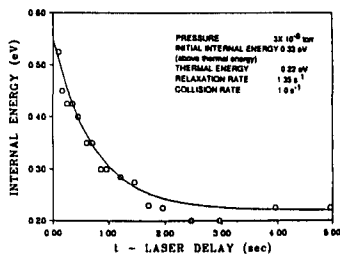
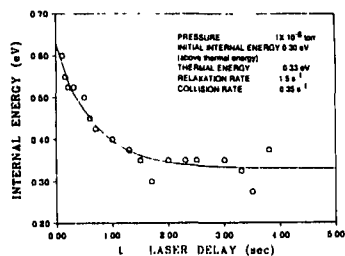
DETERMINATION OF INTERNAL ENERGY AND ENERGY
DISSIPATION RATES IN N-BUTYLBENZENE IONS
BY COMPETITIVE PHOTODISSOCIATION

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THE COMPETITIVE PHOTODISSOCIATION OF N-BUTYLBENZENE IONS INTO FRAGMENTS AT m/e 91 AND 92 IS WELL KNOWN AS A CALIBRATED AND RELIABLE THERMOMETRIC METHOD FOR MEASURING THE ION INTERNAL ENERGY AT THE MOMENT OF PHOTON IMPACT(1). USING THIS METHOD THE RELAXATION OF INITIALLY ENERGETIC IONS RESULTING FROM ELECTRON IMPACT WAS FOLLOWED AT DIFFERENT PRESSURES.

N-BUTYLBENZENE IONS PRODUCED BY ELECTRON IMPACT CONTAINED AN AVERAGE OF 0.55-0.60 eV OF INTERNAL ENERGY. AT LOW PRESSURES ($1-3 \times 10^{-8}$ TORR) APPROXIMATELY 0.3eV WAS LOST AT A RATE OF $1.35 - 1.5 \text{ s}^{-1}$. SINCE THE ION-NEUTRAL COLLISION RATES WERE LOWER THAN THESE RELAXATION RATES, INFRARED RADIATIVE COOLING MUST HAVE DOMINATED OVER COLLISIONAL COOLING. IN CONTRAST, THE RELAXATION RATE AT THE HIGHER PRESSURE ($2-4 \times 10^{-7}$ TORR) WAS COMPARABLE TO THE ION-NEUTRAL COLLISION RATE. ENERGY LOSS AT THIS HIGHER PRESSURE WAS PRIMARILY BY COLLISIONAL QUENCHING.

THE MONITORING OF THE RATIO OF N-BUTYLBENZENE ION'S PHOTOFRAGMENTS, m/e 91 AND 92, HAS PROVENTO BE A CONVENIENT METHOD TO PROBE THE INTERNAL ENERGY OF THE PARENT ION. DYNAMICAL PROCESSES SUCH AS RADIATIVE AND COLLISIONAL COOLING OF INTERNAL ENERGY HAVE BEEN STUDIED USING THIS POWERFUL THERMOMETRIC TOOL.



¹CHEN, J.H.; HAYS, J.D.; DUNBAR, R.C. J. PHYS. CHEM., 1984, 88, 4759-4764. BAER, T.; DUTUIT, O.; MESTDAGH, H.; ROLANDO, C.; J. PHYS. CHEM., 1988, 92, 5674-5679.

TIME RESOLVED PHOTODISSOCIATION OF THIOPHENOL IONS IN AN ICP MASS SPECTROMETER
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44107

Objectives:

Photofragmentation of thiophenol cation into cyclopentadiene cation and CS requires an extensive rearrangement (See reaction 1). This process is therefore a likely candidate for a slow fragmentation. In this study we were interested in:

- 1) Measuring the rate of this photofragmentation at 308nm and
- 2) Comparing it with the rate predicted by RRKM theory.

Introduction:

A time resolved study of the photofragmentation of thiophenol cation was carried out at 308nm. The fragmentation rate was compared to the rate predicted from RRKM theory. Two models were considered; a "somewhat tight" transition state and a "very loose" transition state. The "somewhat tight" transition state is the loosest we considered plausible in view of the severity of the rearrangement required for fragmentation. The very loose transition state was arbitrarily chosen to give agreement with experiment, in order to determine the extent that the transition state had to be loosened to yield the observed rate.

RRKM Calculations:

The RRKM calculations were carried out using the equation

$$k=c(W(E-E_0)^n/p(E))$$

where $c=3 \times 10^{10} \text{ cm/s}$, E = total internal energy (photon plus thermal) of the energized parent ion, E_0 = energy of activation, $p(E)$ = density of states of the parent at E and $W(E-E_0)^n$ = number of states of the activated complex between E_0 and E .

The table compares the parent ion vibrational frequencies with those of the two transition states.

Experimental:

All rate measurements were done on an icr using capacitance bridge detection (non-FTICR). The light source was the 308nm line of an excimer laser. The pulse sequence is shown in figure 1. The fragmentation was monitored by following the appearance of the fragment ion with time.

As pointed out in reference 1 the time resolved appearance curves of the photofragment must be fitted using the following equation:

$$S=C\{\Delta+(1/k)e^{-k\tau}(e^{-k\Delta}-1)\}$$

where S is the fragment ion signal strength, Δ is the length of the effective Rf excite pulse, k is the fragmentation rate, τ is the time from the laser pulse to the beginning of the Rf excite pulse and C is a constant adjusted to make S equal to the long delay time fragment ion signal.

This equation predicts that if $1/k$ is comparable to Δ there will be a noticeable zero time ($\tau=0$) fragment signal as was observed on all of the time resolved plots. All plots were satisfactorily fitted by this equation.

Results:

The photofragmentation rate was found to be about $1.0 \times 10^5 \text{ s}^{-1}$ (see figure 2), 200 times faster than predicted by the "somewhat tight" transition state RRKM model. It was possible to increase the RRKM rate enough to agree with the experimental rate using an unreasonably loose transition state (compare the first and second columns in the table).

Discussion:

It seems that this process requires tightening (rather than loosening) of the molecular geometry in going from the energized parent ion to the activated complex. The somewhat tight transition state used here, which is similar to the energized parent, is already a looser transition state than seems reasonable and the "very loose" transition state seems unrealistic. Therefore we will discuss and rule out two possible reasons for the observed rate being much faster than the rate calculated by the more realistic "somewhat tight" transition state.

I. Hot ions with a total internal energy greater than that assumed in the RRKM calculation will fragment with a correspondingly faster rate. Through a series of cooling experiments

we have shown that excess internal energy of the ions was not the cause of the unexpectedly fast fragmentation observed here. We have shown that after thorough thermalization thiophenol ions (with around 0.2 eV of thermal energy) still fragment with a rate of about $1 \times 10^5 \text{ s}^{-1}$ nearly 200 times faster than the calculated rate.

II. Satisfactory agreement between the experimental rate and that predicted by RRKM theory can be achieved by setting the activation energy for this process at 2.66 eV. However we were able to show that the photodissociation threshold is at least 0.05 eV higher than this and accurate Photoionization AE measurements place this threshold between 3.35 eV and 3.65 eV. In addition heat of formation values yield a thermochemical threshold of 3.2 eV.

We therefore conclude that the unexpectedly fast observed fragmentation rate can not be accounted for by postulating hot parent ions or a dissociation threshold that is too low. A reasonable interpretation of these results requires further study.

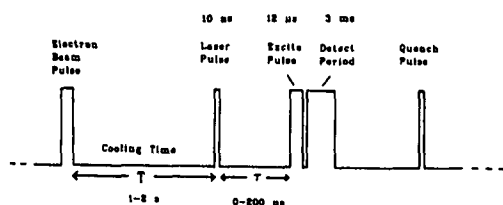
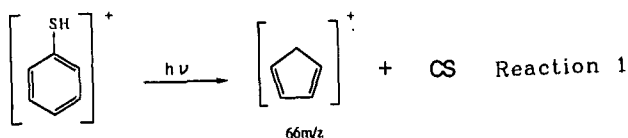


Figure 1

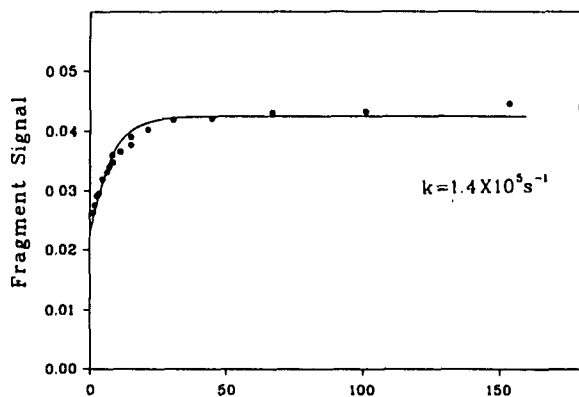


Figure 2

Parent Ion	Tight Transition State	Loose Transition State
185	185	185
278	300	200
412	412	412
412	412	412
464	464	464
616	616	616
689	689	689
698	698	200
736	736	736
834	834	834
901	901	901
914	1000	200
970	970	970
991	991	991
1002	1002	1002
1026	1026	1026
1072	1072	1027
1093	1093	200
1158	1158	1158
1182	1182	1182
1272	1272	1272
1329	Reaction coordinate	
1443	1443	1000
1480	1480	1000
1582	1582	1000
1582	1582	1000
2575	2575	1000
3037	3037	3037
3048	3048	3048
3056	3056	3056
3086	3086	3086
3150	3150	3150
250	300	15 (Rotation)

Fragmentation rate for thermalized ions 380 s⁻¹ 1x10⁵ s⁻¹

FRAGMENTATION MECHANISMS FOR HIGHLY FUNCTIONALIZED IONS:
THE FAB MASS SPECTRA OF SOME CARDIAC GLYCOSIDES REVISITED

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Michigan State University, East Lansing, Michigan 48824

When electron ionization (EI) was the dominant ionization method in mass spectrometry, and analyte molecular weights were below 1000, mass spectral interpretation was frequently straightforward. Fragmentation mechanisms were established (1) which were extremely useful in understanding the relationship of fragment m/z values and intensities to the structure of the analyte molecule. Desorption/ionization methods have taken us to a situation where much larger, highly functionalized molecules can be studied, however the focus has not been on establishing fragmentation mechanisms for these large molecules. When working with small molecules, many mechanisms are determined through labeling experiments, which are much more difficult to perform for larger molecules such as saccharides and peptides. We evaluate here the use of high resolution MS and MS/MS for providing insights into the mechanisms leading to fragment ions formed in the FAB analysis of cardiac glycosides, and focus on the molecule digoxin, $C_{41}H_{64}O_{14}$.

The compound is protonated in the FAB analysis, using a glycerol matrix. The MH^+ ion, at m/z 781, forms a number of fragment ions due to cleavage of the three glycosidic linkages in the molecule (figure 1). We will use as an abbreviation (2) for digoxin, $A-O-S_1-O-S_2-O-S_3-OH$. We quickly found that nominal mass information on the fragment ions is frequently insufficient for tentative assignments, and used high resolution results on all fragment ions as the starting point in the project. It was determined that most fragment ions contain the aglycone moiety (A), although some, such as the ion current at m/z 391, represent two species - one containing the saccharide chain and one containing the aglycone, with assignments of $C_{23}H_{35}O_5^+$ ($[AOH]H^+$) and $C_{18}H_{31}O_9^+$ ($[HOS_3OS_2OS_1-H]H^+$).

Consider as an example, a major fragment ion that is observed at m/z 651. It corresponds to the $AOS_1OS_2O^-$ part of the molecule, plus 2 H's in the form $[AOS_1OS_2OH]H^+$. We considered a number of mechanisms by which this fragment may be formed. The simplest mechanism is charge-initiated fragmentation (CIF) which follows protonation of the glycosidic oxygen between S_2 and S_3 with accompanying H shift from the departing neutral. The H-shift could follow a 1,2 or 1,3-elimination mechanism, which would yield the same ionic fragment, presumably with the initial site of protonation also being the site of protonation in the product. We also consider a remote-site (RS) mechanism, in which protonation of the aglycone occurs, and a 1,2 or 1,3-elimination about the glycosidic linkage in question follows. To investigate the structure of this ion, a B/E linked scan was performed on m/z 651, on a JEOL HX-110 double-focusing instrument. The resulting spectrum is shown in Figure 2. If the CIF mechanism is operative, and the site of protonation in m/z 651 is a terminal OH group, we would expect H_2O loss to dominate in the linked-scan spectrum, however this is not the case. The ion fragments in much the same way as does the protonated digoxin molecule - possibly suggesting a RS mechanism. Linked-scan data for the fragment ions are very informative, and show that multiple water losses, which are frequently observed, may be due predominantly to the hydroxy groups on the aglycone ring, rather than those on the sugars. The results suggest that both the CIF and the RS mechanisms may be operative. The combination of high resolution data, and MS/MS analysis of fragment ions formed by FAB can provide some insights into the fragmentation mechanisms of large molecules (when fragment ions are observed in the FAB mass spectrum), however labeling studies (or possibly MS/MS/MS) are still required to sort out the many details that control the fragmentation of protonated, highly functionalized molecules such as cardiac glycosides.

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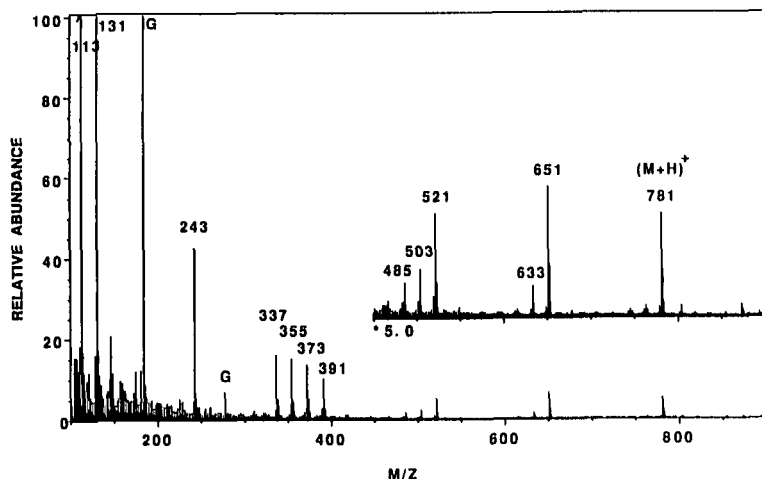


Figure 1. FAB mass spectrum of digoxin in glycerol

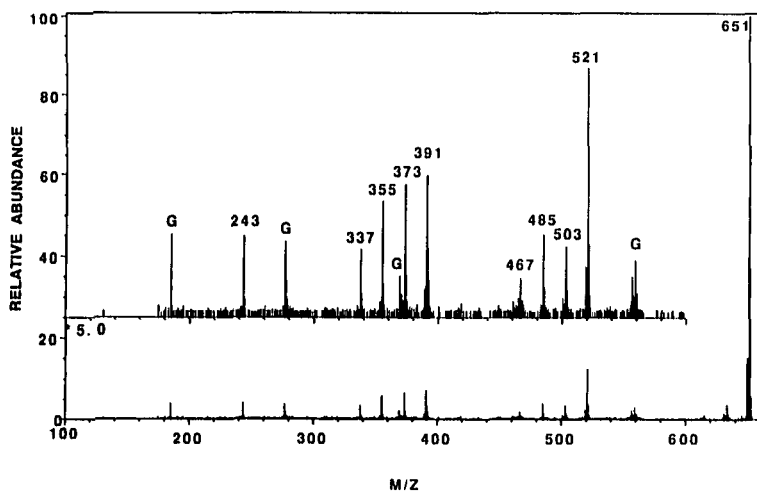


Figure 2. B/E linked scan of the digoxin fragment ion at m/z 651. The successive loss of H_2O from m/z 651 (e.g. at m/z 633, 615) is not as prevalent as the cleavage of the glycosidic bonds (e.g. at m/z 521, 391).

LASER ABLATION MASS SPECTRA OF HIGH
MOLECULAR WEIGHT POLYMERS DETERMINED
BY FOURIER TRANSFORM MASS SPECTROMETRY

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Endicott, New York 13760

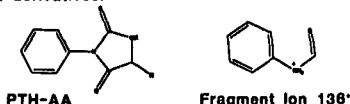
Laser microprobe mass spectrometry (LAMMS) is one of the more promising methods for identification of polymeric or organic particulates on surfaces. Almost a decade ago, TOF-based LAMMS was first applied to polymer analysis. However, the observation of extensive rearrangement products and the limited selectivity of TOF spectrometers militate against the usefulness of the technique. Last year, three groups (including ourselves) reported design and construction of the first FTMS-LAMMS instruments (1 - 3). High mass resolution and multi-stage MS capability of FTMS are expected to be invaluable in studies of complex rearrangement products produced by laser ablation. We report here a survey of industrially important polymers analyzed in the broadband mode of FTMS. The goals of this work are to determine: 1) the extent to which FTMS-LAMMS (spot size $10 < \mu\text{m}$) can be used to identify polymer particles on surfaces; 2) if differences exist between LAMMS spectra from TOF and FTMS analyzers; 3) trends in the observed ion distributions which may yield insight into formation mechanisms and interpretation of unknowns.

Mass resolution sufficient to achieve exact mass measurement was not possible with polymers as analyzed in this study. This contrasts with our previous experience with laser ablation of metals in which mass resolution of $> 4 \times 10^5$ was obtained at $m/e = 206$. Recent experiments indicate the insulating property of the polymers dramatically shorten the transient in our configuration, and high mass resolution studies are currently being pursued. Nevertheless, the spectra indicate a number of trends in FTMS-LAMMS polymer analysis.

Positive ion spectra: 1) FTMS spectra usually show drastic differences from literature TOF spectra. FTMS spectra are usually higher in mass and show more rearrangement products. 2) A number of FTMS spectra exhibit odd mass ions series not observed in TOF spectra. We *speculate* on the origin of these peaks: It is known that the laser atomizes a substantial proportion of the ablated material. In the 3 T magnetic field of the FTMS, protons (and ions with exchangeable protons) will be confined at the ab-

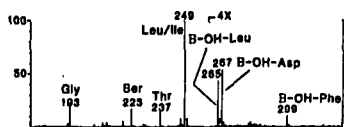
Amino Acid Analysis by DCI MS/MS

Procedure: Amino acid residues obtained from acid hydrolysis are reacted with PITC to form PTH-derivatives.

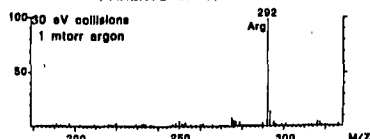


Most PTH-amino acids yield a prominent 136⁺ ion under MS/MS conditions. By acquiring a DCI parent ion spectrum only species that dissociate to produce the 136⁺ fragment are observed, permitting identification of the MW of the PTH-AA's. PTH-arginine and PTC-lysine do not yield intense 136⁺ by MS/MS but they may be screened for by searching for parents of 174⁺ and 306⁺, respectively. After the molecular weights have been determined, a daughter ion mass spectrum is obtained from each component. The resulting spectra are compared against our PTH-AA library for positive identification.

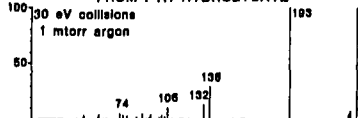
PTH-DERIVATIVE OF LYSOBACTIN HYDROLYSATE DCI PARENTS OF 136⁺ MASS SPECTRUM



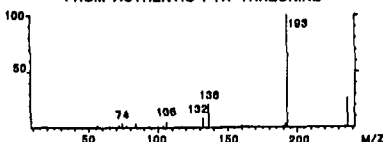
PARENTS OF 174⁺ ION



DCI DAUGHTER ION MASS SPECTRUM OF 237⁺ ION FROM PTH-HYDROLYSATE



DCI DAUGHTER ION MASS SPECTRUM OF (M+H)⁺ ION FROM AUTHENTIC PTH-THREONINE



Edman Degradation: Identification of Products by FAB & CI MS/MS

Ile-BLeu-Thr-Ser-BLeu-Abu-Ser-Trp-Orn-Phe

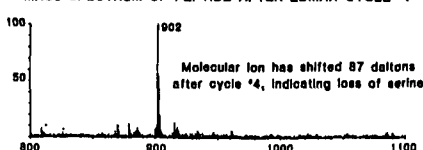
reduced acyclic Janthiomycin C

BLeu = B-hydroxy-Leu

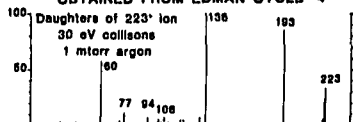
MASS SPECTRUM OF PEPTIDE AFTER EDMAN CYCLE '3 POSITIVE FAB IONIZATION



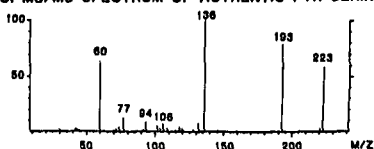
MASS SPECTRUM OF PEPTIDE AFTER EDMAN CYCLE '4



CI MS/MS SPECTRUM OF PTH-AMINO ACID OBTAINED FROM EDMAN CYCLE '4



CI MS/MS SPECTRUM OF AUTHENTIC PTH-SERINE



Conclusion Peptides produced by micro-organisms pose special problems for sequence determination. These peptides may contain unusual or novel amino acids whose chirality may be D or L, erythro or threo. The identity of these unusual amino acids and their stereo-chemistry can be determined by GC-MS. Alternately, for rapid amino acid analysis, the PTH derivative of the hydrolysate can be examined by DCI MS/MS. The presence of D-amino acids also limits peptide susceptibility to enzymatic cleavage. Mild acid hydrolysis is useful for producing fragment peptides when MS/MS and enzymatic digests fail. Also Edman degradation followed by FAB-MS and CI MS/MS is an extremely valuable tool for sequence determination of peptides with a free N-terminus.

G.E. Unger, B.M. Warlock, *Spectroscopy*, 3, 33-38 (1988)

FOURIER TRANSFORM MASS SPECTRA OF POLYBUTADIENES AND POLYISOPRENES

A. Kasem Chowdhury and Charles L. Wilkins

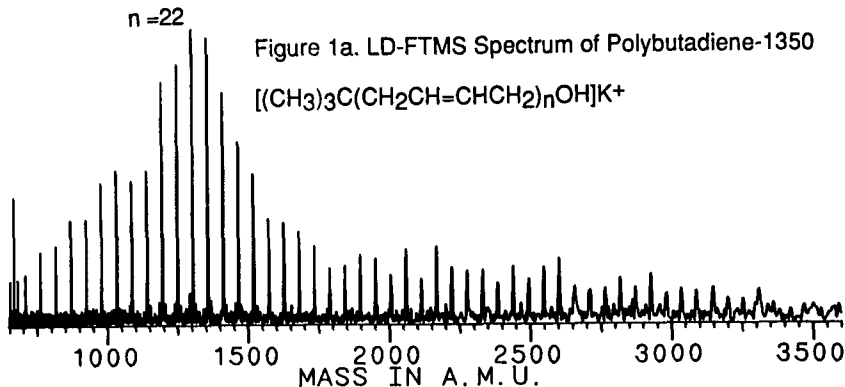
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Introduction. Although laser desorption Fourier transform mass spectrometry (LD-FTMS) has emerged as an alternative soft ionization method for analysis of non-volatile polymer samples, very few non-polar polymer samples have been analyzed to date. The majority of samples examined thus far have been polymer samples containing oxygen and nitrogen. Here, we report the first characterization of polybutadienes and polyisoprenes using LD-FTMS.

Experimental. All mass spectra were obtained with a Nicolet FTMS-2000 mass spectrometer equipped with a 7.0 tesla superconducting solenoid magnet and a CO₂ laser. Polybutadienes and polyisoprenes were obtained from Polysciences, Inc. Samples were loaded onto a probe tip either as a pure compound or dissolved in dichloromethane. Addition of a few drops of gold oxide/methanol solution appears to assist ionization of polymer samples. For thermal desorption studies, water was used as the CI reagent to obtain thermal desorption CI (TD-CI) spectra. The polymer samples were thermally desorbed at 75-150 °C. Water was ionized by a 10-us (for negative CI 100-ms) beam of 50 eV (for negative CI 6.75 eV) electrons with filament emission current of approximately 10 uA. The ions were trapped in the cell for about 5 seconds, prior to observation, to allow ion-molecule reactions with the desorbed polymers.

Results and Discussion. LD-FTMS spectra were obtained for the following polymers; polybutadiene-1000; polybutadiene-1350 (OH terminated); polybutadiene-1300 (phenyl terminated) and polyisoprene -3280. Figure 1 shows mass spectra of polybutadiene-1350 and polyisoprene-3280. The major peaks in the spectra are those arising from potassium attachment to the individual oligomers differing by 54 amu for polybutadiene and 68 amu for polyisoprene. Although no significant fragmentation is observed for the polybutadienes, polyisoprene shows small secondary envelope corresponding to loss of two methyl groups. Figure 2 shows TD-CI mass spectra of polybutadiene-1350 (OH terminated). The thermal desorption CI methods produce a series of low mass ions differing by 54 amu corresponding to losses of $(n \times 54 + H_2)$



and $(n \times 54 + 57)$ for positive and negative CI, respectively. The loss of mass 57 in addition to the loss of $n \times 54$ in the negative CI spectrum appears to be a terminal $(CH_3)_3C$ group. Thus, TD-CI method can provide complimentary information for analysis of polymers. This study demonstrates that certain non-polar polymers can be analyzed using the FTMS technique.

Figure 1b. LD-FTMS Spectrum of
Polyisoprene-3280

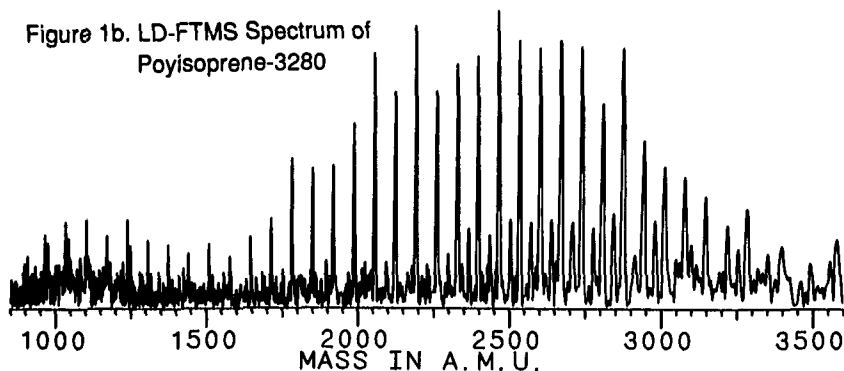
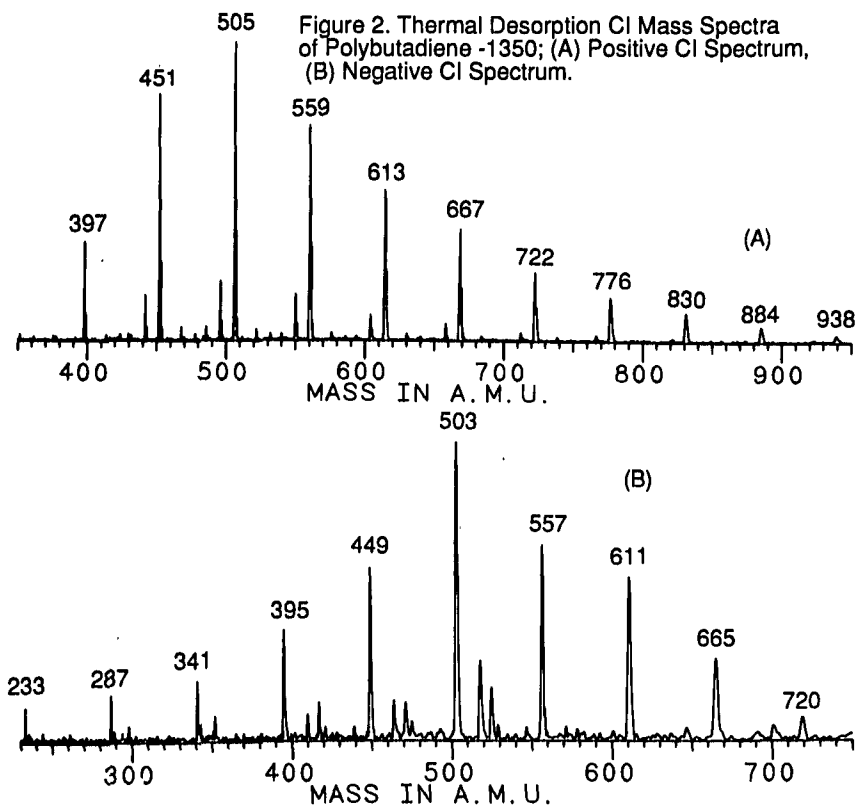


Figure 2. Thermal Desorption CI Mass Spectra
of Polybutadiene -1350; (A) Positive CI Spectrum,
(B) Negative CI Spectrum.



LASER DESORPTION FT/ICR MASS SPECTRA OF POLYAMIC ACIDS

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INTRODUCTION

Polyimides have gained increased attention in recent years due to their excellent thermal stability and property retention following high temperature processing (1). Because of the intractable nature and high thermal stability of polyimides, direct analysis of their structure by mass spectrometric methods is difficult. Recent descriptions of laser desorption mass spectra obtained from polyimides are not encouraging as the spectra were dominated by carbon clusters and only small, structurally insignificant, organic ions were observed (2). Polyamic acids are common precursors to polyimides and are generically poly(ortho-carboxylic acid)amides. Previous work on polyamides (3) suggests that polyamic acids would yield analytically meaningful spectra using laser desorption mass spectrometry. If successful, the approach would provide identification of the constituent monomers in the resin.

EXPERIMENTAL

Laser desorption mass spectra were obtained using a dual-cell Nicolet FTMS 2000 spectrometer interfaced with a Tachisto 215G carbon dioxide laser (10.6 μm). The spectra were obtained using standard pulse sequences. The trapping potential was + 1.0 V. The excitation band width was 800 KHz and a sweep rate of 800 Hz was used. When analyzer side detection was performed, an ion transfer time of 400 μs was used. The transients from single laser pulses were digitized at 64K, zero filled twice, and transformed using three-term Blackman-Harris apodization. Accurate mass measurements were made using calibration tables obtained from laser desorption spectra of a mixture of monomeric polyimide model compounds as an internal or external calibrant.

Polyamic acids were either synthesized by standard methods (4) or were purchased from suppliers. Four different polyamic acids were studied. Two different methods were used to prepare samples for analysis. In the first method, the viscous polyamic acid solutions were precipitated in methanol using a Waring blender. The precipitate was collected and vacuum dried at ambient temperature to yield a powder which was applied directly to the probe tip as a smear. In the second method, the polyamic acid solution was diluted 100:1 in NMP solvent and the diluted solution applied to the laser target using a microliter syringe following by drying in vacuum for 2 hours at 60 C.

RESULTS AND DISCUSSION.

Polyamic acids can be viewed as polymeric phthalate half amides. and their positive ion LD spectra follow a pattern that is very similar to the patterns observed from the fragmentation of phthalate derivatives. Figure 1 shows a source side, positive ion spectrum obtained from a polyamic acid derived from 3,3',4,4'-benzophenone tetracarboxylic acid dianhydride (BTDA), 1,4-oxydianiline (ODA), and 1,3-phenylene diamine (mPDA). All of the information required to determine the monomer composition of the polymer is present in the spectrum. For example, the ions at m/e 108, 200, and 323 (Figure 1) correspond to the molecular ion of mPDA (in a mass doublet with an ODA fragment), the molecular ion of ODA, and the protonated pseudomolecular ion of BTDA. The higher mass ions at m/e 504, 696, and 990 correspond to polymer repeat units and are formed by a process involving both N and O acyl bond cleavages and a series of dehydrations. This process results in a series of amine-anhydride terminal ions corresponding to BTDA-ODA (504 amu), ODA(BTDA)ODA (696 amu), and ODA(BTDA)ODA(BTDA) (990 amu) combinations. Ions representing more than two repeat units were not observed despite numerous attempts involving varied laser power, pulse sequence modifications, and cationization with alkali metal salts. The spectra from the other polyamic acids studied gave spectra following the same pattern as the BTDA-ODA-mPDA polymer. The only difference in source vs. analyzer side spectra was that the source spectra showed higher abundances of ions in the 500 to 1000 amu range.

The accurate mass data obtained from Figure 1 are shown in Table I and are averages from ten consecutive laser pulses with internal calibrant. The values measured were in good agreement with those calculated from the postulated structures. The accurate mass measurements were reproducible using the external calibration method. For example, ten repetitive measurements of the ODA molecular ion showed: calculated for $C_{12}H_{12}N_2O=200.0944$ amu; measured mean=200.0938 amu and standard deviation=0.0012 amu. As expected, internal calibration gave a somewhat better result with mean=200.0948 amu and standard deviation=0.00064 amu.

SUMMARY

Laser desorption FT/ICR spectra of polyamic acids provide structural information on the polymers and allow monomer identification. Polymer fragment ions of up to two repeat units are routinely observed. Mass accuracy with internal calibrant was routinely less than 5 ppm.

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Figure 1.

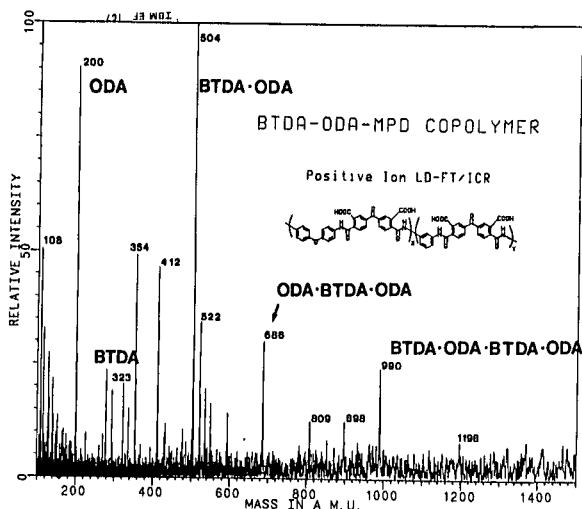


Table I

Accurate Mass Data For BTDA-ODA-MPD Polyamic Acid

Ion/Charge	Measured Mass amu	Calculated Mass amu	Error ppm
MPDA M^+	108.0684	108.0681	2.8
MPDA $+H^+$	108.0782	108.0782	1.8
ODA M^+	200.0948	200.0944	2.0
ODA $-CO_2H^+$	108.0437	108.0444	0.3
BTDA $+H^+$	323.0178	323.0186	2.5
BTDA M^+	322.0115	322.0118	1.2

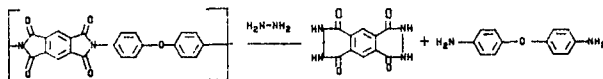
IDENTIFICATION OF POLYMER SYSTEMS BY MASS SPECTROMETRY

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Identification of the component monomers of a polymer system can be achieved by mass spectral analysis after hydrolytic cleavage of the polyimide into its monomers. Hydrolytic cleavage (1,2,3) of the polyimide can be carried out in aqueous hydrazine or ammonium hydroxide. Cleavage of a polyimide by hydrazine will produce diamine and hydrazide components (4). Cleavage by ammonium hydroxide will produce diamine and tetraacid components.

This study involved the use of direct inlet probe (DIP) FTMS and GCMS for the analysis of hydrolysis products. DIP-FTMS allowed direct analysis of all hydrolytic species resulting from hydrazinolysis and NH_4OH hydrolysis without additional sample preparation. Due to their low volatility, hydrazides were not detectable by GCMS. Chemical derivatization of tetraacids for enhanced volatility was necessary for detection by GCMS.

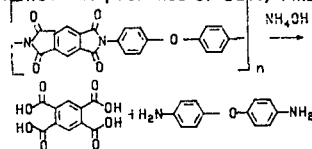
HYDRAZINOLYSIS



Hydrazide standards were prepared from two common dianhydrides, Pyromellitic dianhydride (PMDA) and Benzophenone dicarboxylic dianhydride (BTDA). Reaction of the dianhydride powder was completed in 40% aqueous hydrazine at 85°C for 12 hours. The hydrazide and diamine components in the hydrolysis medium were analyzed directly using heated DIP-FTMS. The sample was heated in the DIP to 400°C. The PMDA bishydrazide showed a molecular ion at $M=246\text{amu}$ and fragment ions at 189amu ($M-\text{N}_2\text{H}_2$, CO) and 160amu ($M-\text{N}_2\text{H}_2$, 2CO), see Fig. 1. The reaction of BTDA with hydrazine produced two components: BTDA bishydrazide, $M=350\text{amu}$ and BTDA bishydrazide-hydrazone, $M=364\text{amu}$. The BTDA bishydrazide spectra (Fig. 2) showed a large molecular ion at 350amu and fragment ions at 189 ($M-\text{C}_8\text{H}_5\text{O}_3\text{N}_2$) and 160amu ($M-189$). The BTDA bishydrazide-hydrazone showed a relatively small molecular ion at 363amu ($M-\text{H}$) and a large fragment ion at 336amu ($M-\text{N}_2$).

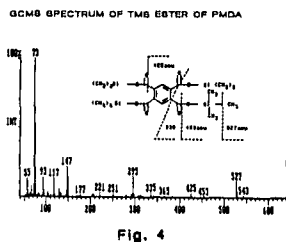
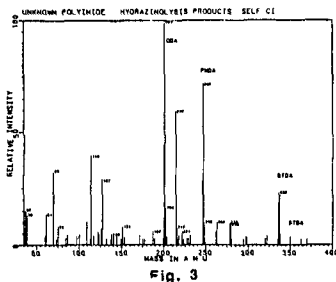
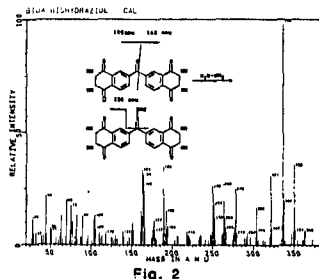
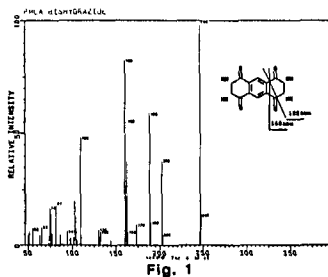
A polymer of unknown composition was hydrolyzed in aqueous hydrazine. The same procedure used in the preparation of the hydrazide standards was used in the hydrolysis of the unknown. Probe FTMS analysis of the hydrolyzate showed the presence of ODA, PMDA and BTDA in the unknown. (see Fig. 3).

HYDROLYSIS WITH AMMONIUM HYDROXIDE

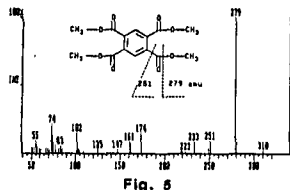


Methyl ester and trimethylsilyl (TMS) ester derivatives were prepared from the tetraacids of PMDA and BTDA. GCMS detected the tetramethyl ester of PMDA (Fig. 4) but not of the larger BTDA. TMS derivatization further increased the volatility of the tetraacids. TMS esters of both PMDA and BTDA were detectable by GCMS. The TMS esters were prepared by reacting the tetraacids with hexamethyldisilazane (HMDS) in a solvent medium of HMDS/pyridine (1/4). Figure 5 shows the spectrum of PMDA tetra(TMS) ester, with molecular ion of $M=542\text{amu}$ and fragment ions at 527 ($M-\text{CH}_3$), 425 ($M-\text{TMS ester}$), and 73amu (TMS). BTDA tetra(TMS) ester (Fig. 6) exhibits a molecular ion of $M=646\text{amu}$ and fragment ions at 631 ($M-\text{CH}_3$) and 573amu ($M-\text{TMS}$, OTMS).

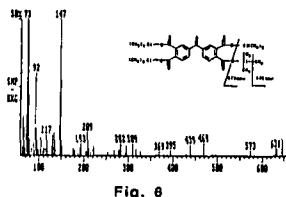
1,4-Oxidianiline (ODA) and 1,3-bis(aminophenoxy)benzene (APB), two commonly used amines, were readily detected using both probe FTMS and GCMS techniques. ODA produced a molecular ion of $M=200\text{amu}$ and a large fragment ion of 108amu . APB produced a molecular ion of $M=292\text{amu}$ and a fragment ion of 183amu .



GCMS SPECTRUM OF TETRAMETHYL ESTER OF PMDA



GCMS SPECTRUM OF TMS ESTER OF BTDA



CONCLUSIONS:

Positive identification of the monomeric components of a polyimide system can be successfully achieved using either GC/MS or probe FTMS. Hydrolytic and hydrazinolysis products of polyimide cleavage can be analyzed directly using heated solids probe MS. Hydrazinolysis products could not be analyzed by GCMS due to low volatility. Diamine components of hydrolytic cleavage are easily detected by GCMS. Tetraacid components of hydrolytic cleavage require additional derivatization, preferably silylation, to be detected by GCMS.

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2. J. Jones, J of polymer Sci., Part C, 22,773 (1969).
3. R. Dine-Hart, D. Parker, W. Wright, Br. Polymer J.,3, 226,(1971)
4. H. R. Linde, S.Meeks, Jr., Anal. Chem., 55, 1443, (1983)

EI AND CI SFC/MS OF ISOCYANATE PREPOLYMERS

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Isocyanate prepolymers are very difficult to analyze by mass spectrometry. Their lack of volatility prevents their analysis by GC/MS, and their reactivity in the presence of free hydrogen precludes their analysis by conventional thermospray liquid chromatography/mass spectrometry. Other types of ionization for non-polar LC methods are also problematic. Supercritical fluid chromatography / mass spectrometry (SFC/MS) provides a very good method to separate and analyze these compounds quickly and easily. Both electron ionization (EI) and chemical ionization (CI) information can be obtained by capillary column SFC/MS. A sample of 4,4'-diisocyanatodiphenylmethane was analyzed by both EI and CI capillary SFC/MS. From the EI results, it is possible to identify the three components of the sample as the 4,4'- and 2,4'- positional isomers of the diisocyanate prepolymer and one polymerization moiety. Methane-CI SFC/MS corroborates the EI result, but is an order of magnitude more sensitive for these compounds. Three additional peaks found in the CI chromatogram represent another positional isomer of the polymerization product, the next expected polymerization product, and a methanol reaction product. These results show that SFC/MS is an excellent method for understanding the exact nature of contaminants in polymer starting material of this type, which are very difficult to analyze by other methods.

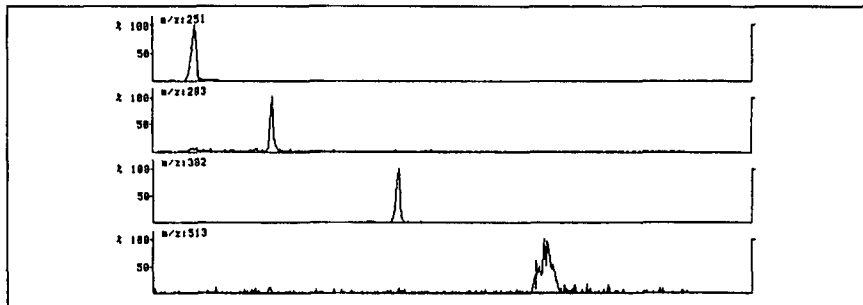
Methods

A sample of 4,4'-diisocyanatodiphenylmethane was analyzed by capillary SFC/MS on a Lee Scientific Model 602 supercritical fluid chromatograph interfaced to a Finnigan MAT SSQ™ 70 single quadrupole mass spectrometer.

Chromatographic Conditions:

Injector:	Valco timed split with 2-m retention gap
Column:	SB-Biphenyl-30, 50 μ m
Supercritical Fluid:	CO ₂
Pressure Program:	100 to 300 atm at 10 atm min ⁻¹
Temperature:	100°C
Restrictor Conditions:	Frit restrictor at 325°C Guthrie restrictor at 250°C

The sample was analyzed under both EI and methane-CI mass spectrometer conditions. There is some evidence that under CO₂ SFC conditions, EI is, in fact, CO₂ charge-transfer ionization. However, spectra yielded under these conditions are very similar or identical to classical EI spectra and are library-searchable using standard library-searching algorithms and the NBS (NIST) library of mass spectra.



Results

The three peaks shown in the EI results correspond to two positional isomers of the monomer and one polymerization moiety. The molecular ion is predominant in all the spectra and is the base peak in both monomer spectra. The primary peak is most likely the nominal 4,4'-diisocyanatodiphenylmethane. Its primary fragment ion is m/z 208, which corresponds to the loss of an isocyanate group (i.e., 42 u). The smaller monomer peak is the 2,4'-positional isomer. The loss of 29 u results from the *ortho* effect in which CHO is lost through rearrangement. In any case, m/z 221 and m/z 208 are present in both spectra and differ only in their relative abundances. The third peak is m/z 381 from the polymerization product, 3,4-di(4'-isocyanatotolyl)phenylisocyanate.

The CI chromatogram shows the three major peaks, and the spectra corroborate the EI results by showing the M+H ions expected for each of the molecular ions found in the EI spectra. It is interesting to note that the sensitivity of the analysis by CI is about an order of magnitude better than EI, which is probably due to the increased ionization efficiency of methane CI as compared to CO₂ charge transfer. Also, three additional small peaks are found. One is the second expected polymerization product, with a molecular weight of 512 u, as shown by the M+H ion at m/z 513. A second small peak, showing a molecular weight of 282 u, represents the urethane formed in reaction with methanol, which can probably be found in trace amounts in the methylene chloride used as the solvent in this analysis. A third very small peak represents the 2,4'- isomer of the di(isocyanatotolyl)phenylisocyanate polymerization product.

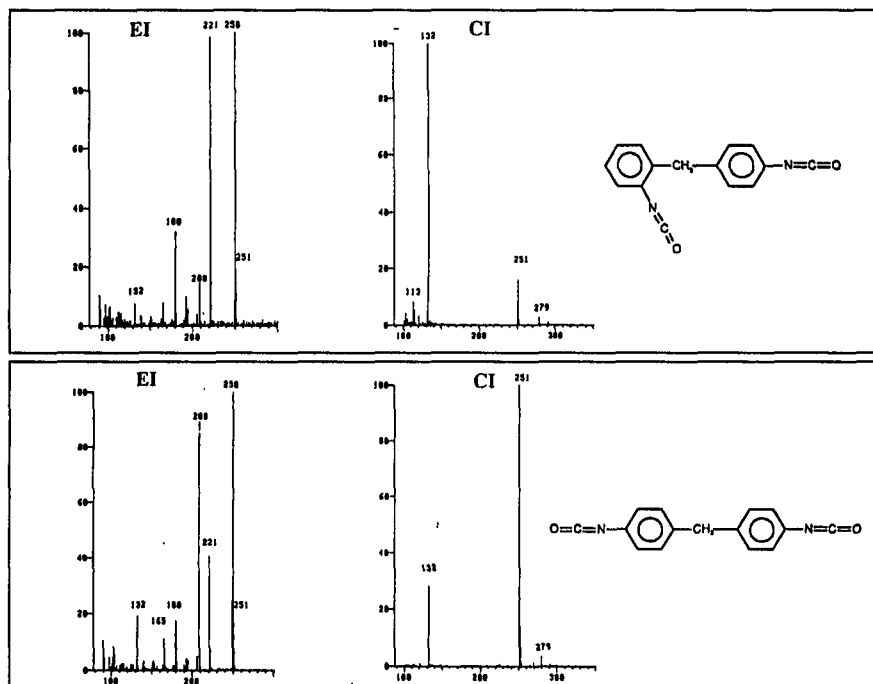


Photo- and Thermal Degradations of Iodonium Salts:
'Complications' within Secondary Ion Mass Spectrometry and
Fast Atom Bombardment

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Previous work with iodonium salts has shown that liquid secondary ion mass spectrometry (SIMS) and fast atom bombardment (FAB) produce good quality mass spectra for iodonium salts, containing abundant signals for the intact cation and some degree of structurally informative fragmentation. Diaryliodonium salts are used extensively as photocatalysts of cationic polymerizations. Upon irradiation with ultraviolet light, they form strong Bronsted acids that initiate cationic polymerizations, used most often in the cross-linking of polymers. The presence of certain ions in the spectra suggest that some amount of photodegradation of the salts may be occurring within the mass spectrometer. This study is concerned with determining whether the ions seen in the mass spectra of these salts are related to their thermal and photodegradations or if these reactions are completely unique to the mass spectrometer.

The ion and atom sources in FAB and SIMS are good sources of light in the visible and ultraviolet wavelength regions. This would suggest that photoinduced reactions could occur within the mass spectrometer to form the products seen in thermal and photodegradations. Therefore, thermal- and photo- reactions were performed outside the mass spectrometer, their products analysed and compared to the liquid SIMS spectra of the salts in glycerol. Both reactions were performed using 0.01M solutions of diphenyliodonium chloride in acetonitrile or diglyme. For the photodegradations, the solutions were irradiated with ultraviolet light for 1-3 hours, and then analysed. The thermal reactions were also performed on 0.01M solutions of the salt, but these solutions were refluxed for 1-3 hours, and then analysed.

Ions formed from loss of iodine from the intact cations, and the product iodobenzene, were detected in the liquid SIMS and FAB mass spectra of the salts, and in the FAB mass spectra and GC/MS analysis of the reaction mixtures. Polymerization products were seen in the FAB mass spectra of the photo- and thermal experiments, in addition to the regular mass spectrum of the iodonium salt. The secondary ion yield for iodonium salts is very high, and only a short time is required for measurement of the mass spectrum. If the monomer is present along with the iodonium salt in the sputtered solution, the formation of polymerization products is not expected to be a "complication" in the interpretation of the mass spectra. With longer irradiation times in the source of the mass spectrometer, a similar reaction to that observed in the external irradiation experiments may occur. Experiments with extended irradiation/sputtering times are underway.

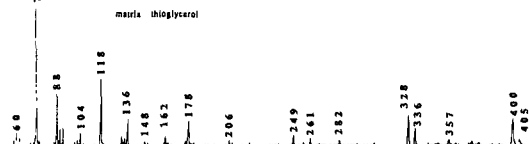
Ions that were detected in the FAB mass spectra and the GC/MS analysis of the reaction mixtures differed with ionization method and reaction. In the thermal and photodegradations of diphenyliodonium chloride in acetonitrile, the FAB spectra indicate a tropylium ion which is not seen in the EI spectra. Additionally, ions corresponding to polymerization products are seen in FAB, the highest molecular weight corresponding to a polymer of seven monomer units ($m/z = 289$). The main difference between the thermal and photodegradations is in the intensity of the ions that are related to polymeric species. This indicates that the photolyzed sample polymerizes to a greater extent than the thermal sample.

In the EI mass spectra of these reactions, the more volatile species chlorobenzene and iodobenzene are seen along with diphenyliodonium and biphenyl, though they were not observed with FAB.

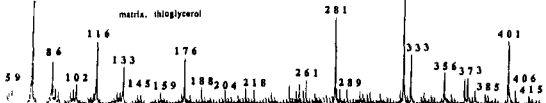
When the same reactions were performed with diglyme, only FAB spectra were recorded because the diglyme was too polar for separation by GC. The products seen were iodobenzene (small relative abundance), diphenyliodonium and polymerized diglyme for both the photo and thermal reactions. The photo reaction, however, produced higher molecular weight products.

FAB

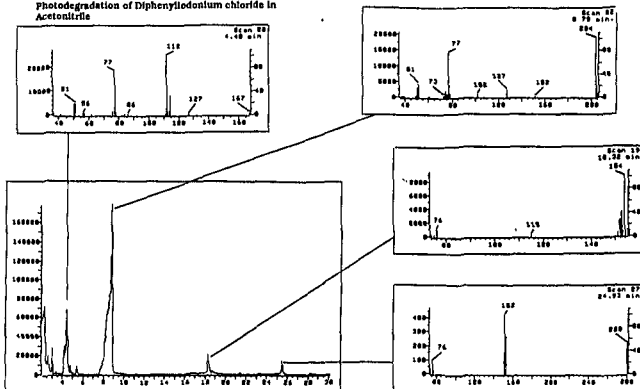
Photodegradation of Diphenyliodonium chloride in Diglyme



Thermal Degradation of Diphenyliodonium chloride in Diglyme



Photodegradation of Diphenyliodonium chloride in Acetonitrile



IDENTIFICATION OF POLYMER INTERMEDIATES BY LINKED SCANS
FAST ATOM BOMBARDMENT MASS SPECTROMETRY.

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The curing of epoxy resins with amines to create high performance composites with high heat resistance is of considerable commercial interest. In order to understand the properties and behavior of these materials, a fundamental knowledge of their chemistry is required. This overall understanding of the bulk properties is facilitated by the identification and characterization of the many intermediates which are involved in the polymerization process. Since, however, the most often used epoxy resins are polyfunctional, such as N,N,N',N'-tetraglycidyl-4-4'-diamino diphenylmethane (TGDDM) and they are cured with polyfunctional primary amines, the final product and its intermediates are difficult to characterize. As a means of simplifying the problem a model system, diglycidylaniline (DGA) and aminodiphenyl sulfone (ADS), has been used in identifying the key polymerization processes. Moreover, because the structure of the first intermediate (1:1 DGA/ADS) will define all further reactions, the structural identity of this product is of considerable interest.

It is known that the addition of the DGA to the ADS occurs *via* the opening of the epoxy ring to create a secondary amine and free hydroxy. However, the relatively high reactivity of the second glycidyl group and its close proximity to both the amine and the hydroxy functionalities allows for several intramolecular reactions to occur. In order to determine the correct structure of this product the material was isolated and subsequently identified using linked scans fast atom bombardment mass spectrometry. By performing linked scans on the major fragment ions, the structural relationship between the ions was determined which made structure identification easier and more confident. It has been shown that although the possibility exists for several intramolecular reactions, only one of the epoxies initially reacts.

In addition to the DGA/ADS system, linked scans were used to determine the structure of an impurity formed during the synthesis of another polymer starting material, 1,1'-(methylene-di-4,1-phenylene) bismaleimide (APO-BMI). The linked scans of both the compound of interest, as well as the impurity are presented in Figures 1a and 1b respectively.

Many of these peaks were not seen in the normal FAB spectra because of the high abundance of background peaks.

A wealth of information may be obtained by following the metastable transitions which a particular molecule undergoes in the first field free region of a double focusing mass spectrometer. The added selectivity of the linked scans allows for the determination of low intensity fragmentations which would normally be lost within the background chemical noise. These fragmentations, which are normally higher energy processes, help identify and characterize the molecular structure.

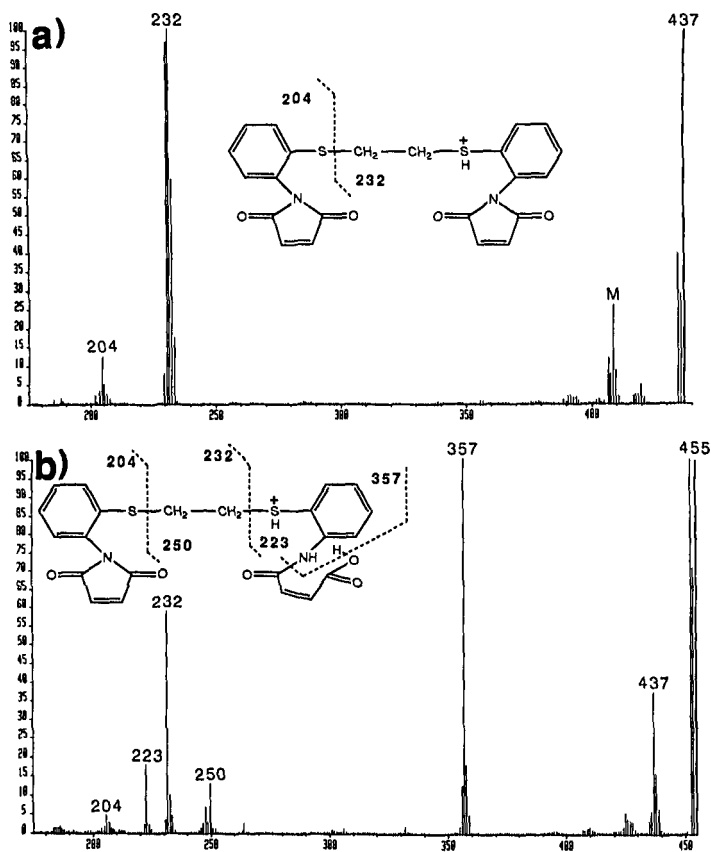


Figure 1 Linked scans of protonated molecular ions of (a) APO-BMI (m/z 437) and (b) impurity (m/z 455).

THE CHARACTERISATION OF AROMATIC POLYMERS BY FIELD DESORPTION MASS SPECTROMETRY

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Cleveland, England

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INTRODUCTION

The importance of non-polar synthetic molecules of >1000 daltons has significantly increased in recent years with the use of sophisticated formulations in the general chemical and polymer fields and with the advent of low molecular weight (5-20,000 daltons) aromatic polymers used in high performance applications such as composite manufacture.

We have reevaluated FD techniques for the characterisation of these molecules and have found that this approach affords significant advantages over pyrolysis, laser desorption and high temperature probe techniques previously used.

DEMULSIFIERS

Among a range of complex chemical formulations we have studied using FD are a range of products manufactured by ethoxylating and/or propoxylating alkyl phenol formaldehyde resins. These materials, which are extremely difficult to characterise, are used as demulsifiers in the oil industry. Molecular weight distribution, both of the resin and the product, and degree of ethoxylation/propoxylation are key parameters in determining the end properties of the product.

Fig 1 shows the FD spectra of two typical products together with their GPC traces. RA 00/50 is an ethoxylated octylphenol formaldehyde resin and RC 15E/05 is an ethoxylated and propoxylated butyl phenol formaldehyde resin. The spectra indicate, at a glance, whether the sample is ethoxylated, propoxylated or both and gives excellent molecular weight distribution information. Closer inspection of the spectra indicate the nature of the resin employed.

AROMATIC OLIGOMERS

Fig 2 shows the FD spectra of two oligomeric systems A and B which are both based on the system $\text{ClOSO}_2(\text{OSOSO}_2)_n\text{OCl}$ (where O indicates a para substituted benzene ring). The first point to notice is the abundant multiply charged ions in the lower part of the spectrum. The spectra show that sample B has a higher molecular weight distribution than sample A indicating that the polymerisation has gone further. Of even more interest however is the presence in the spectrum of sample B of ions to the left of the major oligomeric ions. These correspond to molecules in which the end group is different. The differences indicate that the substitution is F for Cl and OH for Cl. The distributions for these mixed end group species can also be measured giving important information on kinetics and reactivity. Note that the identification of OH and F end groups requires a resolution of two daltons.

AROMATIC POLYMER

Modern aromatic polymer systems such as aryl ethers, ketones, sulphides and sulphones have a molecular weight range of ~5-25,000 daltons. In the past mass spectrometry could only contribute to the characterisation of these materials after chemical or thermal degradation. This approach has only limited utility. Developments in ionisation technique, mass range and sensitivity of mass spectrometric instrumentation was required in order for these systems to be studied intact. The combination of FD with high performance double focussing instruments has made this a reality. Figure 3 shows the FD spectrum of an aromatic polymer of the structure indicated. This is typical of a wide range of polymers studied where ions up to 10,000 daltons can be easily seen.

CONCLUSIONS

Field desorption ionisation coupled with high mass range double focussing mass spectrometers has been shown to be a powerful approach to the characterisation of non polar molecules of >1000 daltons mass range. Providing information on structure and molecular weight distribution difficult to obtain by other methods.

FIGURE 1

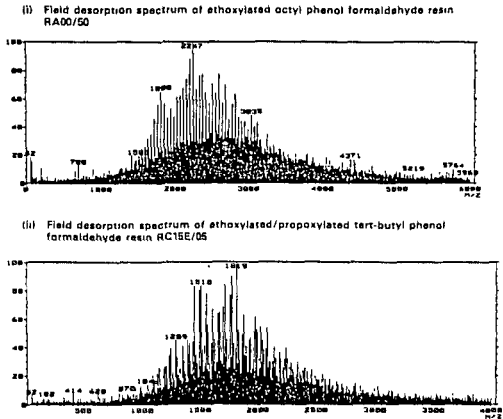
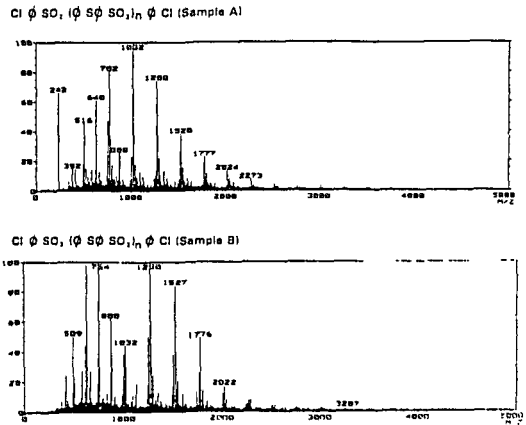


FIGURE 2



THE CHARACTERISATION OF SYNTHETIC LUBRICANT FORMULATIONS BY FIELD DESORPTION MASS SPECTROMETRY

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The analysis of complex mixtures in difficult matrices is a recurring requirement for the industrial mass spectrometry laboratory. This provides a considerable challenge to the mass spectroscopist since such samples can often contain compounds of widely differing polarity, thermal lability and molecular weight. A recent example of this type of work in our laboratory has been the analysis of commercial synthetic lubricant samples. These formulations typically consist of up to three major components; a synthetic or natural oil, a polyolefin and a synthetic ester.

The initial approach to this problem was the analysis of the lubricant formulations by conventional GC-MS and direct insertion probe methods. However, the combined problems of overwhelming hydrocarbon background signal which results in extreme difficulty in the extraction of useable information coupled with the involatility of the increasingly complex high molecular weight esters used make the use of both of these techniques in this area inappropriate. For these reasons, the techniques of field desorption (FD) and field ionisation (FI) were evaluated as possible solutions.

The FD/FI spectra were obtained using a VG Analytical ZAB-2SE mass spectrometer of reverse geometry and the standard combined VG FAB/FD/FI source. 13 μ m FD emitters were prepared using the high temperature indene FD-emitter activation process of Rabrenovic et al (1).

Three examples of the utility of the FD-MS technique over a range of three different types of mixtures are presented here. The first example, shown in Figure 1, is the FD spectrum of a synthetic oil-based lubricant of molecular weight distribution 300 to 600. Figure 2 shows a second example in which the oil base has been supplemented by the addition of a polyalphadecene additive as shown by the peaks appearing at a spacing of 140 daltons. The final example shown in Figure 3 is a good illustration of a lubricants package comprising a synthetic oil base of molecular weight distribution 300 to 500, again the polyalphadecene additive (peaks at M/Z 421, 561, 701, 841 and 979) and proprietary esters at M/Z 424 and 1110. A further point worth noting from the spectrum is the distribution of peaks at M/Z 1110 $\pm$ 14 daltons which provides information on the alkyl chain length distribution of the feedstock acids/alcohols used in the preparation of these esters. This again provides a convenient method for ester characterisation.

The use of FD/FI techniques clearly provides an elegant approach to the characterisation of complex mixtures. The ability to investigate a wide range of compound types of varying polarity and thermal stability all within a single mass spectrum is an extremely attractive feature. The installation of a four sector tandem MS-MS system of BEBE geometry (the VG Analytical ZAB-T) in late 1989 will be used to extend the present work to the use of FD-MS-MS is the characterisation of synthetic lubricants.

Reference

- 1 M. Rabrenovic, T. Ast and V. Kramer, Int. J. Mass Spectrom. Ion Phys., 37, 297 (1981).

FIGURE 1 : FD SPECTRUM - SYNTHETIC OIL BASED LUBRICANT

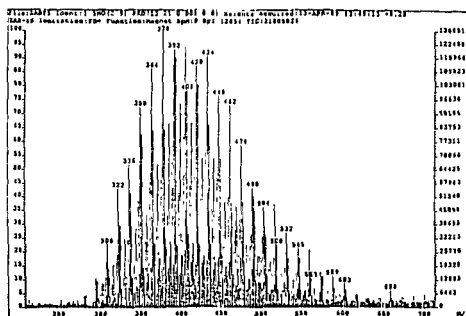


FIGURE 2 : FI SPECTRUM - OIL/POLYALPHADECENE LUBRICANT

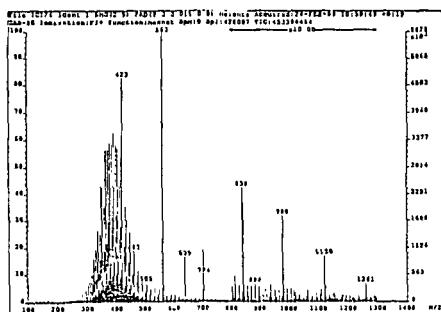
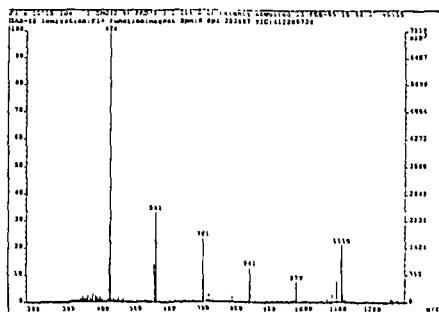


FIGURE 3 : FI SPECTRUM - OIL/POLYALPHADECENE/ESTERS LUBRICANT



MASS SPECTRAL CHARACTERIZATION OF COMPOUNDS FORMED DURING HIGH TEMPERATURE OXIDATION OF STYRENE BASED POLYMERS USING THERMAL CHROMATOGRAPHY

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Three styrene based polymers, polystyrene, poly[4-chlorostyrene], and poly[4-t-butylstyrene] were thermally decomposed by heating to 600°C in both helium and air in a Ruska Laboratories' Thermal Chromatograph-Mass Spectrometer (TC-MS). In this turn-key heating/GC/MS instrument, the identification and detection of compounds eluting from a capillary GC column was accomplished by using a Hewlett-Packard 5970 MSD mass spectrometer equipped with a 59940 HP-UX data system.

Different experiments were performed in order to assess the dependance of relative amounts of oxidation upon various experimental parameters. The polymers were heated at either 5°C/min or 30°C/min in either helium or air from 30°C to 600°C. In the oxidation experiments the effect of flow rate of air past the heated polymer was studied as well. Thermal degradation compounds were trapped in a cold trap at -80°C. They were desorbed by heating the trap to 340°C at 30°C/minute and were subsequently cryogenically focused on the head of a 30m x 0.32mm DB-5 capillary column at -45°C. The GC column was heated from -45 to 305°C at 5°C/min. Simultaneously with the initiation of GC column temperature programming, the Hewlett-Packard MSD was turned on. It scanned a 34 to 450 amu mass range once every 1.35 seconds.

For each polymer, the major thermal decomposition product formed by heating in helium was the monomer: styrene, 4-chlorostyrene, and 4-t-butylstyrene (Figure 1, for example). The most abundant oxygenated thermal decomposition product formed by heating polystyrene in air was benzaldehyde. Other oxygenated compounds formed included phenol, benzene acetaldehyde, 1-phenylethanone, phenylpropenal isomers, and benzoic acid (Figure 2). The 4-chloro and 4-t-butyl analogs of these compounds were formed by heating poly[4-chlorostyrene] and poly[4-t-butylstyrene] in air.

THERMAL DEGRADATION OF POLYSTYRENE BEAD IN HELIUM

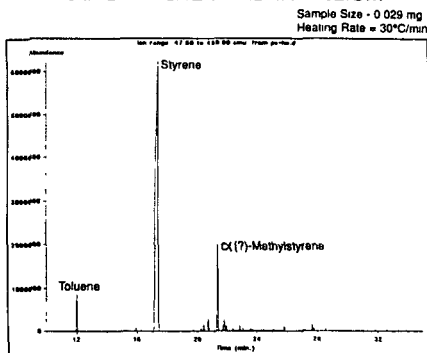


Figure 1

THERMAL DEGRADATION OF POLYSTYRENE THIN LAYER IN AIR

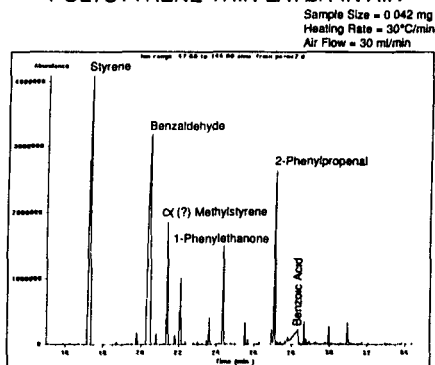


Figure 2

One way in which the relative degree of oxidative thermal degradation can be assessed is by the ratio of the aldehyde to the corresponding styrene based monomer (Table 1). For one set of experimental conditions, this ratio is 3.83 for polystyrene, 8.01 for poly[4-chlorostyrene], and 1.53 for poly[4-t-butylstyrene]. Note that the benzaldehyde to styrene ratio of 19.3 in Table 1 may be anomalously high because of loss of styrene to benzaldehyde caused by inefficient trapping in that experiment. Other

parameters such as the ratios of benzoic acid to benzaldehyde, 4-chlorobenzoic acid to 4-chlorobenzaldehyde, and 4-t-butylbenzoic acid to 4-t-butylbenzaldehyde also indicate oxidation. Interestingly, the oxidation of 4-chlorostyrene was accompanied by the formation of significant amounts of hydrochloric acid. Further experiments indicated that the most important experimental parameter affecting the relative amount of oxidation was the heating rate from 30 to 600°C: slower heating rates led to more oxidation. The surface to volume ratio of the heated polymer and the flow rate of air through the porous, fused quartz crucible appear to be of less importance. Finally, the susceptibility of the styrene based polymer to oxidation depended upon the nature of the substituent group on the benzene ring. An oxidation index was formulated by dividing the ratio of aldehyde to monomer for thin layer data at a 5°C/minute heating rate by the same ratio for thin layer data at a 30°C/minute heating rate for each polymer. Larger values of this index indicate increased susceptibility to oxidation. Based upon this index, poly[4-chlorostyrene] appears to be more susceptible to oxidation than polystyrene which, in turn, appears more susceptible than poly[4-t-butylstyrene]. In this respect the oxidation index correlates well with the Hammett substituent constant, sigma.

TABLE 1
ALDEHYDE TO MONOMER RATIOS INDICATE OXIDATIVE THERMAL DEGRADATION OF SUBSTITUTED POLYSTYRENES

EXPERIMENT	POLYSTYRENE	POLY[4 CHLOROSTYRENE]	POLY[4 t BUTYLSTYRENE]		
	BENZALDEHYDE ¹ STYRENE ²	4-CHLOROBENZALDEHYDE ³ 4-CHLOROSTYRENE ⁴	4-t-BUTYLBENZALDEHYDE ⁵ 4 t BUTYLSTYRENE ⁶		
Helium 30°C/min	0.0	0.0	0.0		
Air, Bead 30°C/min Air = 30 ml/min	0.78	1.60	1.12		
Air, Thin Layer 30°C/min Air = 30 ml/min	0.72	0.69	0.68		
Air, Bead 5°C/min Air = 30 ml/min	19.3	6.50	1.63		
Air, Thin Layer 5°C/min Air = 30 ml/min	3.83	8.01	1.53		
Air, Bead 30°C/min Air = 4 ml/min	0.73	1.45	0.98		
Air, Thin Layer 30°C/min Air = 4 ml/min	0.86	0.74	0.76		
1. m/z = 106	2. m/z = 104	3. m/z = 139	4. m/z = 138	5. m/z = 147	6. m/z = 145

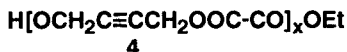
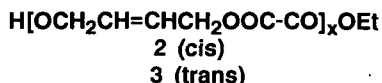
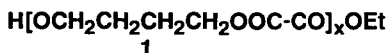
Although this study focused on low molecular weight thermal decomposition products, various dimers and trimers were also identified along with corresponding oxygenated derivatives formed by heating the polymers in air.

Mass Spectral Study of the Thermal Behavior of Polymers of Oxalic Acid

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This project involves the design of polymers that decompose cleanly to gaseous products. One class of polymer that appears promising in this regard is the unsaturated aliphatic polyoxalates, which may be expected to decompose to carbon dioxide and conjugated polyenes. In order to test the concept, a series of polyoxalate esters was prepared by transesterifying diethyl oxalate with the following C4 alcohols: 1,4-butanediol, *cis*- and *trans*-2-butene-1,4-diol, and 2-butyne-1,4-diol. The structures of the resulting polymers (1-4) are as follows:



The polymers were characterized by IR and NMR spectroscopy. The latter technique showed that the degree of polymerization was generally low for each product; the highest DP (~ 32) achieved with polymer 4. Thermal behavior of the four products was investigated by means of TGA, DSC, and EI- and CIMS.

TGA showed that polymers 1-3 decomposed quite cleanly, leaving residues ≤ 10 percent at 450°C. Polymer 4 left a residue of 20-30 percent at that temperature. DSC results were consistent with our initial hypothesis: while the saturated polymer 1 decomposed endothermically as expected, polymers 2-4 decomposed exothermically. This result agrees with formation of stable fragments by a simple bond cleavage mechanism.

In order to investigate the decomposition mechanisms in detail, MS was used for product identification. The literature contains no report on the MS of polyoxalates and few on simple oxalate esters. The latter are "very unstable upon electron impact." [1] For this reason, both EI- and CIMS (methane reagent gas) were applied. The former was useful for identifying low molecular weight fragments, the latter for detecting oligomeric products. Samples were decomposed in the direct insertion probe of a Finnigan 4500 mass spectrometer while the temperature was raised from 50 to 350°C. The probe was held at several intermediate temperatures (~ 140, 200, 250, 300) for periods up to 1 minute with rapid change between temperatures.

Three major processes were identified for the decomposition of 1. At temperatures up to 140°C, the main reaction is further polymerization, indicated by production of the alcohol end groups, ethanol and butanediol (see Table). A temperature increase leads to appearance of small linear oligomers, also a result of further transesterification. At temperatures above 300°C, depolymerization through formation of cyclic oligomers becomes important. [2]

Polymers 2 and 3 also undergo transesterification and evolve alcohols and short chain linear oligomers below 300°C. Unlike 1, however, 2 and 3 lose alcohols by heterolytic cleavage of the labile allylic and propargylic O-CH₂ bonds. This reaction produces HOOC-COOR end groups, evidenced by the detection of protonated oxalic acid (*m/z* 91) and of their thermal products, fragments with formyl end groups, HCOOR. Above 300°C 2 and 3, like 1, depolymerize to cyclic oligomers, but this process becomes secondary to homolytic fragmentation to CO₂ and 1,3-butadiene, as suggested by DSC. Both 2 and 3 produce very similar spectra, suggesting similar thermal fragmentation paths.

THERMAL PRODUCTS

Polymer	$T < 125^{\circ}\text{C}$	$100^{\circ}\text{C} < T < 300^{\circ}\text{C}$	$T > 300^{\circ}\text{C}$
1	$\text{HO}(\text{CH}_2)_4\text{OH}$ EtOH	$\text{HO}(\text{CH}_2)_4\text{OOCOR}$ $\text{EtOCCOO}(\text{CH}_2)_4\text{OR}$	$[-\text{O}(\text{CH}_2)_4\text{OCCO-}]_n$
2,3	$\text{HOCH}_2\text{CH:CHCH}_2\text{OH}$ EtOH	$\text{EtOCCOOCH}_2\text{CH:CHCH}_2\text{OR}$ $\text{HOCCOOCH}_2\text{CH:CHCH}_2\text{OR}$ $\text{HCOOCH}_2\text{CH:CHCH}_2\text{OR}$	$\text{CH}_2\text{:CHCH:CH}_2$ $[-\text{OCH}_2\text{CH:CHCH}_2\text{OCCO-}]_n$
4	$\text{HOCH}_2\text{C:CCH}_2\text{OH}$ EtOH	$\text{HOCH}_2\text{C:CCH}_2\text{OCCOOR}$ $\text{HOCCOOCH}_2\text{C:CCH}_2\text{OR}$ $\text{HCOOCH}_2\text{C:CCH}_2\text{OR}$	$\text{CH}_2\text{:C:C:CH}_2$ $\text{HOCCOOCH}_2\text{C:CCH}_2\text{OR}$

Polymer 4 continues the pattern of transesterification and alcohol loss at low temperatures, and fragments to butatriene at high temperatures. Formation of cyclic oligomers is restricted by conformational considerations and no compounds larger than dimers were detected. Cross-linking of the triple bonds at elevated temperatures probably explains the large residue.

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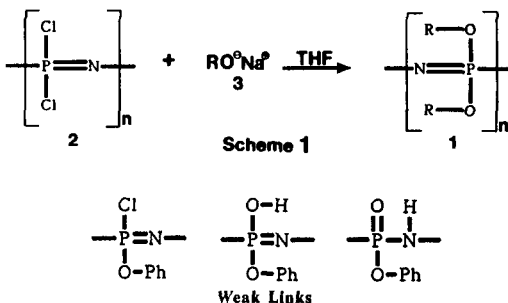
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DEGRADATION OF POLY[BIS(PHENOXY)PHOSPHAZENE] POLYMERS

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Poly(organophosphazenes) (**1**) are made by the reaction of poly(dichlorophosphazene) (**2**) with alkoxides (**3**), shown below in Scheme 1, where OR is an alkoxy or an aryloxy group^{1,2}. They are elastomers or film-forming thermoplastics, and have superior physical and mechanical properties. These polymers are currently being used where physical flexibility or chemical durability are needed in extreme temperature regimes³. As a result of the high technology applications of poly(organophosphazenes), thermal and hydrolytic degradation of these materials has been, and continues to be of interest⁴. One of the main conclusions drawn by several studies is that the degradation of poly(organophosphazenes) is greatly affected by "weak links", as shown below. To date, however, a systematic study of the effect that weak links have on the thermal or hydrolytic degradation of poly(organophosphazenes) has not been done. This study focuses on the effects that P-Cl weak link sites have on the thermal and hydrolytic degradation of poly[bis(phenoxy)phosphazene] (PBPP). A series of polymers that have varying concentrations of P-Cl weak link sites were prepared and subjected to several degradation conditions⁵. This report will discuss only one of these degradation conditions -- dynamic degradation.

Compounds: Three samples of PBPP were prepared and studied in detail, and were designated based on their chlorine contents: PBPP-0.00 contained less than 0.005% (by weight) chlorine, while PBPP-2.40 and PBPP-9.90 contained 2.40% and 9.90% chlorine, respectively. The chlorine content in the latter two polymers corresponds to one [N=P(Cl)(OPh)] unit for every six [N=P(OPh)₂] units in PBPP-2.40 and one [N=P(Cl)(OPh)] unit for every [N=P(OPh)₂] unit in PBPP-9.90. All evidence is consistent with the distribution of chlorine along the backbones of the polymers being random.



Dynamic Degradation Procedure: Mass spectral analyses were performed on a VG Analytical 70-S mass spectrometer⁶ using 70 eV electron impact ionization. Samples of PBPP-0.00, PBPP-2.40, PBPP-9.90, or model compounds were introduced by direct insertion probe and heated from ambient temperature to ca. 480°C at ca. 26°C/min. Mass spectra of volatilized materials were concomitantly acquired by repetitive scanning.

Dynamic degradation of PBPP samples. Mass spectrometry was used to identify the degradation products evolved during dynamic degradation of these samples. Figure 1 shows the total ion current (TIC) profiles as a function of probe temperature. These curves are similar to differential thermogravimetric curves, in that their maxima characterize the temperature at which maximum depolymerization of the polymers occurs under these experimental conditions. Figure 2 shows representative mass spectra corresponding to the maxima of the TIC profiles in Figure 1. Identification of products was supported by comparison with the mass spectra of authentic samples. The presence of structurally significant fragment ions, such as [M-OPh]⁺ and M²⁺, that evolved concomitantly with their respective molecular ions were used to support identification^{7,8}. Chlorine isotope patterns were used to identify chlorine-containing products.

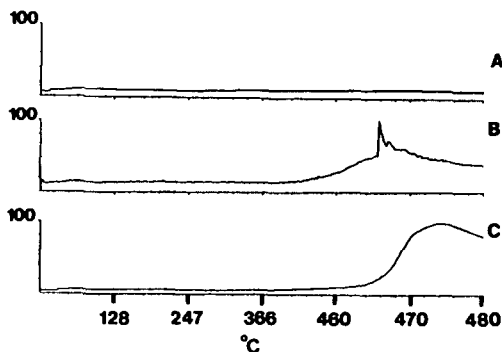


Figure 1:

The TIC profile in Figure 1a shows that depolymerization of PBPP-0.00 was negligible between 25–480°C. Traces of phenol (m/z 94) and $[\text{NP}(\text{OPh})_2]_3$ (M^+ , m/z 693, and M^{2+} , m/z 346.5) were evolved from the sample at 200°C and 480°C, respectively. Traces of monochlorotrimer were also observed at 145°C.

Unlike PBPP-0.00, large amounts of volatile degradation products were evolved from PBPP-2.40, which began to degrade at 375°C (Figure 1b). The species that evolved (Figure 2b) were identified as $[\text{NP}(\text{OPh})_2]_3$ (m/z 693), $[(\text{NP})_3\text{Cl}(\text{OPh})_5]$ (m/z 635), $[(\text{NP})_3(\text{Cl})_2(\text{OPh})_4]$ (m/z 577), $[(\text{NP})_4\text{Cl}(\text{OPh})_7]$ (m/z 866), and $[(\text{NP})_4(\text{Cl})_2(\text{OPh})_6]$ (m/z 808). The corresponding M^{2+} were observed at m/z 346.5, m/z 317.5, m/z 462, m/z 433, and m/z 404, respectively.

Likewise, PBPP-9.90 evolved large amounts of volatile degradation products at 350°C (Figure 1c). The species that evolved (Figure 2c) were identified as $[\text{NP}(\text{OPh})_2]_3$ (m/z 693), $[(\text{NP})_3\text{Cl}(\text{OPh})_5]$ (m/z 635), $[(\text{NP})_3(\text{Cl})_2(\text{OPh})_4]$ (m/z 577), $[(\text{NP})_4\text{Cl}(\text{OPh})_7]$ (m/z 866), $[(\text{NP})_4(\text{Cl})_2(\text{OPh})_6]$ (m/z 808), and $[(\text{NP})_4(\text{Cl})_3(\text{OPh})_5]$ (m/z 750). The corresponding M^{2+} were found at m/z 346.5, m/z 317.5, m/z 259.5, m/z 433, m/z 288.5, and m/z 375.

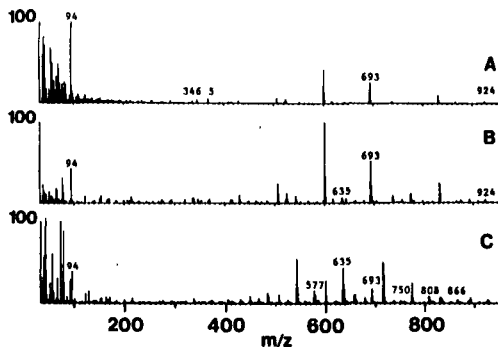
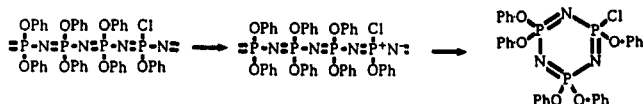


Figure 2:

Traces of monochlorotrimer observed during the dynamic degradation of PBPP-0.00 show that mass spectrometry is uniquely able to identify the presence of residual chlorine by identification of cyclic trimer phosphazene compounds that contain chlorine, even at this very low concentration. The observation of monochlorotrimer indicates that the phosphorus-nitrogen bond from the P-Cl weak link site undergoes a heterolytic bond cleavage to form monochlorotrimer by cyclization depolymerization (Scheme 2). However, there was not an increased amount of trimer or tetramer observed when monochloro trimer was examined. Therefore, depolymerization by cyclization was not significant for PBPP-0.00. This indicates that it is important to identify and quantify the residual chlorine content in a phosphazene polymer prior to characterizing its thermal behavior, in order to obtain an accurate evaluation of its thermal stability. Allcock et al. observed chlorobenzene produced from degradation of PBPP and suggested it to be a sensitive test for identifying residual chlorine. In this study, where PBPP was intentionally prepared with residual chlorine, no chlorobenzene was detected under these conditions.



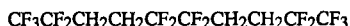
Scheme 2

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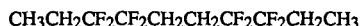
THERMAL STABILITY OF ETFE COPOLYMER SYSTEMS *, John J. Morelli, Charles G. Fry, Clarence J. Wolf, Michael A. Grayson, and Arthur C. Lind, McDonnell Douglas Research Laboratories, St. Louis, MO, 63166.

This report focuses on evolved gas mass spectrometric (EGAMS) analysis of an irradiated (X) poly(ethylene tetrafluoroethylene) (ETFE) insulation and neat unirradiated ETFE resin. ¹³C-NMR and IR data are presented to complement the EGAMS findings and allow a more complete understanding of chemical changes occurring within the resin during thermal aging in air. This combination of techniques allowed both evolved gases and bulk polymer constituents to be characterized.

Figure 1 is typical of the total ion current (TIC) profile obtained from ETFE and X-ETFE systems before and after nine days of thermal aging in air at 200°C. The major change observed between the evolution profiles of aged and unaged systems is in the region of scans 15 to 45 of Figure 1 (corresponding to 200 and 350°C). The EGAMS spectra obtained in this temperature interval from the unaged systems indicate that the materials evolved are low-molecular-weight oligomers of ETFE. Figure 2 is an example of the spectra obtained. Although the spectrum is a mixture of compounds, major ions can be rationalized by comparison with reference spectra of tetradecafluorodecane (I) and octafluorodecane (II). Spectra of I and II indicate m/z 69, 77, 127, 177, and 197 are ions associated with fragmentation from the CF₃ terminus, whereas m/z 79 characterizes fragmentation from the CH₃ terminus.



I



II

The following series of ions were observed below pyrolysis temperatures in EGAMS spectra of ETFE systems: (a) 69 + n128, 77 + n128, 127 + n128, 177 + n128 due to fragmentation from the CF₃ terminus; (b) 79 + n128 due to fragmentation from the CH₃ terminus; (c) 147 + n128, m/z 147 is seen in both fluorinated decane spectra. The spectrum in Figure 2 and the reference spectra of I and II indicate fragmentation preferentially involves the bond between consecutive fluorinated carbons. We assume that ions which do not fit the above series but can be rationalized as displaced by 28 mass units indicate that an extra ethylene unit is present in the fragment. Preliminary data indicate that such ions are direct evidence of consecutive ethylene units in an otherwise alternating chain of ethylene and tetrafluoroethylene units. Similarly, ions displaced by 50 or 100 from these series indicate extra tetrafluoroethylene units. For example, m/z 205 is included in series (a); therefore, this signal is not attributed to be m/z 177 + 28. Alternatively, m/z 227 and m/z 297 are not members of series (a), (b), or (c); thus, these ions are assigned to m/z 177 + 50 and m/z 197 + 100, respectively. In general, series (d) is assigned to ions characteristic of consecutive ethylene or tetrafluoroethylene units.

An additional species was detected in the unaged X-ETFE wire insulation between 200°C and 350°C and was identified as triallyl isocyanurate (TAC; III). This species was not detected during EGAMS analysis of the aged wire, suggesting that it was lost during thermal aging.

Figure 3 shows the solid-state ¹³C cross-polarization/magic-angle-spinning (CP/MAS) NMR spectra of unaged and thermally aged wire insulation. Resonances from tetrafluoroethylene (3) and ethylene (5) units, and TAC resonances (1, 2, and 4) were observed in the unaged wire. The "s" contributions are spinning sidebands, that is, artifacts due to the sample spinning used to maximize spectral resolution from solid-state materials. In the NMR spectrum of the thermally aged insulation, the allyl moiety resonances, 2 and 4, of TAC are missing. Together, the NMR and EGAMS data indicate TAC is present in unaged wire as two separate species. We conclude that these forms are "free" TAC and crosslinked TAC. During thermal aging, "free" TAC is lost. In addition, a new resonance (6) is observed by NMR after thermally aging insulation and neat ETFE resin and indicates the polymer is undergoing crosslinking reactions during thermal aging.

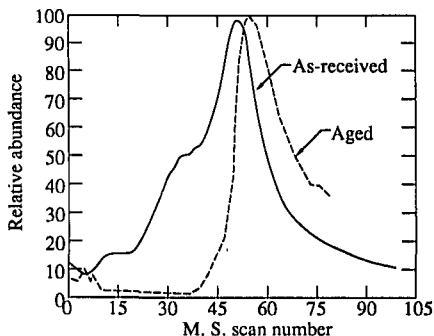
Figure 4 shows the IR spectra obtained from unaged and thermally aged insulation between 1800 cm⁻¹ and 1550 cm⁻¹ and confirms that two carbonyl species are present (1700 cm⁻¹ and 1740 cm⁻¹). IR spectral examination also shows the preferential loss of one species after thermal aging of the wire.

The pyrolysis mass spectra of all systems obtained in the temperature region above 400°C show the ETFE ion series (a) - (d) described above and many low-intensity ions as yet unidentified. The wire insulation also evolved antimony oxide and antimony fluoride at pyrolysis.

Reference

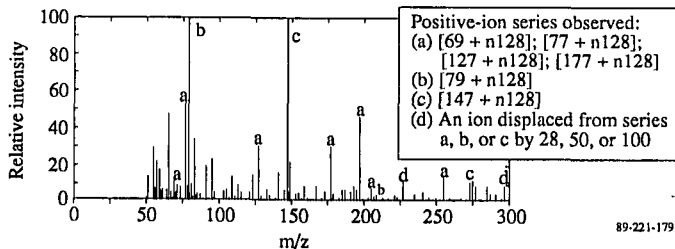
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*This research was conducted under the McDonnell Douglas Independent Research and Development program.



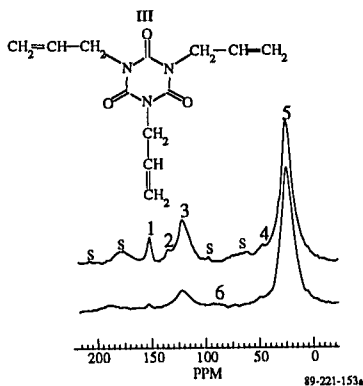
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Fig. 1 EGAMS (total ions) of as-received and aged electrical wire insulation.



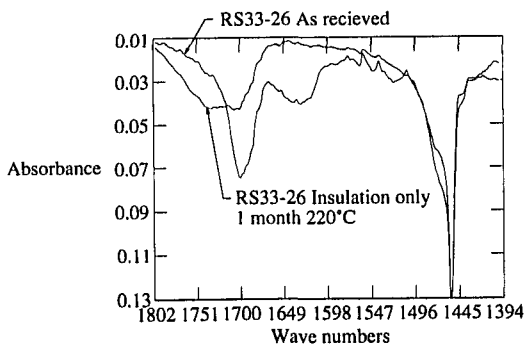
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Fig. 2 EGAMS of ETFE film; T = 250°C.



89-221-153a

Fig. 3 ^{13}C CP/MAS spectra of X-ETFE as received (top) and aged 1 month at 220°C (bottom).



89-223-40

Fig. 4 IR spectra of ETFE wire systems.

LIQUID CARBON DIOXIDE VS. SOLVENT EXTRACTION
OF ORGANIC POLYMER ADDITIVES
FOR MASS SPECTRAL ANALYSIS

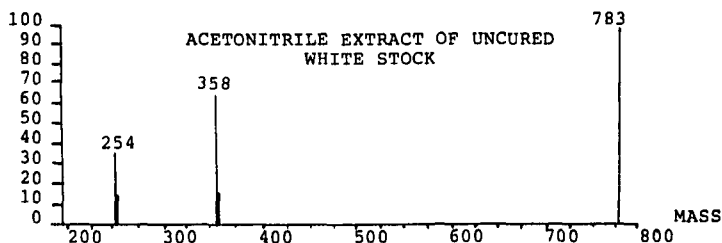
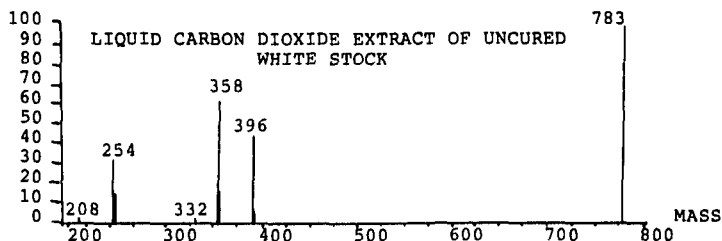
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The analysis of polymer additives is an important part of product formulation or failure analysis. Traditionally, organic additives in rubber formulations have been analyzed by characterization of isolated extracts from solvent extraction techniques. A common method involves the use of Soxhlet-type apparatus, where the additives are exposed to boiling solvent for long periods of time and are diluted by a large volume of solvent. Typically, organic polymer additives are used at a level of less than 1%. Consequently, the loss of analyte to reaction, degradation, and volatilization during extraction or sample concentration makes the task of analysis much more difficult. Additionally, degradation of the polymer and the volume of solvents involved can introduce artifacts. Uncured compounded rubber presents an additional complication in the analysis of samples because the reactive curatives are temperature sensitive. This paper presents a comparison of liquid carbon dioxide extraction versus soxhlet-type solvent extraction of organic polymer additives for mass spectral analysis. The advantages of liquid carbon dioxide extraction are the low temperature to which the additives are exposed and that the analytes are obtained as a solvent free residue. Mass spectrometry is a highly sensitive and proven technique for the identification of organic additives in polymers (1).

Experimental: The liquid carbon dioxide extractions were carried out in a J&W high pressure soxhlet extractor for 3 hours using dry ice as a source of carbon dioxide. The solvent extractions were carried out in a Soxtec extraction apparatus using 50 ml of acetonitrile as solvent for a period of one hour and were concentrated under nitrogen flow with heat. Both types of extraction were performed on uncured and cured rubber samples consisting of a white stock and a black stock based on synthetic polyisoprene. One gram samples in thin sheets were extracted. Field desorption mass spectrometry was carried out by dipping the carbon emitters in an extract solution at a concentration of 20 mg/ml.

Results/Discussions: The extracts were run by FD/MS since it has been demonstrated this technique gives the most information in the least amount of time (2). The white stock sample contained the following organic additives: mercaptobenzothiazole disulfide (MBTS); tetramethylthiuram monosulfide (TMTM); 4,4'-thio-bis-(2-methyl-6-t-butyl phenol) (TMBP); and tris(3,4-di-t-butyl-4-hydroxybenzyl) isocyanurate (BHBI). The black stock sample had the following organic additives: t-butylamine-2-benzothiazylsulfenamide (TBBS), N-(cyclohexylthio) phthalimide (CTP) and N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD). Molecular ions for the curatives, TBBS and CTP were observed at 238 and 261 amu in the uncured black stock extracts by liquid carbon dioxide and solvent extraction. However, molecular ions for the curatives, TMTM and MBTS were observed (208 and 332 amu) only in the liquid carbon dioxide extract of the uncured white stock. Rheological curves of the black and white stock samples indicate the white stock is vulcanized in half the time of the black stock. This indicates the white stock sample is too reactive for soxhlet-type extraction, but the

mild conditions of liquid carbon dioxide extraction isolates intact curatives. The antidegradant molecular ions of 6PPD, TMBP, and BHBI were observed at 268, 358, and 783 in cured and uncured samples by both extraction techniques. Ion intensities of all the additives were similar in the mass spectra of the liquid carbon dioxide and acetonitrile extracts even though in every case the liquid carbon dioxide extract weights were lower than the equivalent acetonitrile sample. This suggests the liquid carbon dioxide extraction efficiency is comparable to the acetonitrile extraction for the range of polarities and molecular weights of organic additives in this study.



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**STEPWISE PYROLYSIS OF POLYMERIC MATERIAL
BY ON-LINE PYROLYSIS/GC/MS**

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Pyrolysis (PY) is the thermal dissociation of material into simpler fragments. Pyrolysis under controlled conditions can be used to obtain characteristic fragmentation of non-volatile materials into volatile components. Pyrolysis, followed by GC/MS, is a particularly powerful tool for the separation and identification of polymeric materials.

An on-line PY/GC/MS system has been constructed and modified from commercially available instruments. A Chemical Data Systems (CDS) Pyroprobe Model 122 was coupled to a Hewlett-Packard (HP) 5890 Gas Chromatograph/5970 Mass Selective Detector (GC/MS) system. A four-way ball valve was installed between the inlet and the outlet of the GC interface of the Pyroprobe, so that the GC carrier gas could either sweep or by-pass the interface after the pyrolytic process. A small amount of sample was placed in a quartz tube. The quartz tube was inserted into a platinum coil probe. The temperature of the probe was set at different temperatures. The same sample was pyrolyzed for 10 seconds at each temperature. The temperature of the probe chamber was 200°C. Helium was used as the carrier gas. The flow rate was 15 ml/min over the pyrolytic zone. After the pyrolytic process, the volatile pyrolysis products were transferred directly into the GC injector and were analyzed by GC/MS. A 60-m DB-1701 fused silica capillary column was used for GC separation of the pyrolysis products. The GC oven temperature was set at 35°C, held for 5 minutes and then programmed to 220°C at 3°C/min. The GC/MS interface was an open-split interface at 250°C. The temperature of the ion source was 200°C. The mass spectrometer was operated in the EI mode at 70 eV energy. The mass spectral scan range was from 29 to 500 amu. The GC/MS system was interfaced to an HP 1000 computer with the RTE-A series data system. During the course of such successive Pyroprobe treatments, the number of the pyrolysis products detected was seen to increase with increasing temperatures up to a maximum, after which the number decreased with each successive treatment until only a few or no further pyrolysis products could be detected. In the example studied, the temperature of the probe was set at 400°C, 500°C, 550°C, 600°C, 650°C, 750°C, 850°C, and 950°C, respectively. The maximum number of pyrolysis products occurred at about 550°C with only a few components being seen above 950°C. Mass spectra of the pyrolysis products revealed the presence of derivatives of furan, 2-cyclopenten-1-one, furanone and phenol. Since such products are components of pyrolyzed cellulose, it is thus shown that the stepwise pyrolysis technique as performed by the described system can satisfactorily assist in the identification of pyrolysis products and give a full profile of the polymeric materials.

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BULK POLYMER ANALYSIS USING LASER IONIZATION MASS SPECTROMETRY (LIMS) AND
PATTERN RECOGNITION TECHNIQUES

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Laser ionization mass spectrometry (LIMS) is a microanalytical materials analysis technique which employs a focused, high power density pulsed laser irradiation and time-of-flight mass spectrometry (TOF-MS) for the chemical analysis of a wide range of solid materials. The LIMS technique is capable of performing both elemental or inorganic microanalysis as well as organic microanalysis. This paper describes continuing research performed in our laboratories in which pattern recognition processes are employed to classify and identify complex LIMS mass spectra produced from organic polymers.

The present study had two main objectives:

1. Determine how well principal component analysis/discriminant analysis (PCDA) routines can distinguish mass spectra produced from chemically similar polymers and
2. Evaluate whether partial least squares (PLS) techniques[1] can quantitatively relate physical testing data with mass spectra.

These evaluation were performed on an epoxy system comprised of various stoichiometries of the 4, 4'-diaminodiphenyl sulfone (DDS) hardener to the tetraglycidyl 4,4'-diaminodiphenylmethane (TGDDM) resin. The DDS/TGDDM epoxy resin is one of the most important bonding matrices utilized for carbon fiber materials in the aviation industry and its physical properties are strongly dependent on the DDS/TGDDM stoichiometries[2]. Thus, the ability to predict the stoichiometry and hence the physical properties of this important epoxy system *in situ* on the microanalytical scale typical carbon fibers could represent a unique method for certifying the integrity of these reinforced components.

Previous studies on this epoxy system demonstrated that the most unique LIMS mass spectra of the different compositions were produced in positive ion analyses at relatively low laser irradiance ($\sim 10^6 \text{ W/cm}^2$). Thirty positive ion mass spectra were acquired from thirty distinct locations on each sample and the data was processed using the RESOLVETM principal component analysis/discriminant analysis software which has been previously described[3]. Twenty-nine principal components described >94% of the raw data and cross validation of the canonical variates produced in the linear discriminant analysis yielded a cross validation accuracy of ~ 60%. Thus, the PCDA analysis could accurately describe 60% of this data which is considered reasonably good considering the similarities in the composition of the samples. Figure 1 is canonical variate score plot obtained in this analysis in which each letter in the plot represents an individual mass spectrum and the different letters represent different hardener to resin ratios. With the exception of the 1.5 (Sample E) data, this score plot illustrates a nearly linear variation of score value and sample stoichiometry. Thus, this type of component score plot can be used to predict the stoichiometry of an unknown sample.

This data was also analyzed using a PLS routine. The correlations investigated in this analysis were between sample stoichiometry, mechanical properties (tensile strength and peak strain) and the LIMS mass spectra. Figure 2 is a plot of the calculated relationship between the mechanical properties and the LIMS data obtained in the PLS analysis. The 0.2 stoichiometry (C Sample) is not included in this plot because there was no mechanical data for this stoichiometry. The variation in the LIMS data indicates the reproducibility of the raw data in the PLS analysis. This data does illustrate an almost linear relationship between the mechanical data and the LIMS mass specibility of the raw data in the PLS analysis. This data does illustrate an almost linear relationship between the mechanical data and the LIMS mass spectra (sample stoichiometry) indicating that the LIMS technique in conjunction with these data analysis procedures can provide useful information on this materials' mechanical properties at the microanalytical dimensions.

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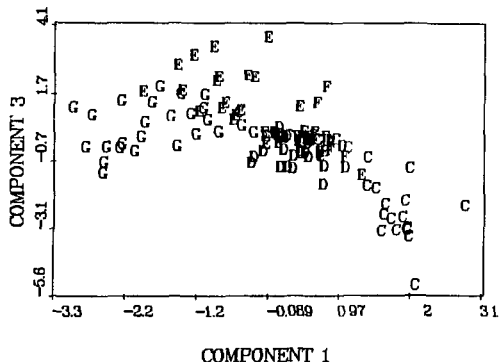
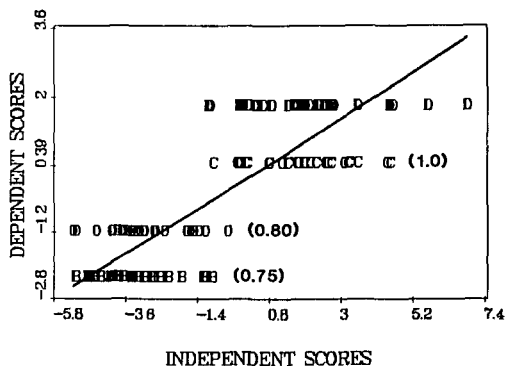


FIGURE 1

FIGURE LEGEND: HARDENER (DDS) TO RESIN (TGDDM) RATIOS
C: 0.2, D: 0.75, F: 0.8 (Production Material), G: 1.0 and E: 1.5

FIGURE 2



Study of Redox Properties of Poly-3-methylthiophene Films by
Cf-252 Plasma Desorption Mass Spectrometry

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Poly-3-methylthiophene (PMeT) films are stable in both the oxidized and reduced forms. The oxidation of PMeT films is accompanied by negatively charged counter ions diffusing into the film to balance the charge of polymer chains. If the film is reduced, counter ions are expelled from the film. These processes can be reversibly accomplished in an electrochemical cell. The oxidation of the film introduces electronic states in between the band gap known as bipolarons, which contribute the conductivity of the polymer. In this study, we made thin PMeT films for Cf-252 Plasma Desorption Mass Spectrometry (CF-252 PDMS) studies. The desorbed counter ions were detected as a function of oxidation potential in the cycling processes either from the oxidized state to the reduced state or vice versa. Electronic states are formed in between the band gap due to oxidation and can be studied by UV-Vis spectrophotometry.

Figure 1(a) shows the intensity of the counter ion CF_3SO_3^- from PMeT films which were held at different potentials as the film was reduced in the electrochemical cell. The counter ion intensity was very high for the oxidized state (1.00 V vs. Ag/AgCl). As the potential decreased, the counter ion intensity remained at a fairly constant value and then dropped to a much lower intensity from 0.4 V to 0.20 V. At this stage, the PMeT film was reduced. We repeated the same measurement as PMeT films were cycled from the reduced state to the oxidized state. The mass spectra are shown in Figure 1(b). The intensity of CF_3SO_3^- peak increased rapidly as the potentials went from 0.4 V to 0.6 V. The PMeT film was oxidized.

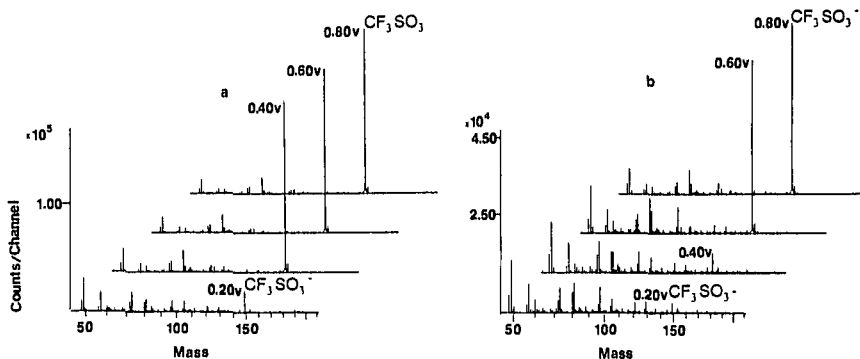


Fig. 1 Mass spectra of PMeT, CF_3SO_3^- is the counter ion. The intensity of the counter ion changes as the potential held the for the PMeT film varies (Ag/AgCl as the reference). (a) the PMeT is cycled from the oxidized state to the reduced state. (b) The film is cycled from the reduced state to the oxidized state.

Figure 2 shows the UV-Vis absorption spectra of PMeT films held at different potentials. The intense absorption band for the reduced PMeT film at 400nm is the band gap transition for PMeT. As the film is oxidized, a new absorption band appears at about 800nm which is a transition from the valance band to the bipolaron states. The intensity of this peak increases gradually as the PMeT film is oxidized. There is no big difference in absorption

spectra whether the PMeT film is cycled from the oxidized state to the reduced state or vice versa.

From mass spectra of the PMeT film (Figure 1), we can see that the potential range at which the counter ion intensity changes depends on the cycling processes. If the PMeT is cycled from a higher potential (oxidized) to a lower one (reduced), the intensity drop is in the 0.4 V to 0.2 V range. If the film is cycled from the reduced state to the oxidized state, the intensity increase occurs in the 0.4 V to 0.6 V range. There is no such difference in UV-Vis absorption spectra. Compared with the cyclic voltammogram (Figure 3), the potential range corresponding to the change of counter ion intensity in mass spectra is seen as two peaks on the cyclic voltammogram. One of these appears at 0.6 V as the film is cycled from the reduced state to oxidized state. Another is at about 0.25 V when the film is cycled from the oxidized state to the reduced state. The diffusion of counter ions into or out of the PMeT film involves kinetic factors not seen from the UV-Vis absorption spectra which mainly reflect oxidation levels. The following mechanism is suggested to explain the phenomenon. The reduction of the PMeT film occurs layer by layer starting with the layer closest to the Au electrode substrate. As counter ions are being expelled into the solution during the reduction, the counter ion concentration is kept almost constant at the surface layer where the 252 Cf-PDMS is most sensitive until the whole film is reduced. That is why a fairly constant intensity of counter ion peaks is observed as the film is reduced. In the re-oxidation process, counter ions diffuse to the inner layers first and the surface layer is filled last. After the whole film is filled with counter ions, a constant intensity of counter ion peak will be observed.

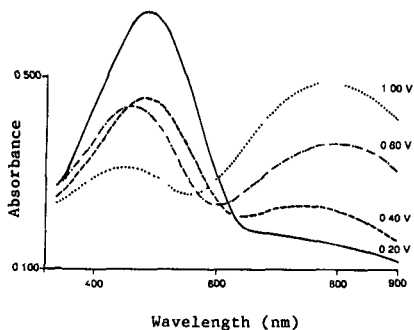


Fig. 2 UV-Vis absorption spectra of PMeT films. The PMeT films were held at different potentials in the electrochemical cell.

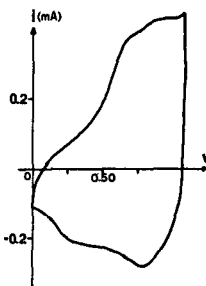


Fig. 3 Cyclic voltammogram of PMeT film. Ag/AgCl is the reference electrode.

WATCHING REACTIONS WITH K⁺IDS:

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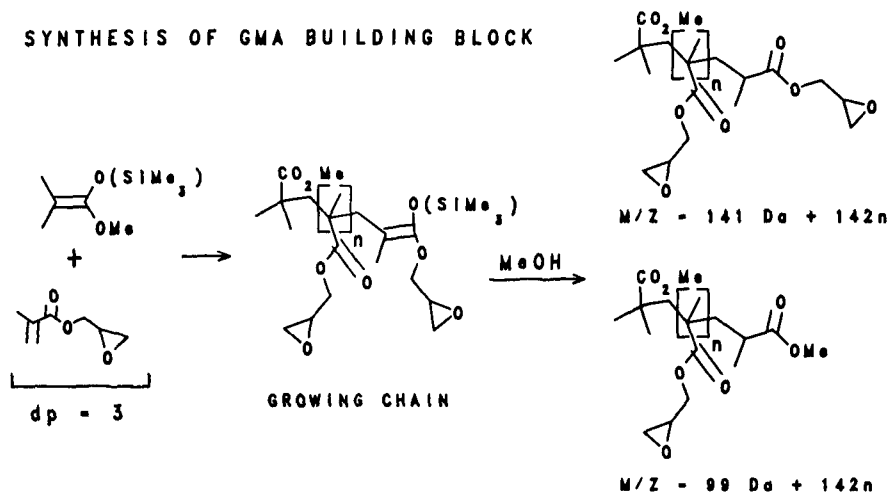
Many of today's architecturally designed high performance polymers are synthesized using oligomeric building blocks which possess molecular weights of less than 1000 Dalton (Da). The chemical microstructure of these oligomers is critical to the final properties of the high performance polymer. We have used the molecular weight data furnished by potassium ionization of desorbed species (K⁺IDS) to characterize several oligomers. Based upon the molecular weight data and a knowledge of starting materials, we have identified and quantified multifunctional oligomers synthesized by group-transfer polymerization (GTP). Furthermore, K⁺IDS analysis can also be used to identify differences in product distributions resulting from catalyst changes or in the polymerization conditions.

The K⁺IDS technique uses rapid heating to desorb large intact oligomers (>1500 Da). Once in the gas phase, ions are generated by potassium attachment. The K⁺IDS mass spectrum consists of [M]K⁺ for each desorbed molecular species, hence a molecular weight snapshot of the sample is produced. The picture takes only ten minutes to develop. All experiments were performed on a Finnigan 4615B quadrupole GC/MS system operated in the electron impact mode. No equipment modification or capital investment was necessary to perform K⁺IDS.

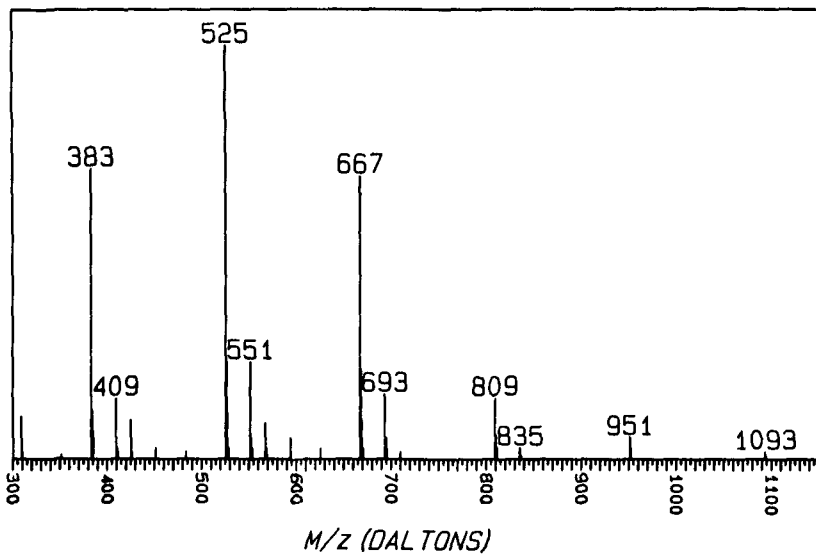
Figure 1 is the K⁺IDS MS of the GTP reaction targeted at the synthesis of a glycidyl methacrylate (GMA) building block. The reaction sequence is given below. Initially, we compared the reaction products from a batch versus a continuous feed polymerization. The K⁺IDS data showed no significant differences in the products. However, we did not expect the ions seen in Figure 1. The expected ions are 425 Da (degree of polymerization (dp) = 2), 567 Da (dp = 3), and 709 Da (dp = 4). The 142 Da spacing results from the GMA repeat unit. We observe a lower intensity envelope corresponding to the expected ions, but the major ions are 383 Da, 525 Da, and 667 Da. These ions are 42 Da less than the expected products. A transesterification by a methoxy (-OCH₃) (m/z = 31 Da) for a glycidyl group (-OCH₂CH(O)CH₂) (m/z = 73 Da) could account for the -42 Da mass change. To test this hypothesis, a batch reaction was quenched with CD₃OD. If transesterification occurred, a shift of four Da would be expected in the CD₃OD quenched transesterified GMA oligomers. Our results support the hypothesis because an increase in four Da was seen in the base ion envelope. This results from the incorporation of both the deuterium and the -OCD₃. Furthermore, we found more than one transesterification had occurred on some of the oligomeric GMA chains. For example, the 551 Da ion seen in Figure 1 was shifted 6 Da higher in mass to 557 Da in the CD₃OD quenched reaction. This results from incorporation of two -OCD₃ groups. We observed as many as three transesterifications on some GMA chains.

The loss of the epoxide functionalities can be detrimental to product performance. K⁺IDS provides a quick and simple method to monitor this loss because a functionality change results in a change in molecular weight. Furthermore, using the molecular weight data afforded by K⁺IDS we have been able to characterize the specific structures of GTP oligomers functionalized with nitro, amine, or diol groups. Finally, the total time necessary to obtain this information was under 10 minutes which included sample preparation.

SYNTHESIS OF GMA BUILDING BLOCK



K⁺IDS MASS SPECTRUM
FIGURE 1. - GMA OLIGOMERS



PYROLYSIS GAS CHROMATOGRAPHY MASS SPECTROMETRY
OF POLYARYLACETYLENE RESINS

M. P. Easton and D. Gilmartin

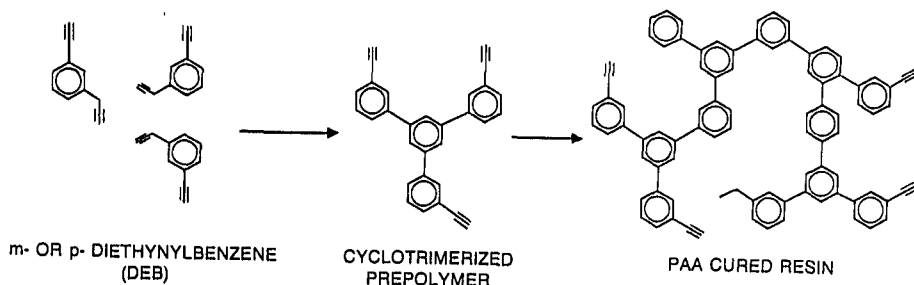
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Los Angeles, CA 90009

ABSTRACT

Polyarylacetylene (PAA) resins are being considered as matrix precursors in the fabrication of carbon (C-C) composites and for use as spacecraft structural materials. These resins leave 82-86% carbon residue when pyrolyzed to 1000°C. Resins presently in use have much lower char yields. PAA resins are prepared by the polymerization of diethynylbenzene using a Ni catalyst which favors cyclotrimerization. The polymer structure contains mainly polyphenylene units; acetylinic, benzylic and ethylbenzylic end groups; and possibly olefinic units due to linear polymerization. Thermogravimetric analysis shows that the resins lose weight in two steps at about 600 and 750°C. Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC-MS) was performed to determine the thermal decomposition products and to characterize the chemical structure. The cured resins analyzed were prepared from meta diethynylbenzene (DEB) or a 60/40 ratio of meta to para DEB. Phenylacetylene (PA) was added as a chain terminator to some of the resins. Ethylethynylbenzene a 5% wt impurity in DEB also acts as a chain terminator. Py-GC-MS was obtained at 400, 600, 700, 800, and 1000°C. The Py-GC-MS analysis shows that above 700°C the main product is hydrogen. Below this temperature a number of aromatic hydrocarbons are detected. The differences observed in the pyrograms of the different cured resins can be correlated to the polymer structure.



PHOTOIONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY OF ORGANIC COMPOUNDS FROM MANTLE MINERALS

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Sensitive detection of abiotic organic molecules in geological samples has been achieved using thermal desorption combined with surface analysis by laser ionization (SALI), which uses nonselective photoionization combined with reflecting time-of-flight mass spectrometry. Photoionization was accomplished by single-photon ionization with 118 nm light (10.5 eV) generated coherently by tripling the third harmonic of a pulsed Nd:YAG laser; this method has recently shown attomole sensitivity when combined with pulsed stimulated desorption (1).

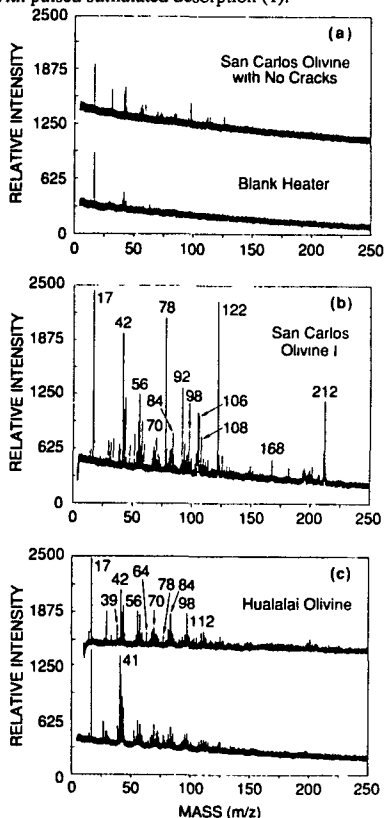


Figure 1. Temperature-integrated thermal-desorption mass spectra; a, blank heater (lower) and tumble-polished olivine from San Carlos with no cracks or inclusions; b, interior fragment cleaved from San Carlos olivine with microcracks; c, interior portions from two separate Hualalai olivine porphyroblasts. Prominent mass peaks are indicated. Mass assignments discussed in text.

Volcanoes bring samples of the Earth's mantle to the surface in which olivine, $(\text{MgFe})_2\text{SiO}_4$, is the most abundant mineral. Carbon x-ray microscopy and x-ray photoelectron spectroscopy have revealed that 1-10 nm thick carbonaceous films exist on many microcrack surfaces in mantle samples (2,3). Thermal desorption SALI yielded more detailed chemical information on these films.

We sampled interior portions of large single crystals of olivine and other silicates from rock samples by fracture in the laboratory and then immediately introduced them into the ultrahigh vacuum chamber for analysis. Spectra were accumulated during 200-2000 pulses of the 10 Hz laser as a function of sample temperature which was ramped over the range 25-750°C. Representative temperature-integrated thermal-desorption mass spectra are shown in Fig. 1 for olivine from two different localities, San Carlos, Arizona, and Hualalai, Hawaii. Significant variation among samples from the same locality has also been observed (3). Peaks observed in these spectra have been assigned to NH_3 , an alkene series [butene, $m/z = 42, +n \times (14 \text{ amu})$], and some relatively simple aromatic compounds (e.g., benzene, toluene, phenol, cresol, benzaldehyde, benzoic acid, and thiophene). Alkyl radicals are probably responsible for some masses, such as $m/z = 43$ and 55. The observed volatile compounds were released primarily above 300°C, and hence probably were pyrolysis products. For these temperature-integrated thermal desorption spectra, the sensitivity for a given mass is estimated to be 40 ppb for samples weighing ~5 mg.

To assess the extent of laboratory contamination, crack-free specimens were similarly analyzed; these showed negligible organic molecular ion signals. Field or environmental contamination also has been eliminated. We determined that the microcracks on which the organics occur have been apparently sealed; analysis of crystals immersed in solvents doped with a tagging molecule showed no evidence of penetration. The organic-bearing microcracks were produced during eruption. Catalytic activity of freshly exposed silicate surfaces for condensation reactions of partially reduced

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species found in volcanic gases, such as CO and H₂, has been proposed as the origin of the organic films (2,3).

In summary, nonselective photoionization TOF mass spectrometry of thermally desorbed species originating mostly above 300°C from fractured olivine crystals has shown the presence of various abiotic organic compounds consistent with pyrolysis of known very thin carbonaceous films on microcracks. Contamination could be ruled out, and from the petrography of the cracks where these films occur it is concluded that these films represent the products of condensation reactions of reduced volcanic gases on fresh-fractured silicate surfaces produced during eruption.

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HYDROCARBON-TYPE ANALYSIS BY FIELD IONIZATION
MASS SPECTROMETRY

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This work proposes and demonstrates a field ionization mass spectrometric, FIMS, method of analysis designed specifically to replace the six-component group-type matrix calculation method now being run on our CEC 103 mass spectrometer in the electron impact mass spectrometric, EIMS, mode. This analytical method is for hydrocarbons in the C_6 to C_{14} molecular weight range. The FIMS method adds molecular weight information to the analysis. It also evaluates the contribution from all the molecular ions, unlike the electron impact matrix calculation method which selects specific fragment ions and ignores others.

A VG ZAB mass spectrometer was used to generate the data presented here. A resolution $M/\Delta M$ of 2500 was used to separate the $Z=+2$ from the $Z=12$, etc.

A special open FI source was designed for the ZAB to minimize as much as possible fragmentation of the molecules through contact with hot surfaces. A schematic diagram of this source is shown in Figure 1.

The vapor from the batch inlet sees only the emitter prior to ionization. The emitter is pulse heated between acquisition scans to clean and activate the emitter. The extractor plate is heated to about 250°C.

The structural candidates for each Z category are shown in Figure 2. The sensitivities determined for the various categories are shown in Figure 3. A typical output from the FIMS method is shown in Table I. The statistics on a standard sample for FIMS and EIMS are shown in Table II.

TABLE I
HYDROCARBON-TYPE AND MOLECULAR
WEIGHT DISTRIBUTION (AS WEIGHT PERCENT)

File - ZFI2468 Scans 1-20 Summed
Sample - LHS2 No. 6
Conditions - FD
Date - 07/18/88 13:31:16 Weight Fraction 100.0%
Carbon Range - C_6 to C_{14} Z Range -12 to 2

Z Series: -12 -10 -8 -6 -4 -2 0 2

Mass	C No.	M	M + 2	M + 4	M + 6	M + 8	M + 10	M + 12	M + 14
72	6	0.000	0.000	0.000	0.000	0.000	0.028	0.020	0.013
86	7	0.000	0.000	0.013	0.546	0.000	0.011	0.010	0.000
100	8	0.000	0.012	0.005	1.304	0.000	0.000	0.066	0.062
114	9	0.000	0.000	0.211	4.603	0.000	0.614	5.627	1.618
128	10	2.143	0.030	3.879	5.834	0.142	9.728	16.612	9.840
142	11	3.901	0.010	0.023	0.217	0.079	4.842	10.893	10.300
156	12	0.006	0.000	0.069	0.083	0.053	1.019	1.782	2.623
170	13	0.027	0.000	0.000	0.000	0.088	0.137	0.399	0.408
184	14	0.000	0.009	0.000	0.000	0.046	0.000	0.000	0.015

Wt % Of Total Sample:

100 6.08 0.06 4.20 12.59 0.41 16.38 35.41 24.88

Number Averages:	Mol. Wt	140.77	Weight Averages:	Mol. Wt	142.17
	Z Number	-1.85		Z Number	-1.67
	Carbons	10.19		Carbon	10.27
	Hydrogens	18.53		Hydrogens	18.88
	H/C Ratio	1.82		H/C Ratio	1.84

TABLE II

STATISTICS ON SAMPLE STANDARD LHS2 NO. 6
SEPTUM INLET
F1 MASS SPECTROMETRY

Sample No.	2473	2472	2471	2469	2470	2468	X	Sigma	6 X 6 GT	Sigma
+2	23.5	23.3	25.4	25.7	24.3	24.9	24.5	0.99	24.3	0.41
0	36.4	35.0	35.1	33.3	35.7	35.4	35.1	1.04	35.6	0.75
-2	17.8	17.5	17.5	17.6	17.8	16.4	17.1	0.94	17.9	0.33
-4	0.2	0.3	0.4	0.6	0.5	0.4	0.4	0.14	-	-
-6	11.9	13.1	12.3	11.2	11.3	12.6	12.1	0.74	11.8	0.40
-8	4.8	4.3	4.9	4.8	4.5	4.2	4.6	0.3	4.5	0.20
-10	0.1	0.3	0.1	0.1	0.1	0.1	0.1	-	-	-
-12	5.4	6.1	6.5	6.8	5.9	6.1	6.1	0.48	5.8	0.40

MW: 142.0 141.9 142.2 142.9 143.2 142.2 142.4 0.50
Z Number: -1.64 -1.80 -1.73 -1.74 -1.64 -1.67 -1.70 0.06
H/C Ratio: 1.84 1.82 1.83 1.83 1.84 1.84 1.83 0.01

FIGURE 1

FIMS SOURCE DETAIL

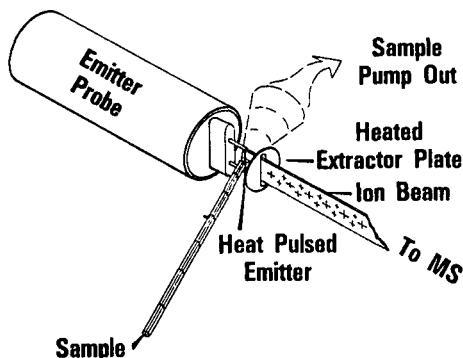


FIGURE 2

C₆-C₁₄ FIMS HYDROCARBON AND
MOLECULAR WEIGHT DISTRIBUTION
ANALYSIS STRUCTURAL CANDIDATES

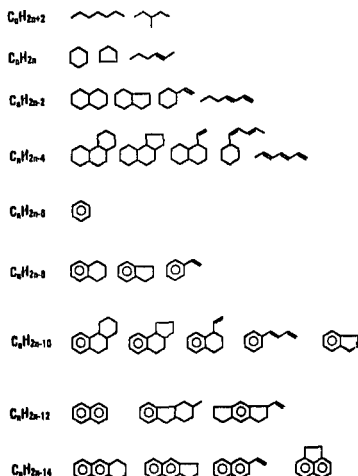
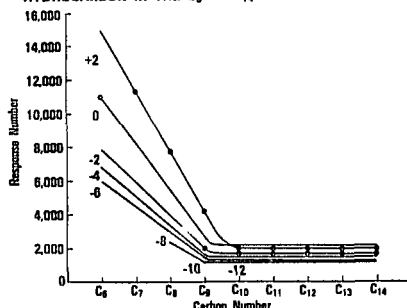


FIGURE 3

RESPONSE FACTORS FOR Z = +2 TO -12
HYDROCARBON IN THE C₆ TO C₁₄ CARBON NUMBER RANGE



THE ON-LINE DETERMINATION OF WATER BY MASS SPECTROMETRY

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Our triple quadrupole mass spectrometer is used to monitor 62 species on-line while pyrolyzing fossil fuel samples. One of the major oxygen-containing species evolved is water, and it is important to determine it accurately with the same temporal resolution as all other species. In addition, we must determine the amount released or created, not just the concentration. We have succeeded in determining the water by mass spectrometry.

One of the most difficult tasks was developing a reliable method of standardization. A series of four bubblers were used; the first two contain water and the last two are water saturated with NaCl. Argon passes through the bubblers at from 100 to 200 cc/min. The first two bubblers bring the water content to above 50% humidity. The last two bubblers drop the humidity to ~25%, the exact partial pressure depending upon the temperature. This method has been checked by comparison with the integrated water evolution from $\text{BaCl}_2 \cdot 2(\text{H}_2\text{O})$. It was also verified by decomposing propane over hot copper oxide, calibrating for CO_2 , and using stoichiometry to know the water content.

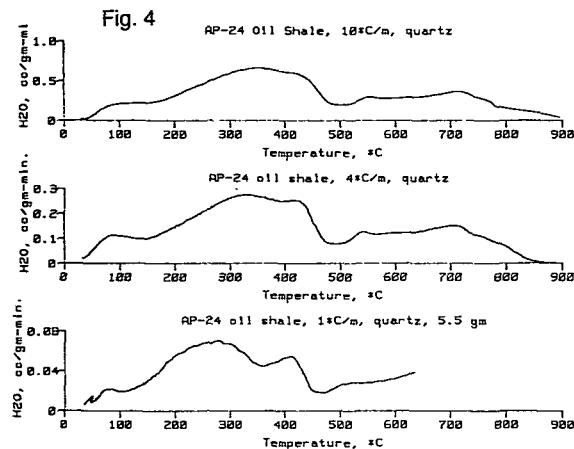
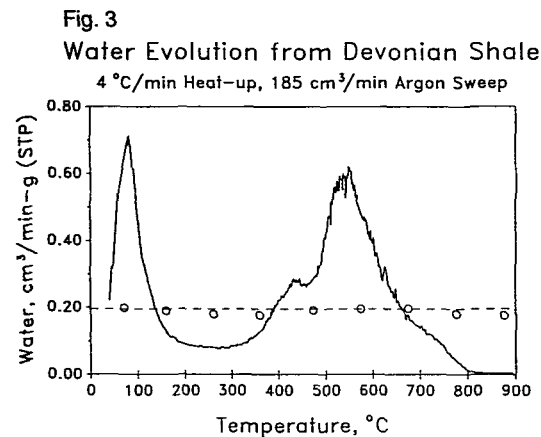
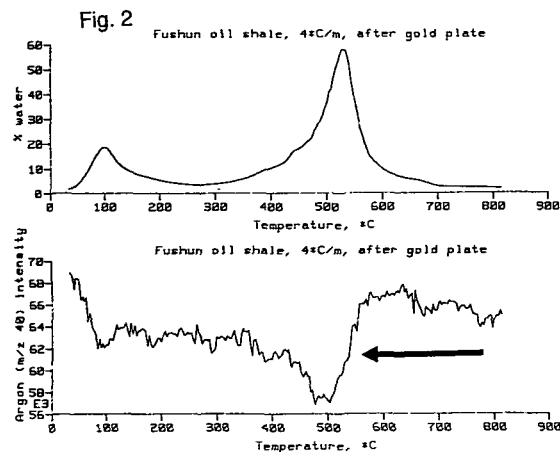
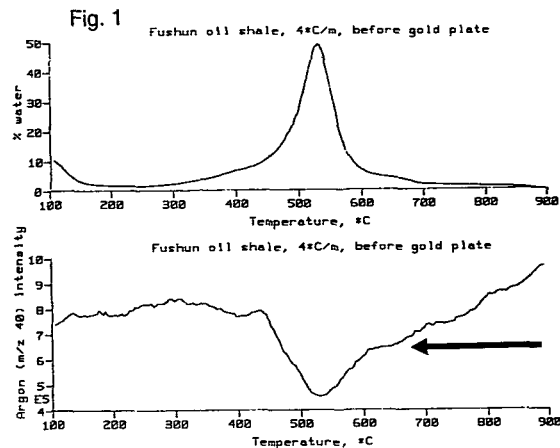
The samples and standards were introduced through a two stage pressure drop, a pumped capillary followed by an orifice into the TQMS source. All lines that see water vapor were heated to ~140°C.

Water vapor reduces the overall sensitivity of the mass spectrometer. Research to date suggests that the water is chemically modifying critical lens elements in the source. This is a critical problem in these experiments. The intensity from the argon, used as a precisely flow controlled sweep gas through the pyrolysis zone, must be determined accurately. The argon signal is used to modify the signal from determined species in order to convert concentration into flow. As can be seen in Figure 1, the argon signal recovered very slowly after a large (50% of the gas) water peak.

Our experience suggested that the repeller was the single most contamination sensitive source element. We applied a thick, electro-plated coating of gold on the repeller. As can be seen in Figure 2, the argon intensity recovered rapidly, after a water peak as concentrated as that in Figure 1. This improvement is not degraded by repeated exposure of the repeller to fossil fuel pyrolysis products.

Figure 3 shows an analysis in which water standards (O) were introduced at intervals throughout the run to verify the calibration with time. Although some variations in the standard can be seen, due mostly to increased background because of the large amount of water evolving from the sample, no serious drift is encountered. Figure 4 shows three runs on the same oil shale but at different heating rates, 1, 4 and 10°C/minute. A major shift in the temperature of maximum evolution is seen for the water coming from the mineral Analcime (one of the minerals in the Green River Formation oil shale), moving from ~280°C at 1°C/m to ~350°C at 10°C/m.

Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.



NAPHTHENIC ACIDS IN PETROLEUM BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY
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The naphthenic acids in the crudes are considered a class of biological markers. Their potential use in source correlation and as indicator of biodegradation have been reported previously (1). The presence of naphthenic acids may also cause corrosion problems and fish toxicity in the waste water at refineries. Detailed structural characterization of these acids has been difficult due to the high complexity of the mixture. This report describes a method using ion exchange column chromatography and FAB analysis. This provides a group type distribution of the acids and was found useful for geochemical correlation purposes.

The resin fraction in the crude oil was first isolated, followed by ion exchange column separation of the resin into fractions of different acidity. The FAB analyses were first dissolved in toluene (approximately 1:5) and analyzed in a TEA matrix (triethanol amine). The analyses were performed in negative ion mode at 8 kV acceleration voltage with a Xe atom beam at 1 mAmp ion current and 9 kV kinetic energy.

Negative ion FAB spectrum of a naphthenic acid standard from Eastman Kodak (Fig. 1) shows only intense deprotonated molecular ions of the general formula $R-COO^-$ from each acid component. The mass assignment is straightforward by calculating the corresponding molecular weight. The peak intensities are used to calculate their relative distributions.

Due to the complicated nature of the naphthenic acid mixtures, the acid components were classified into groups according to Z number. The general formula $C_nH_{2n+2}O_2$ is used to represent carboxylic acids where Z is equal to 0 for all saturated paraffinic acids. For every additional ring or double bond in the molecule, the Z number decreases by 2 due to the loss of 2 hydrogens. Thus a decrease in Z number indicates an increase in number of rings or unsaturation in a molecule.

The negative ion FAB spectrum of an acidic fraction from a Louisiana oil is shown in Fig. 2. High molecular weight naphthenic acids up to 700 amu were observed. Heavy acids have been reported in California crudes (1). The carbon number distribution of three Z groups, -2, -6, and -10 are plotted in Fig. 3. The shift in the curve maximum toward higher carbon number with decreasing Z number can be explained by the fact that a pentacyclic molecule requires a minimum number of carbons (Z = -10) greater than that for a tricyclic (Z = -6) or a monocyclic (Z = -2) molecule.

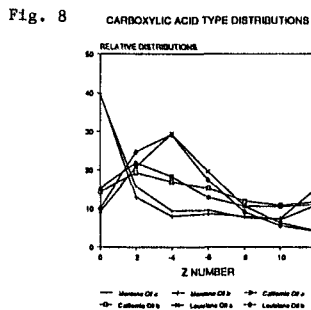
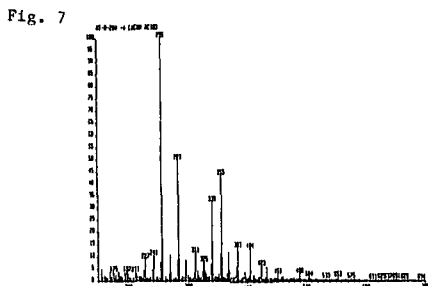
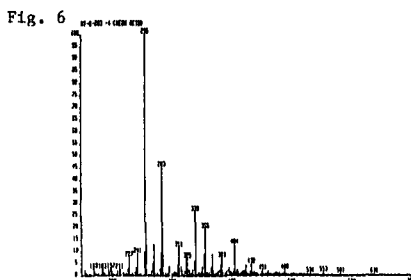
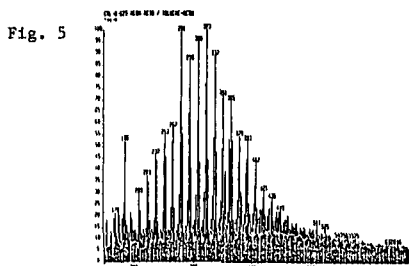
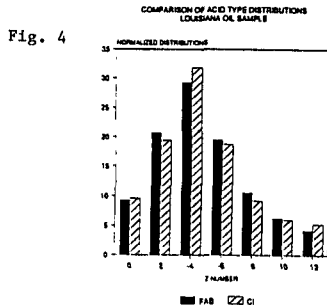
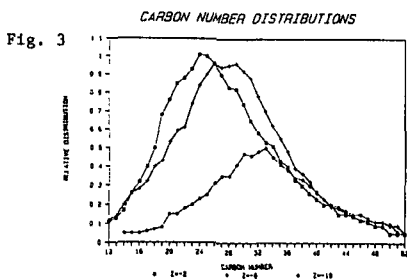
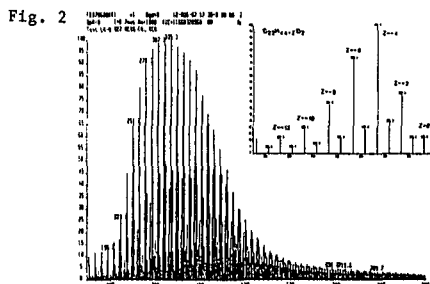
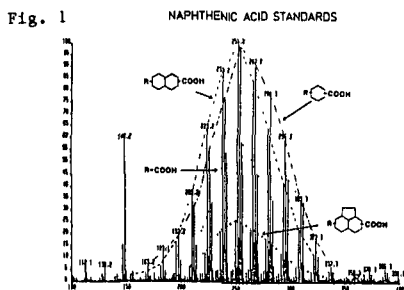
Fig. 4 compares the normalized acid type distributions (based on C12 - C32) obtained from FAB and CI(2) for two California crudes. The agreement between the two methods is excellent considering the techniques are completely different in nature. Reproducibility of the FAB analysis is within 10%.

Figs. 5 - 7 are the FAB spectra of 3 naphthenic acid extracts from California (Fig. 5) and Montana (Fig. 6, 7) respectively. The distribution of acids are similar between samples from the same source, but are significantly different between those from different sources. These can also be clearly observed in the acid group type distribution plot (Fig. 8). The greatest difference among different sources was observed in the distribution of saturated carboxylic acids, which is considered closely related to the degree of oil maturity (1).

The method described here provided a relatively simple routine procedure in characterizing the complicated naphthenic acids in petroleum based on their group types. The results demonstrated the potential in using the naphthenic acid distribution for geochemical source correlations. More data sets will be collected in the future.

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DEVELOPMENT OF ITD BASED GC/MS TECHNIQUES FOR RAPID PROFILING OF
MICROBIAL CELL WALL LIPIDS

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The objective of this study was to develop and compare mass spectrometric techniques suitable for classifying twenty five microbial strains isolated from hydrocarbon rich fossil deposits (viz. oil shales, tar sands, oil seeps and gilsonite) in the Green River region of Wyoming, Utah and Colorado. Three different mass spectrometric methods were compared.

- (1) GC/MS analysis of fatty acid methyl esters (FAMES) of microbial cell walls using a standard saponification, derivatization and extraction (SDE) method [1].
- (2) GC/MS analysis of FAMES obtained by rapid, pyrolytic on-line derivatization (OLD) of whole bacterial cell walls using tetramethyl ammonium hydroxide [2].
- (3) Direct Curie-point pyrolysis MS (Py-MS) analysis of whole bacterial cells.

Typical FAME chromatograms prepared by the SDE and OLD techniques are shown in Figures 1 and 2, respectively. All chromatogram peak areas were integrated. Each data set was then subjected to factor analysis (followed by discriminant rotation of the Py-MS data).

Multivariate analysis (Figures 3,4 and 5) revealed three main clusters: two Gram positive and one Gram negative cluster. The first cluster of Gram positive organisms is identified as the *Nocardia*/*Arthrobacter* group on the basis of comparisons with several reference strains (not shown) and the presence of straight chain fatty acids and tuberculostearic acid (TSA). The second set of gram positive bacteria designated as "coryneforms" are characterized mainly by strong iso- and anteiso fatty acid signals. The third cluster (Gram negative bacteria) is characterized by hydroxy fatty acids. Unfortunately, this class of compounds is missed by the OLD method [2]. Furthermore, the Gram negative group is characterized by unsaturated and cyclic fatty acids.

The Py-MS data provide information about sugar, protein and nucleic acid components in addition to some of the fatty acid patterns of microbial cell walls. The Gram positive bacteria in this study show the highest sugar content, whereas the Gram negative bacteria exhibit higher protein signals.

In order to combine the data obtained by all three methods into a single data set, canonical correlation analysis was performed. Three "significant" canonical variate functions were obtained with canonical correlation coefficients > 0.9 , as shown in Figure 6. The Gram negative organisms appear to start separating into small subgroups (e.g., *Pseudomonas*, *Aeromonas*, *Acinetobacter* and *Flavobacterium*).

In conclusion, similar clustering trends were obtained by all three methods, thus providing stable "natural" clusters. The SDE and OLD methods produce similar FAME profiles with the exception of the hydroxy fatty acids which are destroyed by the OLD method. The Py-MS data set shares more than 30% of its total variance with 50-70% of the OLD and SDE data sets, respectively. Although Py-MS provides much less detailed data on cell wall lipids, useful additional information is obtained on biopolymeric components (viz., proteins, polysaccharides, and nucleic acids).

ACKNOWLEDGEMENTS

The authors wish to acknowledge financial support from the Idaho National Engineering Laboratory, contract #C85-110763.

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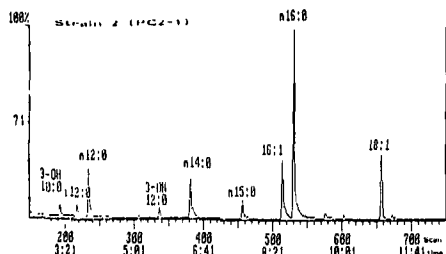


Fig. 1. FAME profile at m/z 74 (SDE method).

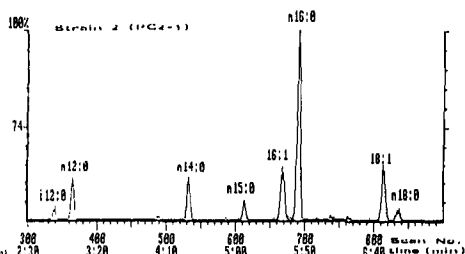


Fig. 2. FAME profile at m/z 74 (OLD method).

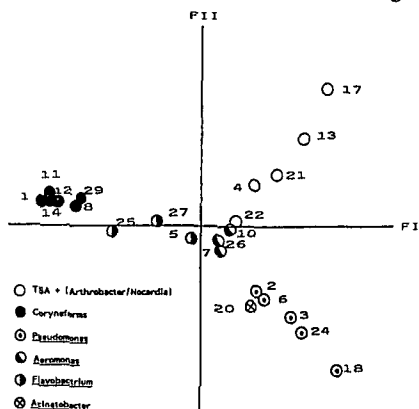


Fig. 3. Factor score plot (SDE method).

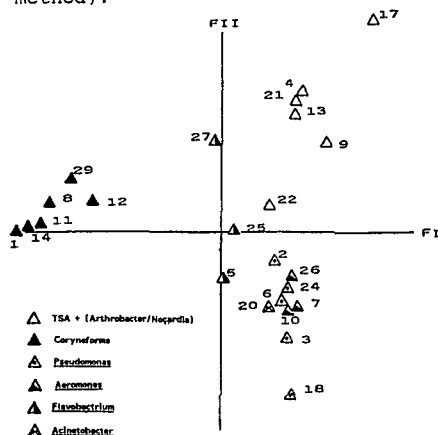


Fig. 4. Factor score plot (OLD method).

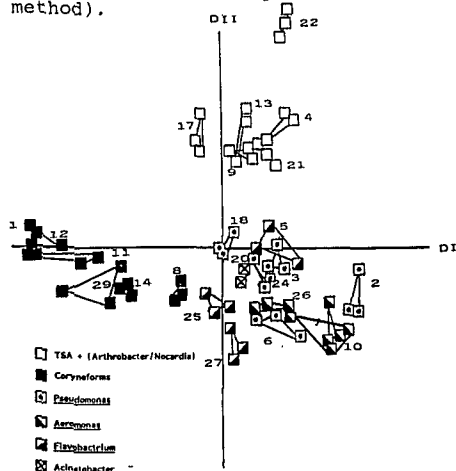


Fig. 5. Factor score (discriminant rotation) plot (Py-MS method).

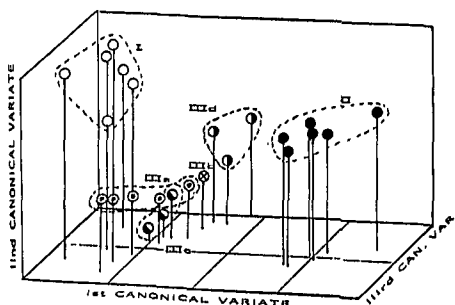


Fig. 6. Canonical correlation score plot (only generalized scores).

^W LASER PYROLYSIS MASS SPECTROMETRY OF SINGLE LEVITATED COAL PARTICLES

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A laser pyrolysis mass spectrometry experiment was designed to study the devolatilization behavior of individual coal particles at high heating rates (10^4 - 10^6 K/s), characteristic of pulverized coal combustion reactors. The experimental set-up (Fig. 1) consists of an electrostatic particle levitation cell, also known as an "electrodynamic balance" [1], a 50 W cw CO₂ laser and a Finnigan-MAT ITMS system.

The particle levitation cell was constructed by modifying a regular ion trap electrode assembly in such a way as to provide line-of-sight access to the center of the cell for the CO₂ laser beam as well as for visual observation by means of a stereo microscope. Typical cell operating parameters for levitating a 120 μ m dia. Spherocharb particle are: ring electrode 3000 V (60 Hz ac), upper end-cap +100 V dc, lower end-cap -100 V dc.

The CO₂ laser (Apollo 3050 OEM) is capable of electronic pulsed beam operation. The 8 mm dia. beam is split equally into 2 opposing beams focussed at the center of the levitation cell (beam waist ca. 400 μ m, power density ca. 4-10 MW/m²), as a co-linear parfocal HeNe laser beam permits positioning the levitated particle in the optical and electrical center of the cell. Two IR detectors measure the integrated pulse and time-resolved pulse energy (Fig. 2).

A heated transfer line column (2m x .18 mm DB5) equipped with a special air sampling inlet [2] enables intermittent sampling of volatiles from the center of the levitation cell into the ITMS vacuum system. Feasibility studies were performed on 120-150 μ m Spherocharb particles impregnated with ng amounts of an alkylnaphthalenes mixture and heated with a single 10 ms CO₂ laser pulse. As shown in Fig. 3, ample signal intensities were obtained with the first laser pulse to permit the recording of "transfer line" GC/MS profiles. By contrast, the second laser pulse produced <10% of the volatiles observed from the first pulse, thereby demonstrating nearly complete devolatilization of the impregnated particle by a single laser pulse. Fig. 4 shows the repeatability between experiments with separate particles.

Finally, a series of experiments was performed with actual coal particles in the 100-130 μ m size range, prepared by careful sieving of two coals from the Argonne National Laboratory Premium Coal Sample bank, namely a Pittsburgh #8 seam (Illinois) coal and a Blind Canyon seam (Utah) coal of comparable rank. TIC profiles for both coals are illustrated in Fig. 5, whereas Figs. 6a and b show selected ion chromatograms representing C₁- and C₂- alkyl phenols. From previous studies of Pitt. #8 coal using time-resolved Curie-point pyrolysis [3], pyrolysis temperatures necessary to produce these phenolic building blocks at heating rates of 10^5 K/sec are estimated to be ca. 900 K. Of course, kinetic parameters obtained at the much lower heating rates of the Curie-point pyrolysis MS experiment (approx. 10^2 K/s) may only be extrapolated to the laser pyrolysis experiment if the underlying reaction mechanisms are comparable. Evidence supporting this assumption is presented in Figs. 6c and d showing similar C₁ and C₂-alkyl phenol profiles for the Pitt. #8 coal obtained by Curie-point pyrolysis GC/MS [4] using a 15 m fused silica capillary with temperature programming.

ACKNOWLEDGEMENTS

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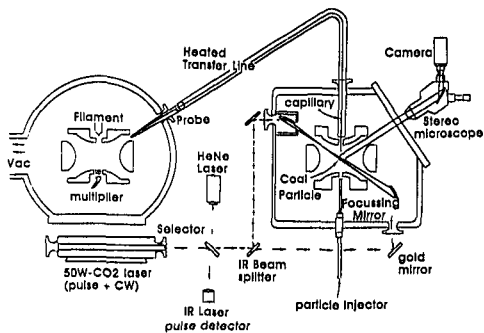
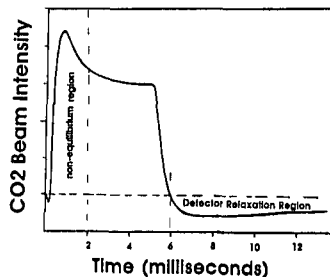


Fig. 1. Experimental set-up.



Laser Beam Energy-Time profile

Fig. 2. Typical CO₂ laser pulse profile.

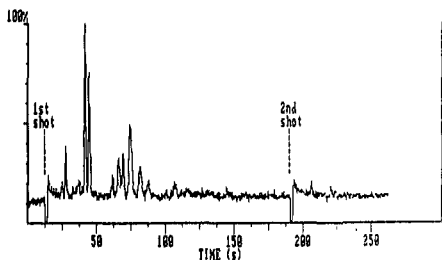


Fig. 3. Comparison of volatile yields from two consecutive laser shots at the same Sphero carb particle (impregnated with alkyl naphthalenes).

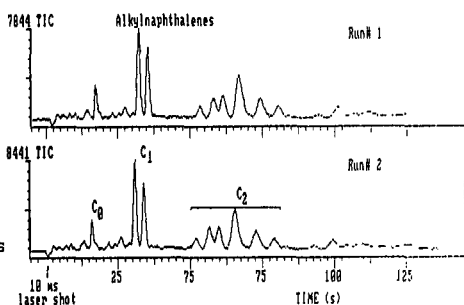


Fig. 4. As Fig. 3, but comparing volatile yields from 2 different Sphero carb particles.

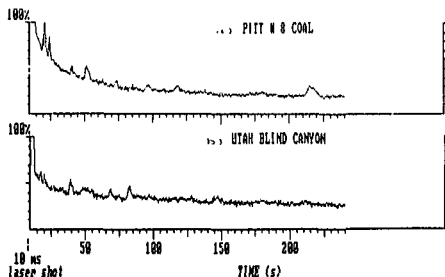


Fig. 5. Comparison of total ion current profiles from single particle. of (a) Pitt. #8 coal and (b) Blind Canyon coal.

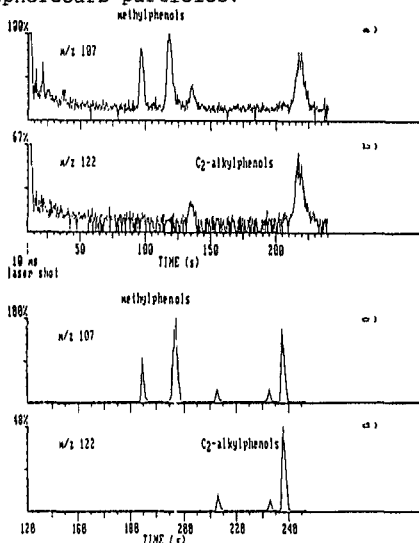


Fig. 6. Comparison of selected ion chromatograms representing alkyl phenols produced by laser pyrolysis (a,b) and by Curie-point pyrolysis (c,d).

THE USE OF A HYBRID MASS SPECTROMETER IN DETERMINING BIOMARKER COMPOUNDS
IN CRUDE OIL EXTRACTS.

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Introduction

The study of "biological marker" compounds in crude oil extracts is now an established method used by Petroleum Explorationists in identifying genetic relationships between various oils and in aiding oil-to-oil and oil-to-source rock correlations.

Polycyclic hydrocarbons such as steranes and triterpanes have been found to be the most useful biomarkers for such studies and their use for the assessment of environment of deposition, maturation, migration and alteration of crude oils is widely documented.

"Typing" of crude oils and source rock extracts is normally carried out by GC/MS, usually with a quadrupole mass spectrometer operated in a unit mass resolution mode. Unfortunately, when monitoring in a selected ion recording mode (SIR), the responses observed are often complicated by other components in the sample. Indeed operating a sector instrument at higher resolving powers is not always a solution as the interfering components may have the same elemental formula. MS/MS analysis solves the analytical problem by greatly enhancing the specificity, even for components with the same elemental formula.

This work illustrates the use of both high and low collision energy environments for this analysis.

Experimental

All the analyses were carried out using a VG Tritech TRIBRID mass spectrometer, whole oil samples were injected in 0.1 μ l aliquots in the split mode onto a 25m bonded phase OV-1 fused silica capillary column (50:1 split). The HP5890A GC was programmed from 60 C to 160 C at 6 C/min, then to 300 C at 3 C/min, holding for 20 minutes. The reactions monitored were from the molecular ions of the steranes and triterpanes to the common fragments for each class of compounds, m/z 217 and 191 respectively. These are shown in table 1. The TRIBRID has the capability of monitoring these reactions both in a "high energy" environment in its first field free region (see figure 1.) or low energy decomposition in an RF only collision quadrupole at low energy.

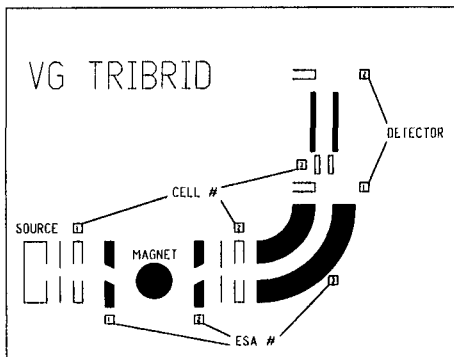
The TRIBRID offers several advantages for this work, in particular the use of an air-core magnet allows rapid, reliable switching of the parent ion mass. No complicated calibration of the mass scale is necessary for the experiment set-up.

Table 1. Reactions monitored

m/z	compound	m/z	compound	m/z	compound
372	C27 steranes	370	C27 triterpanes	426	C31 triterpanes
386	C28 steranes	384	C28 triterpanes	440	C32 triterpanes
400	C29 steranes	398	C29 triterpanes	454	C33 triterpanes
414	C30 steranes	412	C30 triterpanes	468	C34 triterpanes
				482	C35 triterpanes

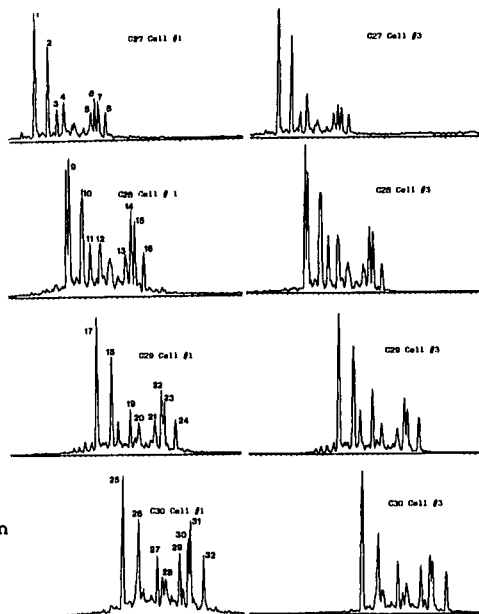
Figure 1. The TRIBRID EBEEQQ geometry. The instrument has three collision cells for MS/MS analyses. (analysers are in black)

The quadrupole cell experiments are set up by tuning with FC43 at m/z 219 - 131 fragment. The Cell 1. does not require any tuning or set up other than the standard source/analyser tune for EI data.



Results

Figure 2. shows the result for the C27 to C30 steranes in each cell. In general the Cell 1 and Cell 3 results are equivalent, although there is perhaps less evidence of the "hump" in the quadrupole case. The relative response of the later eluting isomers is higher in the Cell 1 traces, probably due to the rate constants being different in the two time scales from ion formation. Both experiments clearly isolate the components of interest from the enormous quantities of normal alkanes present in the sample. The absolute responses for the two methods are approximately five times greater in favour of the Cell 3 results. Triterpane results are similar to the steranes, with the exception that an interference seen from the methyl loss from the next eluting homologue is largely unseen in the Quad case (due to the higher parent ion resolution). This is not a practical problem in the Cell 1. results as the GC separation eliminates any direct conflict in response.



Conclusion

The methods of multiple reaction monitoring in either cell yield dramatic selectivity enhancement over other MS separation techniques. The quadrupole method has an advantage in terms of parent ion resolution and absolute sensitivity, however the Cell 1 experiment gives useful results and has the advantage of being available on a standard double focussing instrument as well as being extremely easy to set up.

THERMAL SLICING AS A TOOL TO STUDY THE THERMAL DECOMPOSITION OF COALS & KEROGEN

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It is important to investigate the kinetics of petroleum generation (or kerogen degradation) to improve exploration success. Because of the variety of organic inputs and subsequent disparate burial (temperature) histories of petroleum source rocks, it is desirable to determine kinetic parameters of individual samples from the basin of interest. Furthermore, since kerogen/coals are heterogeneous random polymers, thermal degradation (pyrolysis) techniques can be used to study their various macromolecular structures.

A thermal slice is defined as a temperature interval bounded by a specified lower and upper temperature. With a Ruska Laboratories Thermal Chromatograph-Mass Spectrometer (TC-MS), the temperature range over which a substance thermally decomposes can be subdivided into any number of thermal slices. The relative abundance of thermal decomposition products as a function of thermal slice has been determined using mass spectrometry for two end member geopolymers: the Monterey Shale (sulfur rich kerogen) and a low rank coal (oxygen rich/hydrogen poor organic matter). Both samples were first analyzed using linear temperature programmed heating ($30^{\circ}\text{C}/\text{min}$) directly coupled to a flame ionization detector. The results indicate the decomposition temperature range for the coal and Monterey Shale, and the samples were divided into six thermal slices (as shown in Figure 1) for subsequent TC-MS analysis.

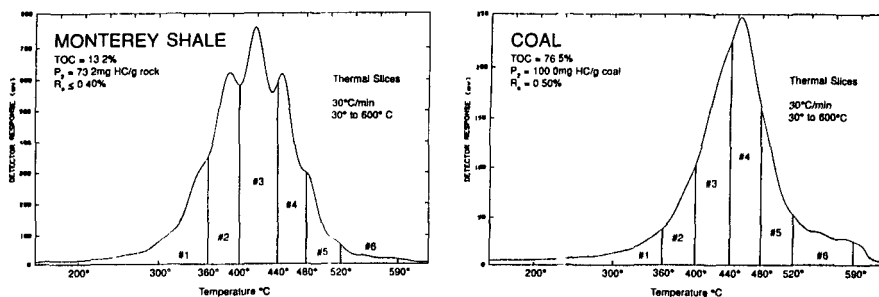


Figure 1. Linear temperature programmed-FID response curves for coal and Monterey Shale.

Distributions (relative abundance as a function of six thermal slices between 360°C and 590°C) of selected individual pyrolyzates (e.g., 2-methylthiophene, phenol, toluene, p + m-xylene, n-octane, 1-octene, n-octadecane, and 1-octadecene) were constructed to compare the thermal degradation behavior. The modes of distributions for heteroatomic molecules and aromatic hydrocarbons occurred about 40°C

lower in the sulfur rich shale than for the coal (see Figure 2). The modes of the distributions of alkanes and alkenes occurred at approximately similar temperatures between the two samples, although 1-alkenes formed in greater abundances at higher temperatures than n-alkanes in the Monterey. The total concentrations for each compound in with respect to the quantity of total pyrolyzable kerogen (P2) are also shown in Figure 2. Calculated activation energies of geopolymer thermal decomposition for individual compounds quantify the observed variations (Table 1). For example, activation energies of 17.9 and 17.2 kcal/mole for 2-methylthiophene and phenol, respectively, from the Monterey Shale are significantly lower than those calculated for the coal (30.0 and 27.6 kcal/mole, respectively). Also, 1-alkene activation energies are slightly higher than corresponding n-alkane activation energies in both samples.

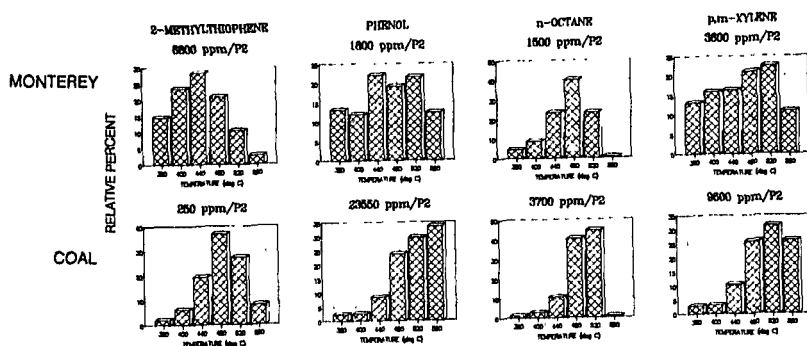


Figure 2. Thermal decomposition product distribution differences between Monterey Shale and Coal over the temperature range 360° to 690°C.

TABLE I
ESTIMATED ACTIVATION ENERGIES
(kcal/mole)

	2-methylthiophene	phenol	toluene	p,m-xylene	n-octane	1-octene	n-octadecane
MONTEREY	17.9	17.2	19.6	16.3	32.1	37.7	26.7
COAL	30.0	27.6	25.3	27.4	42.7	45.2	32.7

MASS-SPECTROMETRIC ANALYSIS OF HIGH-BOILING WAXES SEPARATED
FROM SLUDGE FORMED DURING STORAGE OF CRUDE OIL IN
UNDERGROUND RESERVOIRS

by

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Underground storage of crude oil in salt caverns may result in the formation of a layer of sludge at the brine/oil interface. This sludge is known to consist of inorganic sediment, water, and organic substances (1,2). As part of an investigation into the nature of the organics, waxes from the 850-1050° F, 1050-1250° F, and >1250° F distillates of three different cavern sludges have been isolated and partially characterized. The separation procedure is described in the literature (3). The present discussion is limited to results obtained from a wax isolated from the >1250° F distillate of the sludge recovered from one cavern designated as cavern "B".

Among other techniques, gas chromatography, nuclear magnetic resonance, and mass spectrometry were used to characterize the sludge waxes from all three caverns. Experimental conditions and results for the GC and NMR experiments are given elsewhere (3). Waxes separated from the 850-1050° F and 1050-1250° F distillates were found to consist mostly of paraffins and cycloparaffins (3).

Two methods were used to obtain mass spectra of the >1250° F wax from cavern "B". Initially, field desorption was selected for ion formation based on gas-chromatographic results showing a predominance of aliphatic hydrocarbons in waxes from the lower-boiling distillates. However, the unexpected identification by nominal mass PD/MS of long-chain alkylbenzenes in the >1250° F wax led to further experiments in which 70 eV electron impact was used for ion formation and high resolution (20,000 resolving power) for mass separation. The latter conditions were selected to enhance molecular-ion intensities of aromatic compounds and allow their elemental compositions to be identified. To further facilitate identification of aromatics in the wax, the electron-impact spectra were recorded, not on the wax itself, but on an aromatics fraction separated from it (3).

All mass-spectra were obtained on a Kratos MS-50 mass spectrometer. To record the electron-impact spectra, samples were introduced by probe microdistillation (PMD/MS) over a temperature range from ambient to 600° C. The apparatus and technique are described in the literature (4). Electron-impact data were processed with programs described previously (5,6).

The field desorption mass spectrum of the wax separated from the >1250° F distillate showed prominent peaks in a range extending from m/z 694 to m/z 1128. Based on nominal mass Z series, these peaks were identified as alkylbenzenes. The most prominent member of the series was found at m/z 834, corresponding to C(60)H(114). Other significant compound types

identified included paraffins and cycloparaffins, as shown by peaks extending from m/z 646 to m/z 870 and from m/z 714 to m/z 868, respectively.

The electron-impact spectra recorded for the aromatics fraction showed that the sample distilled over a probe temperature range from approximately 300 to 600° C. Processing of high resolution PMD/MS data produced elimination curves for molecular ions and corresponding numerical results, including areas under the curves. The latter results provided a semi-quantitative analysis of the fraction. In addition to finding the anticipated alkylbenzenes (-6 H) in the fraction, lesser quantities of -8 H and -10 H compound types were found. Homologues of alkylbenzenes were identified in a molecular mass range extending from m/z 708 to m/z 1086. The most prominent member was found at m/z 834, in agreement with results from the FD/MS data recorded for the whole >1250° F wax.

In addition to the aromatic hydrocarbons found in the fraction, benzothiophenes (-10 S) were identified in significant quantity. The most prominent member of this series was found at m/z 834, corresponding to C(58)H(106)S. Owing to very similar volatilities and the same nominal masses, homologues of alkylbenzenes and benzothiophenes were identified in overlapping molecular mass ranges. For these reasons, the latter compounds were not detected in the FD/MS spectrum of the whole wax.

In conclusion, wax separated from the >1250° F distillate of cavern "B" sludge was found to be comprised largely of alkylbenzenes. However, other compound types were present in significant quantities. These included other aromatic hydrocarbons, paraffins, cycloparaffins, and benzothiophenes. In the absence of further information, the relative contributions of these different compound types toward sludge formation is not known.

ACKNOWLEDGMENTS

This project is supported by the U.S. Department of Energy Cooperative Agreement DE-FC22-83FE60149. Appreciation is expressed to F. Tilley and M. White for technical assistance, and to G. Sturm, J. Green, and J. Reynolds for helpful discussion.

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FREE RADICAL AND REDOX CHEMISTRY IN SUPERCRITICAL FLUIDS

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Coal is this country's most abundant resource. The burning of coal is also a major pollution concern. The acid rain in Canada is often contributed to sulfur dioxide emission from the burning of coal by the United States. As other energy sources are depleted, coal will be needed not only as a fuel, but also as a feedstock for the production of chemicals. Consequently, efficient desulfurization of the coal matrix will also become important.

In this study new methods of coal desulfurization are being developed using supercritical fluids as solvents and in some cases reactants. Two different types of reaction processes are under study. One reaction process involves the selective solvation (bond scissioning) of carbon sulfur bonds by the action of supercritical fluids. The other reaction process involves the dissolution of reagents in supercritical fluids that selectively oxidize organo-sulfur bonds.

Several advantages are expected from the use of supercritical solvents. For example, mild thermal conditions should be possible, which should provide minimal loss of heat content. High desulfurization yield should be possible due to the low viscosity of supercritical fluids. Also, the supercritical solvent should be easily recycled.

To carefully monitor the progress of the reaction-processes, an instrumental interface was developed that allows parallel detection by a sulfur detector and a high mass quadrupole mass spectrometer. The details of the instrumental set-up were illustrated in this presentation.

Also, the initial results on the study of the solvation of oxidants in a supercritical fluid mixture were presented. It was shown that ionic species will dissolve in supercritical mixtures at temperatures below 100°C. The use of UV-Vis spectroscopy for determining the solubility of inorganic ions failed. The inorganic ions were found soluble in specific supercritical fluid mixtures, but the electronic transitions of the inorganic ions were not at energies similar to those found in liquids. SFC-ICP analysis of the solutions demonstrated conclusively that the inorganic oxidants were dissolved in the fluids. Future work will involve the mass spectral monitoring of these chemical reactions.

ADDUCTS OF d^5 AND d^{10} TRANSITION METAL CATIONS WITH ALKANES :
AB INITIO AND EXPERIMENTAL STUDIES

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Gaseous transition metal cations, bare or bearing ligands, have been shown to be extremely reactive with various hydrocarbons; for example group 8 metal cations are able to cleave CH and CC bonds (1). We have demonstrated that Cr, Mn, Cu and Zn (d^5 and d^{10}) cations, which are reported to be unreactive under monocollision conditions, lead mainly to $M(\text{alkane})_n^+$ complexes ($n = 1, 2$) under multicollision conditions encountered in tandem quadrupole instruments.

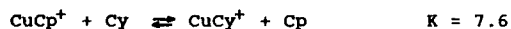
These compounds are very interesting since similar association complexes are in all cases the first intermediates formed in alkane-metal ion reactions. Elucidation of their structure is a key to understand alkane activation either in the gas phase or in the condensed phase.

Using our MS/MS/MS multiquadrupole triple analyzer instrument, various $\text{Cu}(\text{alkane})_n^+$ complexes have been prepared and their structure determined by collisionally activated dissociation (CAD). Alkane loss was the only fragmentation observed, suggesting that the alkane molecule remains as a whole in the complex. Use of a mixture of cyclopentane and deuterated cyclohexane allowed to confirm that the metal ion does not insert into CH bonds, even reversibly : no deuterium exchange was detected in the reaction products, nor in the CAD spectrum of the mixed complex $\text{Cu}(\text{C}_5\text{H}_{10})(\text{C}_6\text{D}_{12})^+$, which is cleaved to $\text{CuC}_5\text{H}_{10}^+$, $\text{CuC}_6\text{D}_{12}^+$ and Cu^+ exclusively.

However, the formation of these adducts cannot be fully understood in terms of electrostatic ion-induced dipole interactions, since K^+ , Ca^+ and Na^+ (which has the same ionic radius as Cu^+) do not react under the same conditions.

Using ion confinement in the collision cell, ligand exchange reactions could be brought to thermodynamic equilibrium, in order to know the influence of alkane structure on adduct stability. For example,

study of the reaction of Cu^+ with a cyclohexane-cyclopentane mixture led to the following equilibrium constants :



Adducts with linear alkanes (pentane, hexane) were observed to be more stable than their cyclic counterparts.

Confinement of copper ion with cyclohexane up to 40 ms allowed complete conversion of Cu^+ and CuCy^+ to CuCy_2^+ . No complex bearing more than two ligands was detected under those conditions.

The rate of association of copper ion with cyclohexane was found to be proportional to cyclohexane pressure, indicating that collisional stabilization of the adduct is not a rate-determining step. The corresponding rate constant ($4.3 \times 10^{-10} \text{ cm}^3 \text{ s}^{-1}$) is very close to the value reported for Cu^+ -ethane association under fast flow reactor conditions (2).

Concurrently, ab initio calculations have been performed on the simplest of these adducts, CuCH_4^+ , using an MP4 method. The bond energy obtained in this way is 52 kJ mol^{-1} (0.54 eV, 12.5 kcal mol^{-1}). The lowest energy structure corresponds to a Cu-C distance of 2.20 Å : the copper is close to two H atoms but is not located in the corresponding H-C-H plane; other structures with only slightly higher energy but more symmetrical were also obtained. In all cases C-H distances remain nearly the same as in the isolated methane molecule, but there is a noticeable distortion of the bond angles, and substantial charge delocalization : the charge on the copper atom is reduced to 0.77 electron unit.

The ethane complex CuC_2H_6^+ , studied in the case of a linear Cu--C-C geometry, gave a similar bond energy (50 kJ mol^{-1}).

The 4s orbitals of the copper ion were found nearly unpopulated : the bonding process essentially involves interaction between occupied orbitals of methane and fully occupied d orbitals of copper. Similar calculations on NaCH_4^+ , using the same geometry as in the optimal CuCH_4^+ configuration, actually led to a repulsive interaction. This might explain why formation of such adducts requires transition metal ions.

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GAS-PHASE ION-MOLECULE REACTION OF Cu^+ AND C_7H_8 ISOMERS: A CA MS/MS STUDY.

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We are interested in exploring the ability of Cu^+ to initiate a valence isomerization of quadricyclane (Q). The gas-phase valence isomerization of Q to norbornadiene (NBD) in the presence of Fe^+ has been demonstrated.¹ The Collisionally Activated (CA) mass spectra of the Fe^+ -NBD and Fe^+ -Q adducts are identical. Because Cu^+ exhibits reactivity similar to that of Fe^+ with alkenes,² we wished to determine whether Cu^+ would convert Q to NBD, or alternatively, differentiate the two isomers. The CA mass spectra of the adducts of Cu^+ with two other C_7H_8 isomers, toluene (T) and cycloheptatriene (CHT), are also presented.

Unlike the CA MS/MS spectra of Fe^+ -Q and Fe^+ -NBD, the CA MS/MS spectra of the Cu^+ -Q and Cu^+ -NBD adducts (Fig. 1) exhibit some similarities, but are distinct. For both Cu^+ adducts, the loss of C_2H_2 predominates, forming an abundant CuC_5H_6^+ ion of m/z 129. Other fragment ions common to both adducts are ions of m/z 63 (Cu^+), m/z 89 (CuC_2H_2^+), and m/z 154 (CuC_7H_7^+). Significant differences are also observed. Cu^+ -NBD loses C_2H_3 , whereas Cu^+ -Q does not. Cu^+ -NBD dissociates to form an ion of m/z 92 (NBD^+), but Cu^+ -Q loses CuH to give an ion of m/z 91 (C_7H_7^+).

The CA MS/MS spectra of the Cu^+ adducts with T and CHT (Fig. 1) suggest that the structures are similar to each other but differ from those of the Q and NBD adducts. The Cu^+ -T spectrum shows two major peaks, m/z 154 and m/z 91, corresponding to the losses of H and CuH , respectively. The Cu^+ -CHT loses CuH to a greater extent than it loses H. Both adducts dissociate to yield ions of m/z 63 and m/z 65 (C_5H_5^+). Cu^+ -CHT also shows successive H losses more abundantly than does Cu^+ -T. Neither, however, show an abundant loss of C_2H_2 observed for the Cu^+ -Q and Cu^+ -NBD adducts.

CA MS/MS/MS spectra were used to further probe the structure of the CuC_5H_6^+ ions. Spectra of the CuC_5H_6^+ ions formed from Cu^+ -Q or Cu^+ -NBD are compared to the CA MS/MS spectrum of the CuC_5H_6^+ ion prepared independently by a reaction between Cu^+ and cyclopentadiene (Fig. 2). The CA MS/MS/MS spectrum of CuC_5H_6^+ from Cu^+ -Q is nearly identical to the CA MS/MS spectrum of the model CuC_5H_6^+ . The most significant fragmentations are the losses of H and C_5H_6 . CuC_5H_6^+ from Cu^+ -NBD similarly exhibits an abundant loss of H. In addition, Cu^+ -NBD loses C_5H_5 to give an ion of m/z 64 (CuH^+). The data suggest that both CuC_5H_6^+ species whether formed by direct condensation or upon fragmentation of the Cu^+ -Q adduct exist as the (cyclopentadiene)copper ion complex, whereas that ion formed from Cu^+ -NBD, exists as a (cyclopentadienyl)(hydrido)copper ion species.

The FTMS data indicate that Cu^+ is the reactive species, rather than C_7H_8^+ ; viz, Cu^+ disappears as the adduct forms (see Fig. 3). A similar result is obtained for Cu^+ -NBD. Additional support for this conclusion is provided by double resonance experiments. Moreover, both systems react with first order kinetics, but Cu^+ reacts more rapidly with NBD than with Q.

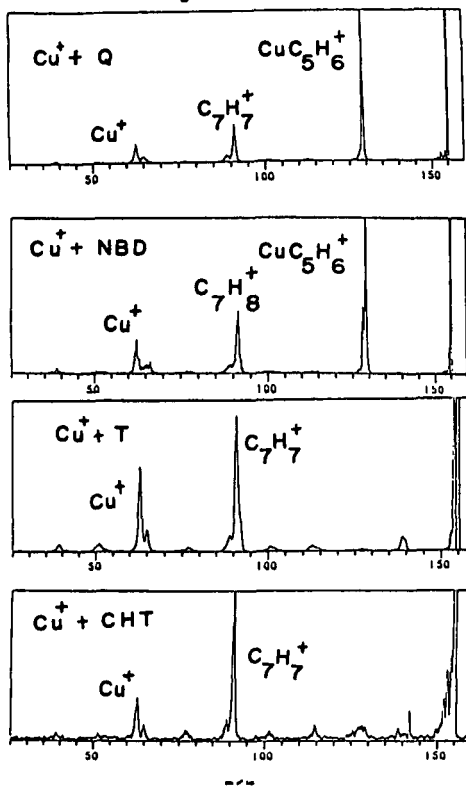
The base peak for the NBD adduct in the FTMS spectrum is an ion of m/z 91 (C_7H_7^+), whereas the ion of m/z 63 (Cu^+) is the major species observed for the Q system. Cu^+ -NBD does not exhibit loss of C_2H_3 , as it does upon CA. The Cu^+ -NBD species adds NBD to form the dimer, $\text{Cu}(\text{NBD})_2^+$ (m/z 247). We did not observe formation of a dimer in the Cu^+ -Q system at reaction times up to 2.110 secs.

The principal form of the Cu^+ -Q adduct is a (cyclopentadiene)(acetylene)copper ion complex produced by a metal-assisted valence isomerization. The rearrangement may be initiated by Cu^+ insertion into a C-C bond, as illustrated in Scheme 1.

Cu^+ catalyzes a retro Diels-Alder rearrangement of NBD to form a (cyclopentadiene)(acetylene)copper ion moiety that is able to reversibly transfer H from the cyclopentadiene unit to the metal center (Scheme 2). Cu^+ -Q, however, does not execute this last step. A similar reaction scheme has been proposed for the reaction of Fe^+ with both Q and NBD.

The Cu^+ -NBD adduct has other minor structural components. One form is a loosely-associated complex that dissociates upon CA to give the C_7H_8^+ fragment. Another adduct structure may involve C-H insertion, followed by rearrangement to a (tropylium)(hydrido)copper ion complex. This species also appears to be a minor contributor to the Cu^+ -Q structure.

Fig. 1



SCHEME 1

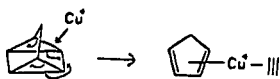


Fig. 2

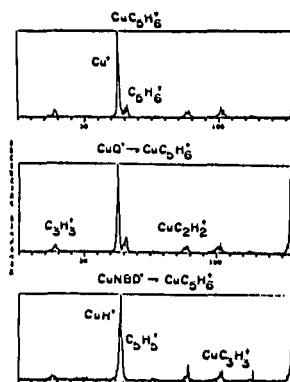
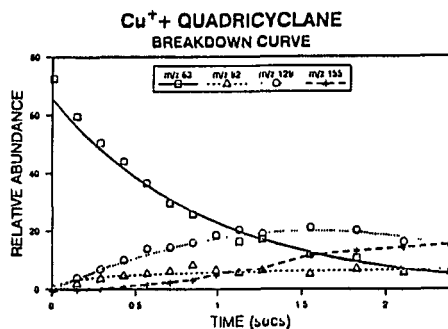
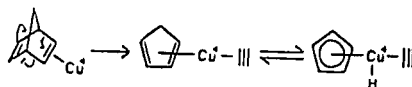


Fig. 3



SCHEME 2



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LIGAND FLUXIONALITY AND METAL-METAL BOND FORMATION FOR $\text{Fe}(^{13}\text{CO})^+$ REACTING WITH $\text{Fe}(\text{CO})_5$

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An FT-ICR two-section cell can be used in a mode such that two different neutral reagents are separated in the differentially pumped regions of the cell. This allows one to compartmentalize reactions performed in the FT-ICR by selecting the location at which a reaction is allowed to occur. For example, $\text{Fe}(^{13}\text{CO})^+$ (m/z 85) can be synthesized by the procedure outlined in Figure 1. $\text{Fe}(\text{CO})_5$ is admitted (ca. 2×10^{-7} torr, static) to one region of the cell and ionized to form $\text{Fe}(\text{CO})^+$ (m/z 84) (Figure 1A). $\text{Fe}(\text{CO})^+$ ions are transferred to the second region of the cell (Figure 1B) which contains ^{13}CO (ca. 5×10^{-7} torr, static). Unwanted ions are ejected from the cell at this point by rf sweeps^{1,2} such that only $\text{Fe}(\text{CO})^+$ remained. The isolated $\text{Fe}(\text{CO})^+$

ions are then allowed to ligand exchange with ^{13}CO to give $\text{Fe}(^{13}\text{CO})^+$ (Figure 1B). Finally, the newly formed $\text{Fe}(^{13}\text{CO})^+$ ions are moved to Region 1 of the cell where subsequent reaction studies are performed without complication from ligand exchange with ^{13}CO (Figure 1C). The transfer of $\text{Fe}(^{13}\text{CO})^+$ from Region 2 to Region 1 was performed under conditions such that residual $\text{Fe}(\text{CO})^+$ was not transferred.³ Such selective isolation of ML_n^+ species allows studies of gas phase reactions of specific $\text{ML}_n^{+/-}$ species without the complication of interfering ions.

The reactions of $\text{Fe}(^{13}\text{CO})^+$ with $\text{Fe}(\text{CO})_5$ to form labeled and unlabeled $\text{Fe}_2(\text{CO})_6^+$ and $\text{Fe}_2(\text{CO})_4^+$ can be studied under the conditions of static admission of $\text{Fe}(\text{CO})_5$ to Region 1. A temporal reaction plot of the normalized ion intensities for this reaction system is found in Figure 2. $\text{Fe}_2(^{13}\text{CO})_6^+$ is the dominant product by a significant margin. In fact, $\text{Fe}(^{13}\text{CO})^+$ forms the labeled $\text{Fe}_2(\text{CO})_6^+$ isomer at about four times the rate of formation of the unlabeled isomer. Assuming that the transition state contains six carbonyls, statistical (fluxional) formation of $\text{Fe}_2(\text{CO})_6^+$ should result in only about 17% labeled product. The dominance of the labeled isomer indicates that, for this reaction, $\text{Fe}_2(\text{CO})_6^+$ is approximately 22 times as likely to retain a labeled carbonyl ligand than an unlabeled one.

In the formation of $\text{Fe}_2(\text{CO})_4^+$ isomers, a somewhat different picture emerges. The labeled version of this species is formed only 1.5 times as fast as the unlabeled version indicating only a ninefold preference for the labeled carbonyl.

One explanation for the observed reactivity for these species is that the formation of $\text{Fe}_2(\text{CO})_6^+$ begins by facial attack by the iron of $\text{Fe}(^{13}\text{CO})^+$ on three carbonyl oxygens of the neutral effectively "locking" the facial carbonyl ligands in place and increasing the Fe- ^{13}C bond strength via enhanced backbonding. In order to increase orbital overlap by increasing molecular symmetry, an unlabeled, "unlocked" carbonyl is lost. Likewise, formation

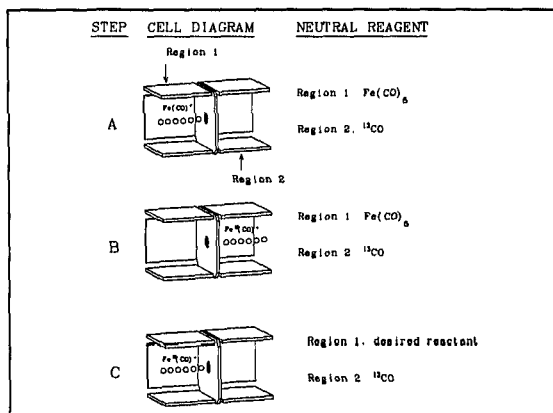


Figure 1. *In situ* synthesis of $\text{Fe}(^{13}\text{CO})^+$ for study of its Fe-Fe bond formation reactions: (A) formation of $\text{Fe}(\text{CO})^+$, (B) formation of $\text{Fe}(^{13}\text{CO})^+$, (C) isolation of $\text{Fe}(^{13}\text{CO})^+$.

of $\text{Fe}_2(\text{CO})_4^+$ isomers could result from edge attack resulting in only two locked carbonyls. For this model, decreased backbonding results in increased lability for the labeled CO. Yet, the drive for increased symmetry still dominates the reaction as indicated by the preference to lose two (presumably unlocked) carbonyls. For both $\text{Fe}_2(\text{CO})_6^+$ and $\text{Fe}_2(\text{CO})_4^+$, the intermediate should quickly rearrange to form an Fe-Fe bonded species.

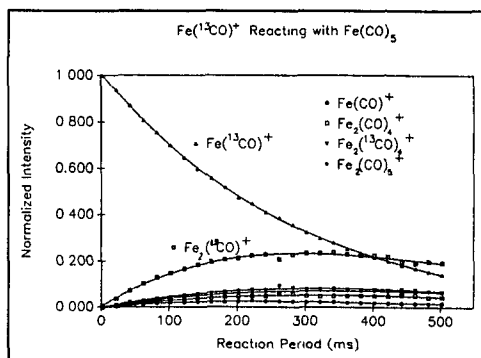


Figure 2. Temporal abundances of ions for the $\text{Fe}^{(13}\text{C})\text{O}^+/\text{Fe}(\text{CO})_6$ reaction system.

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LIGAND EXCHANGE REACTIONS OF IONIC TRANSITION METAL CARBONYL CLUSTERS:
REACTIONS FOR $\text{Cr}_2(\text{CO})_y^+$ AND $\text{Fe}_2(\text{CO})_y^+$

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Ligand substitution reactions have been used extensively to probe the reactivity and chemical properties of organometallic species. Such studies provide information on the coordination saturation/unsaturation of the reaction species, i.e., before coordination of the substrate can occur, a vacant site must be available in the coordination sphere of the neutral. In our laboratory, we are using ligand exchange reactions to probe the reactivity/coordinations unsaturation relationship of ionic cluster fragments of transition metal carbonyls formed by ion molecule reactions. The chemistry of $\text{Cr}_2(\text{CO})_y^+$ ($y = 4-6$) and $\text{Fe}_2(\text{CO})_y^+$ ($y = 3-4$) ions have been studied by using a variety of neutral ligands.

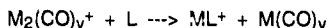
The probe reactions investigated include thermoneutral reactions, e.g., substitution of ^{13}CO for ^{12}CO , and exothermic reactions, e.g., substitution of O_2 for CO . Ligand exchange reactions with ^{13}CO provide insight about ligand lability, e.g., $\text{Cr}_2(\text{CO})_4^+$ readily exchanges all four ^{12}CO ligands, whereas $\text{Cr}_2(\text{CO})_6^+$ does not exchange any ^{12}CO ligands. The relative rate of ligand exchange for $\text{Cr}_2(\text{CO})_y^+$ increases with increasing electron deficiency, e.g., relative reactivity of $\text{Cr}_2(\text{CO})_4^+ > \text{Cr}_2(\text{CO})_5^+ > \text{Cr}_2(\text{CO})_6^+$. Similar differences in ligand lability are observed for $\text{Fe}_2(\text{CO})_3^+$ and $\text{Fe}_2(\text{CO})_4^+$. For example, $\text{Fe}_2(\text{CO})_3^+$ reacts with ^{13}CO to exchange only two ^{12}CO ligands and $\text{Fe}_2(\text{CO})_4^+$ reacts to exchange all four ^{12}CO ligands. The ligand exchange reactions of $\text{Fe}_2(\text{CO})_3^+$ with ^{13}CO indicated that two of the CO ligands of $\text{Fe}_2(\text{CO})_3^+$ are equivalent.

The relative reactivity of the ionic clusters is dependent upon the electron deficiency and the nucleophilicity of the ligand. For example, as the electron deficiency increases (i.e. larger unsaturation) the relative reactivity of the ligand exchange reactions increases. That is, for ligands which are hard bases and produce a large electron density on the metal center, (e.g., NH_3) the rate of the ligand exchange reaction increases. However, for a softer ligand (e.g., CO) which can accept some of the electron density back from the metal center is observed to have a smaller rate of reactivity.

Overall the ligand exchange reactions for the $\text{Cr}_2(\text{CO})_y^+$ and $\text{Fe}_2(\text{CO})_y^+$ systems are similar. For example, most of the ligand exchange reactions observed proceed via the following general reaction.



Although the ligand exchange reactions for the iron and chromium systems have many similarities there are some significant differences observed as well. For example, the chromium dimers undergo metal-metal cleavage with certain ligands.



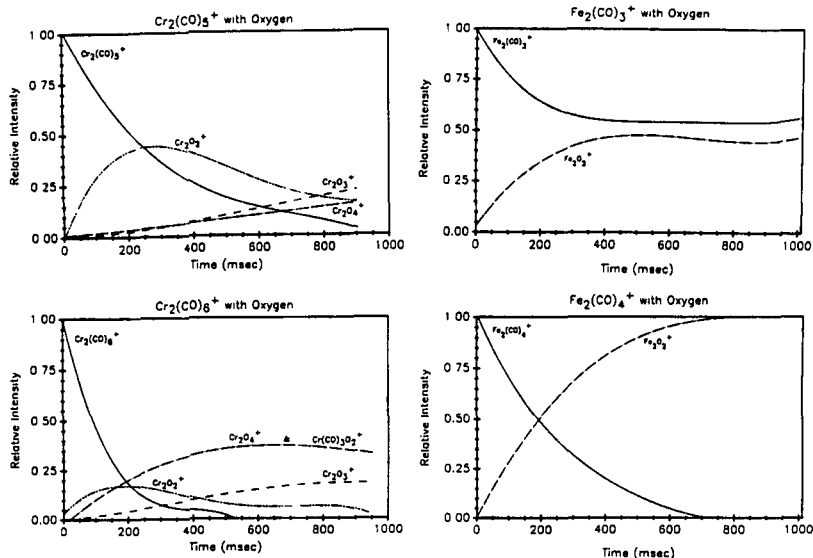
It is not clear why metal-metal cleavage occurs but it is probably due to the binding interaction of the ligand with the dimer. In other words, as the ligand interacts with the Cr dimer ions the dimer undergoes a coordination rearrangement to produce the metal-metal cleavage product. The occurrence of metal-metal cleavage is observed to be dependent upon the electron deficiency as well as the nucleophilicity of the ligands. For example, the product ions which arise by metal-metal cleavage are most dominant for low electron deficiencies, (e.g. $\text{Cr}_2(\text{CO})_6^+ > \text{Cr}_2(\text{CO})_5^+ >$

$\text{Cr}_2(\text{CO})_4^+$). Also, metal-metal cleavage product ions are most dominant for different ligands, (e.g. $\text{NH}_3 > \text{C}_6\text{H}_6 \approx \text{C}_4\text{H}_6 \approx \text{CH}_3\text{OH} > \text{O}_2$). Therefore, it appears that ligand addition to $\text{Cr}_2(\text{CO})_6^+$ corresponds to oxidative addition, reducing the metal-metal bond order.

In addition, different reactions are observed for the $\text{Fe}_2(\text{CO})_y^+$ ions. The ligand exchange reactions of $\text{Fe}_2(\text{CO})_3^+$ are unusual because only 30-40% of the ions react and the remaining 60-70% are unreactive. Freiser observed that only a fraction of the $\text{Co}_2(\text{NO})^+$ ions react with O_2 and suggested that the unreactive ions are of the form $\text{Co}_2(\text{N})(\text{O})^+$; a form where the NO ligand has undergone dissociation. We have no reason to suspect that ligand dissociation occurs for $\text{Fe}_2(\text{CO})_3^+$, e.g., collision induced dissociation yields Fe_2^+ , no Fe_2C^+ or Fe_2O^+ ions are observed. Consequently, we suggest that the reaction between $\text{Fe}_2(\text{CO})_3^+$ and L is subject to a small activation barrier and the reactive fraction developed from the vibrationally excited ion population.

Also, for the ligand exchange reaction between $\text{Fe}_2(\text{CO})_3^+$ and methanol an ion molecule product corresponding to $\text{Fe}_2(\text{CO})_3\text{H}^+$ is observed. This type of ion molecule product is only observed for ligand exchange reactions between $\text{Fe}_2(\text{CO})_3^+$ and alcohols (e.g. methanol and ethanol) and is not observed for any other ionic transition metal cluster or ligand. We tentatively assign the $\text{Fe}_2(\text{CO})_3\text{H}^+$ ion as a formyl ion of the form $\text{Fe}_2(\text{CO})_2(\text{CHO})^+$.

Several example temporal plots are shown below to give an idea of the chemistry observed for both the iron and chromium systems.



DYNAMICS AND THERMODYNAMICS OF LASER ABLATED METAL IONS BY HIGH PRESSURE MASS SPECTROMETRY

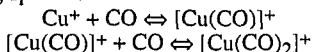
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High Pressure Mass Spectrometry studies were conducted on laser generated metal ions. Two metals were investigated thus far: copper and aluminium.

Both metal ions were ablated with an excimer laser (XeCl @ 5mJ/pulse) in a bath gas pressure of 5 torr. The ions were thermalized and then allowed to react with some ligand of interest. Extensive studies of clustering of CO on Cu⁺ have been carried out. Using a time resolved data acquisition system, we were able to observe the ions for ≈ 3 ms and see equilibrium between the following species:



However, a pressure study showed that the kinetics were not first order. While this may be suggestive of electronically excited states, since we are operating at threshold for ion formation, this seems unlikely. A more probable explanation is that surface effects are important in laser ablation. With copper, we are not able to use nitrogen as a bath gas. Ammonia evolves when we strike the target with the laser. All experiments were conducted in argon. If argon does not quench efficiently the transition state, we would expect this kind of pressure dependence.

Aluminium was studied in the same fashion. Nitrogen was used as the bath gas and CH₃COCH₃ as the ligand. We were not able to measure the binding energy for the first acetone attachment as it is larger than 30 kcal mol⁻¹. However, we have no difficulty measuring the enthalpy and entropy changes involved in the second ligand attachment. A typical time profile is shown in figure 1. A temperature study allowed us to construct a van't Hoff plot shown in figure 2. The enthalpy change is -29.9 kcal mol⁻¹ and the entropy change is -45 e.u. These changes are both quite large. This is suggestive of the structure shown in figure 3 where the second acetone molecule binds to the first acetone and not directly to the aluminium ion.

We are currently building a new apparatus that will extend our mass, pressure and temperature range. Thus far, the rich chemistry exhibited by these metal ions has been a problem but as we learn more, we hope to exploit it!

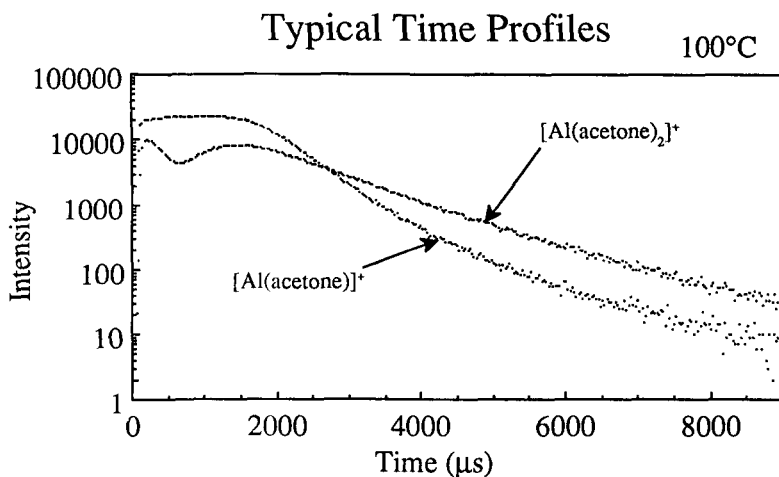


Figure 1

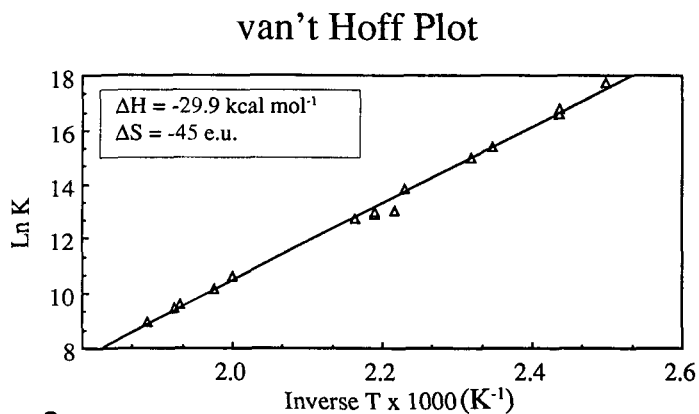


Figure 2

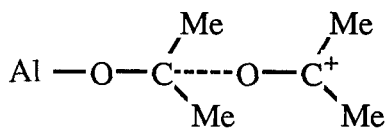


Figure 3

ION-MOLECULE CHEMISTRY OF METHYL CYCLOPENTADIENYL CHROMIUM DI-NITROSYL

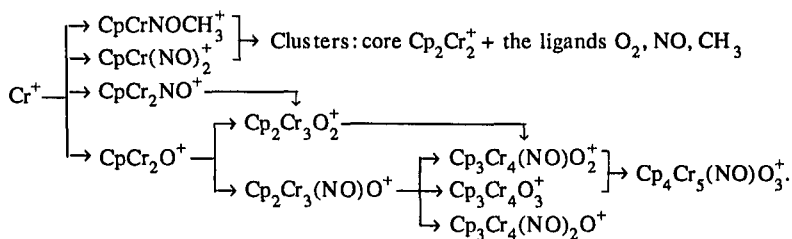
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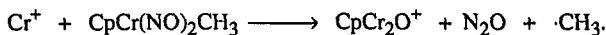
The gas phase condensation chemistry of many organometallic compounds has been investigated by Fourier transform ion cyclotron resonance (FT-ICR) spectroscopy (1, 2). The first demonstration of the utility of FT-ICR for such studies concerned the ion-molecule condensation chemistry of some η^5 -cyclopentadienyl (Cp) organometallic carbonyls(1). However, most of these FT-ICR investigations have been on organometallic carbonyls(2). For both the carbonyls and Cp-carbonyls, the condensation reactions give products in which the integrity of the CO ligand is maintained. In contrast, the ion-molecule condensation chemistry of $\text{CpCr}(\text{CO})_2\text{NO}$ shows many products (1) in which the NO ligand breaks up. In this work we report an investigation of the gas phase ion-molecule chemistry of $\text{CpCr}(\text{NO})_2\text{CH}_3$. This is the first η^5 -Cp, alkyl organometallic compound we have investigated.

Our investigations were carried out on a home-built FT-ICR spectrometer operating at 1.9 Tesla. Ion-molecule reaction pathways were confirmed by FT-ICR double resonance(3), and the results are compared with those of the deuterated compound $\text{CpCr}(\text{NO})_2\text{CD}_3$.

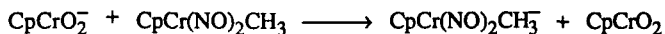
Electron impact ionization (25 eV electrons) on $\text{CpCr}(\text{NO})_2\text{CH}_3$ gives the following ions: $\text{CpCr}(\text{NO})_2\text{CH}_3^+$; Cr^+ , CpCr^+ , CpCrNO^+ , CpCrNOCH_3^+ , and $\text{CpCr}(\text{NO})_2^+$, $\text{C}_6\text{H}_8\text{N}^+$, $\text{C}_6\text{H}_6\text{Cr}^+$ and CpCrO^+ . When these ions are allowed to react with the parent neutral, and followed to fifth kinetic order, many ion-molecule reactions were identified. The following reaction sequences which are derived from Cr^+ are typical:



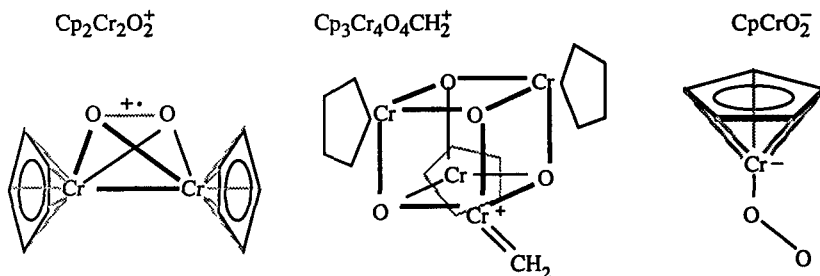
The typical reaction forming highly oxygenated species, for example, is as follow:



In addition to the extensive positive ion chemistry of $\text{CpCr}(\text{NO})_2\text{CH}_3$, negative fragment ions from the parent neutral also undergo condensation. Also, the following electron transfer reaction occurs:



As was true for the condensation chemistry of $\text{CpCr}(\text{CO})_2\text{NO}$ (1), many of the product ions are highly oxygenated. The structures of these ions can be postulated by analogy to the $(\text{CpCrO})_n$ clusters studied by Bottomley (4).



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THE PROPER CONSTRUCTION OF EQUILIBRIUM "LADDER" SCALES

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Dozens of equilibrium affinity scales have thus far appeared in the literature. Creation/ordering of such scales from the original equilibria is tedious. Selection of absolute ΔG 's from the experimental values has been somewhat arbitrary. We have written a program that creates the scale, orders the individual members into an ascending/descending form, gives the best fit of the absolute ΔG 's for each member, and graphs the scale.

For example, from the equilibrium measurements for seven bases a-g:

B ₁ :	a	c	e	a	c	f	h	e
B ₂ :	b	d	g	c	f	g	c	f
ΔG :	0.2	0.1	0.4	0.4	0.5	0.2	0.2	0.2

a 7*7 square skew symmetric matrix is generated:

a	b	c	d	e	f	g	
0	0.2	0.3	xx	xx	xx	xx	where xx = not measured
-.2	0	0.2	xx	xx	xx	xx	
-.3	-.2	0	0.1	xx	0.5	xx	
xx	xx	-.1	0	xx	xx	xx	
xx	xx	xx	xx	0	0.2	0.4	
xx	xx	-.5	xx	-.2	0	0.2	
xx	xx	xx	xx	-.4	-.2	0	

Typically, mostly near diagonal elements are available, due to the limited range of equilibria accessible by the methods. We discard "pendant" values (those linked only to one other base, such as d) and then separate the main matrix into submatrices along the "fusions". These are bases, such as c, in the ladder where no equilibrium arrow crosses a base. The submatrices optimize faster than the whole (ca. $n^{2.9}$ in speed). We average each row and column meeting at an xx to obtain a trial guess for the value of xx. Repeated iteration of this averaging fills the matrix to a constant set of values. The top row is then copied as a monotonically increasing edge vector (x) of absolute ΔG 's, corresponding to the affinity scale. Our actual function that serves as the measure of minimization is an rms function of the difference in the derived values on this edge vector less the measure equilibrium value. A simplex method of optimization is slower than the iterative method, but of slightly better convergence.

Several weighting schemes were considered. The implicit error function in a simple average assumes a constant error in ΔG , say 0.1 kcal/mol, regardless of the size of ΔG . This favors paths with the least number of equilibria, thus often larger ΔG values are favored. A Gaussian weighting function $a \cdot \exp(b \cdot \Delta G^2)$ favors smaller ΔG values in the averaging. We use $a=0.1$, $b=.1733$ to obtain for typical ICR equilibria:

ΔG :	0.0	1.0	2.0	3.0	4.0
$\pm$:	0.10	0.12	0.20	0.48	1.5

It is found that the best scale construction consists of one with few pendants, and fusions at regular intervals, to allow maximal checking of data. Cases in the literature are observed where one additional measurement would considerably reduce the uncertainty in the ladder values. Techniques for detecting data which do not fit well with the rest of the scale are included, to aid in detection of errors in data. The program is available from the authors.

THE STRUCTURE OF THE (M-H) ANIONS OF THE CH_3NO_2 ISOMERS OF
NITROMETHANE AND METHYL NITRITE

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Methyl nitrite, MeONO , is commonly used as a source of methoxide ion in CIMS and ion/molecule reaction studies. Reaction of methoxide with MeONO yields 60^- , the (M-H) ion. This ion can be represented as $\text{O}=\text{NOCH}_2^-$, a dipole stabilized anion.¹ Based on estimated acidities, however, it seemed unlikely to us that this ion could be formed by methoxide. The 60^- could also be the more stable $\text{CH}_2=\text{NO}_2^-$, the (M-H) ion of nitromethane, MeNO_2 .

The (M-H) anions of MeONO and MeNO_2 , formed in the CI source of a ZAB EQ mass spectrometer, fragment differently under CID conditions. The ion from MeNO_2 gives fragments at 59 daltons and 42 daltons, while the ion from MeONO gives three fragment ions at 59 daltons, 58 daltons and 42 daltons. The peak at 58 daltons can be used to identify the different structures. This ion must contain the elements CNO_2^- ; however, the atom connectivity must be $^-\text{OCN}=\text{O}$ since both the CNO_2^- and $^-\text{CON}=\text{O}$ structures are not stable. MIKES was not successful on the 60^- ion from neopentyl nitrite because of its low abundance.

From equilibrium gas phase acidities, the ΔH_{acid} of MeNO_2 is 356 ± 2 kcal/mole. The ΔH_{acid} of MeONO from bracketing experiments (current work) in the ICR is 368 ± 4 kcal/mole, between benzyl alcohol and acetoxime. Neither of the 60^- ions undergo facile deuterium exchange with various ROD, but the (M-H) from MeONO did show small amounts of deuterium exchange with PhCH_2OD which has an acidity close to that of the conjugate acid of 60^- . The ions from MeNO_2 and MeONO do not react with N_2O , CS_2 , OCS or CO_2 . The (M-H) anion of MeNO_2 reacts with dimethyldisulfide (MeSSMe) to produce a characteristic anion at 106 daltons,² while the 60^- anions of MeONO and neopentyl nitrite do not produce 106⁻ under the same conditions.

Ab initio calculations (6-31+G//6-31+G) indicate that $\text{CH}_2=\text{NO}_2^-$, from MeNO_2 , is ca. 51 kcal/mol more stable than $^-\text{CH}_2\text{ONO}$, but comparable in stability to $^-\text{OCH}_2\text{NO}$, another isomer. MeONO is calculated to have an acidity of 405 kcal/mol, 25 kcal less acidic than methanol. HOCH_2NO is at 365 kcal/mol, close to the bracketed value. This calculated acidity agrees with that obtained from experimental HOCH_2X acidities correlated with a linear free energy relationship: the σ_{TP} equation indicates that the nitroso alcohol should be slightly less acidic than $\text{CF}_3\text{CH}_2\text{OH}$ at 362 kcal/mol.

We thus believe that while $^-\text{CH}_2\text{ONO}$ may be formed in a complex on deprotonation by methoxide, it must rearrange to an isomer before the complex separates to methanol and 60^- . The ion is not $\text{CH}_2=\text{NO}_2^-$, based on reactivity with MeSSMe and on acidity measurements. The $^-\text{OCH}_2\text{NO}$ form is consistent with experimental reactivity and calculations. Another possibility is the anion of formyl hydroxamic acid, $^-\text{OCH}=\text{NOH}$, which is calculated to be of comparable basicity to $^-\text{OCH}_2\text{NO}$. We favor the latter alkoxide ion, however, because the hydroxamate should undergo rapid deuterium exchange of a single hydrogen with ROD, which is not observed.

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THERMOCHEMISTRY FOR REACTIVE NEUTRAL SPECIES FROM APPEARANCE
ENERGY MEASUREMENTS IN A FLOWING AFTERGLOW-TRIPLE QUADRUPOLE
INSTRUMENT.

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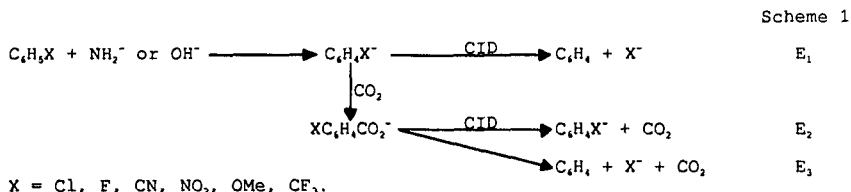
Introduction:

Collision induced dissociation (CID) methods have been used to estimate thermochemical properties for reactive neutral species in a flowing afterglow-triple quadrupole instrument¹. The heats of formation of reactive organic intermediates such as enols, ylides, benzyne, and carbenes can be calculated from the appearance energies (AE) for low energy CID and ion/molecule reactions that produce these neutral species as products. These heats of formation cannot be measured directly by calorimetry nor estimated reliably from thermochemical group equivalents. The experiments presented here provide one of the few methods for acquiring these important thermochemical data.

For a CID experiment, the appearance energy or threshold energy (E_t) corresponds to the activation energy for the dissociation reaction. If there is no significant reverse activation energy, then the threshold energy is equal to the reaction enthalpy. Therefore, the heat of formation for a reactive neutral product from a CID experiment can be estimated using the measured threshold energy (E_t), and the values of the heats of formation of the product ion and the reactant ion from the literature. To illustrate this general technique, we present here our preliminary results for benzyne as well as estimates of the heats of formation for various carbenes.

Discussion:

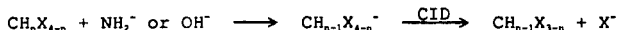
Benzyne are generated from CID of mono-substituted phenide and benzoate ions.



E_1 can also be determined from the difference between E_2 and E_3 , thus providing an additional measure of the desired threshold energy. Our preliminary results reveal some intrinsic difficulties with this experiment. The precursor ions formed by proton abstraction from mono-substituted benzenes appear to be mixtures of ortho, meta and para-substituted phenides. This can lead to variations in the observed CID threshold energy with reaction conditions in the flow tube. For instance, for the fluorobenzene case the direct method gives a value of E_1 which is consistent with the reported literature value for the heat of formation of ortho-benzyne², while the chlorobenzene case gives satisfactory agreement only with the (E_2-E_3) difference method. We are currently studying authentic ortho, meta, and para chlorobenzoate ions. These ions will allow us to acquire heats of formation for isomeric benzyne unambiguously.

Halocarbenes can be formed by CID of halomethide ions.

Scheme 2



X = Halogen, n = 1, 2, or 3

An estimate for the heat of formation of the neutral carbene product can be obtained from the appearance energy of the product halide ion. Other carbenes which have been generated by this method are phenyl, vinyl, and cyano-substituted methylenes. Some of the results of our experiments as well as literature reference values are summarized in Table 1.

Table 1

			CXYZ ⁻ $\xrightarrow{\text{CID}}$ CYZ + X ⁻		
X	Y	Z	AE(X ⁻)	calculated H ₁ (CYZ)	reference ³ H ₁ (CYZ)
Cl	Cl	Cl	32 ± 5	58 ± 5	39 ± 5
Cl	H	Cl	37 ± 5	77 ± 5	71 ± 5
Cl	F	F	<10		
Cl	F	Cl	21 ± 5	2 ± 5	-2 ± 5
F	F	F	62 ± 5	-34 ± 5	-49 ± 3

The CID results for CF₂Cl⁻ suggest the presence of a loosely bonded Cl⁻. Support for this is obtained from the observation that CF₂Cl⁻ transfers Cl⁻ to CO₂ in an exothermic bimolecular reaction. Since the Cl⁻ affinity of CO₂ (D[CO₂-Cl⁻]) is known to be 8.5 kcal/mol⁴, then D[CF₂-Cl⁻] must be less than this. Thus, CF₂Cl⁻ can be regarded as a gas-phase carbenoid. That is, its structure is that of a ground state singlet difluorocarbene loosely associated with a chloride ion. Consequently one might anticipate that this ion could react like a neutral carbene (ie., it might undergo insertion and cycloaddition reactions). However, attempts to observe carbene type reactivity in reactions of CF₂Cl⁻ with various alkenes have thus far failed. Ab-initio calculations for CF₂Cl⁻ are consistent with the assignment of a carbene-chloride complex type structure. For example the C-Cl bond length is 3.09 Å, a value which is much larger than usual C-Cl bond lengths (1.76 Å). Also the negative charge density is largely concentrated on the chlorine atom, making the carbene carbon atom electron deficient.

Conclusions:

1. A useful method for estimating heats of formation for reactive neutral compounds has been demonstrated. The method is based on low energy CID appearance energies, and can be used to derive thermochemical data for carbenes, benzyne, enols, ylides, and other interesting species.
2. The heats of formation of some halocarbenes have been determined using Cl⁻ appearance energies from CID of chloro-substituted carbanions.
3. The CF₂Cl⁻ ion has been shown to possess an extraordinarily weak C-Cl⁻ bond. The Cl⁻ affinity of CF₂ has been estimated to be less than 8.5 kcal/mol from Cl⁻ transfer studies.

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Determination of the Stability and Structure of Cluster ions $\text{Cl}^+(\text{RH})$ (Cl^+ attached with alkane) by High Pressure Mass Spectrometer

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The temperature dependence of the equilibrium for the gas-phase ion-molecule clustering reaction: $\text{Cl}^+ + \text{RH} \rightleftharpoons \text{Cl}^+(\text{RH})$ (Where RH is a normal- or cyclo-alkane) has been investigated by the use of pulsed ionization mass spectrometry. The solvation of Cl^+ by solvent molecules, such as alkanes, is of importance to gain an understanding of the role of solvent on reaction mechanism.

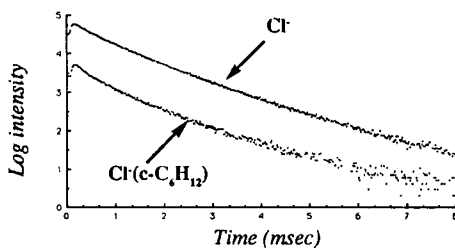
Electron impact of CCl_4 by 2keV 50 μs pulse of electrons produces the reagent ion Cl^+ , which then undergoes association with solvent molecules of interest at the high pressure (3~6) ion source. The hydrogen bond dissociation energies (ΔH°), and entropy changes (ΔS°) have been obtained from the van't Hoff plots. Also, the stabilities and structures of the cluster ions $\text{Cl}^+(\text{RH})$ have been investigated using the Gaussian 86 program and a 4-31G basis set, and compared with experimental results.

Both experimental and theoretical calculations show that the interaction between Cl^+ and alkane molecule is very weak, electrostatic $\text{Cl}^+ \cdots \text{H-C}$ interactions appear to dominate.

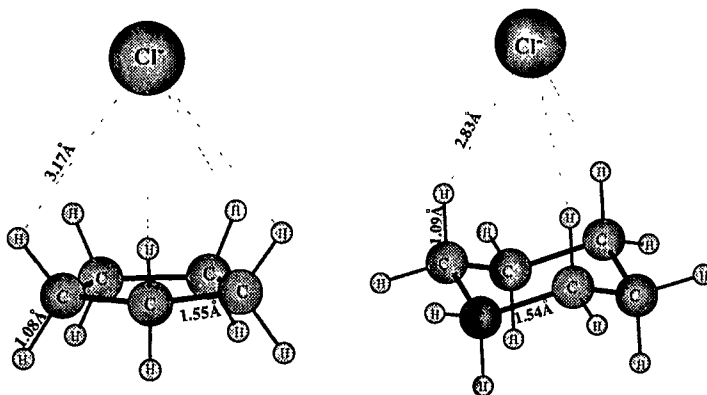
Results are shown in the table below:

Reaction System	$-\Delta E$ (RHF) (kcal.mol ⁻¹)	$-\Delta H^\circ$ (kcal.mol ⁻¹)	$-\Delta S^\circ$ (cal.mol ⁻¹ .K ⁻¹)
Cl ⁻ + n-C ₃ H ₁₂		10.0	24.3
Cl ⁻ + c-C ₅ H ₁₀	7.9	8.5	19.9
Cl ⁻ + n-C ₆ H ₁₄		10.9	25.7
Cl ⁻ + c-C ₆ H ₁₂	11.4	11.2	27.2
Cl ⁻ + n-C ₇ H ₁₆		10.7	24.1
Cl ⁻ + c-C ₇ H ₁₄		10.8	24.3
Cl ⁻ + n-C ₈ H ₁₈		12.5	26.9

The Typical Time Profile of HPMS is shown in the Figure below:



The optimized structures of Cl⁻(c-C₅H₁₀) and Cl⁻(c-C₆H₁₂) are shown below:



FOURIER TRANSFORM ION CYCLOTRON RESONANCE INVESTIGATION
OF HYDROGEN BONDING IN ALCOHOL-FLUORIDE ADDUCT IONS.

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The deuterium isotope effect is a commonly used method for exploring the nature of the potential energy surface for the hydrogen bond¹⁻⁵. There is considerable discussion regarding whether the potential energy surface for ion-molecule hydrogen bond interactions takes the form of a single minimum, a double minimum, or a skewed single minimum.

Hydrogen bond interactions between fluoride ion and alkyl alcohols have been investigated in this research. Strong hydrogen bonding is expected as the acidity of HF is bracketed by the alkyl alcohols in the gas phase. Equilibrium constants for fluoride transfer between isotopic alcohols have been measured for a variety of alkyl alcohols.

All experiments were performed on a Bruker Spectrospin CMS 47 FT-ICR Spectrometer, interfaced with an Aspect 3000 minicomputer. The strength of the magnetic field is 4.7 Tesla.

The low pressures of ICR mean that fluoride ion solvated by one molecule of alcohol must be generated by a sequence of bimolecular reactions (Scheme 1). A typical equilibrium plot is shown in Figure 1; the alcohol is 2-propanol and the pressure is 2.4×10^{-7} mbar.

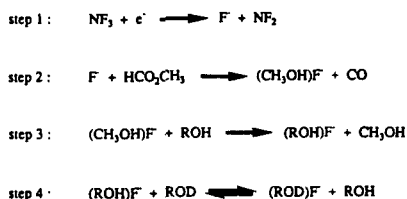
Table 1 shows the results obtained, along with the gas phase acidities of the alcohols. When these results are plotted against the difference in acidity between HF and the alcohol, a linear relationship with zero slope is observed (Figure 2). This suggests that the equilibrium constant for the deuterium isotope effect is independent both of the acidity of the alcohol, and of the nature of the alkyl moiety. The model of potential energy surface for ion-molecule hydrogen bond interactions that is consistent with these results is a skewed single minimum.

Future plans for this project are to (i) investigate the hydrogen bond interactions between alkoxide anion solvated by one molecule of its parent alcohol, (ii) investigate the hydrogen bonding between fluoride ion solvated by two molecules of alcohol, and (iii) perform computer calculations of the equilibrium constants for some of the alcohols. The latter will also allow the evaluation of the relative contributions of translation, rotation, vibration and zero point energy to the equilibrium constant.

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Scheme 1: Sequence of reactions generating (ROH)F⁻



$$K_{\text{eq}} = \frac{[\text{ROD}\text{F}^-][\text{ROH}]}{[\text{ROH}\text{F}^-][\text{ROD}]}$$

Figure 1: Equilibrium plot for 2-propanol.

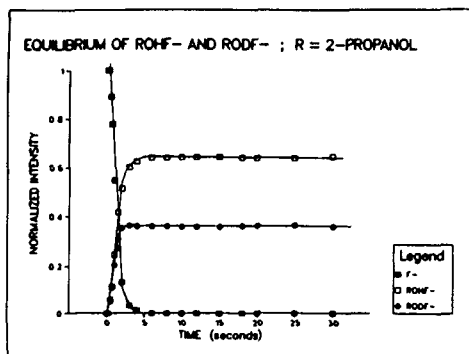


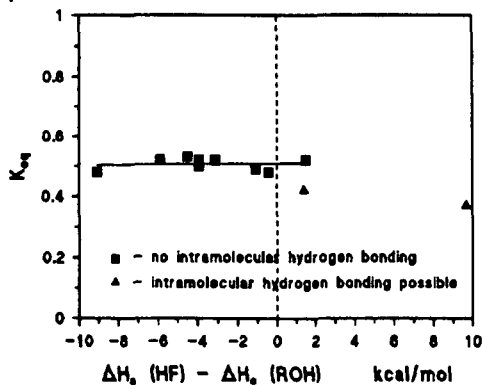
Table 1: Experimental results of K_{eq} , listed with gas phase acidities of the alcohols.

Alcohol	ΔH_a (kcal/mol)	K_{eq}
Methanol	380.6 ± 2.2	0.48 ± 0.08
Ethanol	377.4 ± 2.3	0.52 ± 0.05
1-Propanol	376.0 ± 2.2	0.53 ± 0.03
2-Propanol	375.4 ± 2.3	0.50 ± 0.03
1-Butanol	375.4 ± 2.3	0.52 ± 0.04
t-Butanol	374.6 ± 2.2	0.52 ± 0.05
Neopentanol	372.6 ± 2.8	0.49 ± 0.01
3-Methyl-3-Pentanol	371.9 ± 2.8	0.48 ± 0.05
3-Fluoroethanol	370.1 ± 3.9	$0.42 \pm 0.03^*$
Benzyl Alcohol	370.0 ± 2.8	0.52 ± 0.05
2,2,2-Trifluoroethanol	361.8 ± 3.5	$0.37 \pm 0.03^*$

* - indicates alcohols which can form intramolecular hydrogen bonds

Figure 2: K_{eq} versus difference in acidity between HF and ROH.
 ΔH_a (HF) = 371.5 kcal/mol

K_{eq} versus Difference in Acidity between HF and ROH



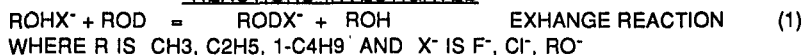
A HIGH PRESSURE MASS SPECTROMETRIC DETERMINATION OF EQUILIBRIUM
ISOTOPE EFFECTS OF ASSOCIATION AND EXCHANGE REACTIONS OF F⁻ AND RO⁻
WITH SIMPLE OH/OD LABELLED ALCOHOLS

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EQUILIBRIUM ISOTOPE EFFECTS [EIE] HAVE BEEN OBSERVED FOR IONS
IN SOLUTION [1] AND IN THE GAS PHASE [2-4.] MOST OF THE PREVIOUSLY
REPORTED WORK WAS OBTAINED BY ICR MS [2,3]. SOME EIE HAVE BEEN
OBTAINED BY PULSED ELECTRON BEAM HIGH PRESSURE MASS SPECTROMETRY
[HPMS] [4] LITTLE BIMOLECULAR EXCHANGE EIE HAVE BEEN REPORTED BY HPMS.

THE EXPERIMENTS WERE CONDUCTED ON TWO DIFFERENT INSTRUMENTS,
A HPMS [5] AND A FOURIER TRANSFORM-ION CYCLOTRON RESONANCE MASS
SPECTROMETER {FT-ICR} [6].

REACTIONS INVESTIGATED



THE DATA ARE SUMMARIZED IN THE TABLES AND SOME van't HOFF PLOTS ARE
SHOWN IN THE FIGURES.

THE FOLLOWING CONCLUSIONS WERE DRAWN FROM THIS STUDY:

- 1) IN THE ALCOHOL SERIES ROH/D, R = CH₃, C₂H₅ OR 1-C₄H₉, SIGNIFICANT EIE
ARE OBSERVED. WITH INCREASING ACIDITY OF THE ALCOHOLS, ΔH INCREASES
AND ΔS DECREASES, IMPLYING A MORE FLAT BOTTOM POTENTIAL ENERGY WELL
FOR THE HYDROGEN BOND AND MAYBE AN INCREASING BARRIER TO ROTATION OF
THE OH/OD GROUPS IN THE NEUTRALS. THIS MAY EXPLAIN THE DISCREPANCY
BETWEEN THE HPMS AND FT-ICR AS THE HPMS DATA WERE OBTAINED ABOVE
410 K. FOR LARGER ΔH VALUES, MOST OF THE EIE IS DUE TO ZERO POINT
ENERGY DIFFERENCES IN THE NEUTRALS (IN THE OH/OD STRETCH).
- 2) THE MULTIPLE SOLVATION THERMOCHEMISTRY OF F⁻ BY CH₃OH HAS BEEN
DETERMINED. THE FIRST THREE SOLVENT MOLECULES ARE MORE STRONGLY
BOUND TO F⁻ THAN FOR Cl⁻. THE FORTH ADDITION IS VERY SIMILAR [7].
- 3) THE 2,3 F⁻ AND THE 1,2 CH₃O⁻ CLUSTERING WITH CH₃OH SHOW AN
UNUSUALLY LARGE ΔS OF -32eu AND -28eu RESPECTIVELY. MOLECULAR MODELS
OF THE CLUSTER IONS PROSESS SOME STERIC LIMITATION TO THE INTERNAL
ROTATIONS. THE TRIPLY SOLVATED F⁻ IS MORE RESTRICTED THAN THE DOUBLY
SOLVATED CH₃O⁻ CLUSTERS.
- 4) NEW THERMOCHEMICAL DATA FOR THE 0,1 AND THE 1,2 CLUSTERING OF
CH₃O⁻ WITH CH₃OH ARE REPORTED HERE WITHOUT THE COMPLICATIONS OF
OXIDATION REACTIONS WHENEVER N₂O OR CH₃ONO ARE USED TO GENERATE
CH₃O⁻

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WATERLOO, WATERLOO, CDN.
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TABLE ONE

HPMS ISOTOPE EXCHANGE REACTIONS

R	REACTION	ΔH	ΔS	K_{eq} HPMS	K_{eq} FT-ICR
CH ₃	ROHF + ROD	0.1	-0.9	0.57	0.48
C ₂ H ₅	"	0.4	-0.2	0.48	0.52
1-C ₄ H ₉	"	0.6	-0.1	0.39	0.52
CH ₃	ROHCl + ROD	0.2	0.4	0.80	
1-C ₄ H ₉	"	0.0	-0.4	0.85	
CH ₃	ROH ¹⁸ O + ROD	0.1	-0.9	0.60	0.35
C ₂ H ₅	"	0.0	-1.0	0.60	0.46*
CH ₃	(ROH) ₂ O + ROD	-0.1	0.6	1.7	
C ₂ H ₅	"	0.0	1.1	1.6	
CH ₃	ROHF ¹⁸ O + ROD	0.0	-1.8	0.44	
C ₂ H ₅	"	0.1	-1.3	0.45	

NOTES

- 1) ΔH kcal mol⁻¹ error +/- 0.2 kcal per mole
- 2) ΔS cal mol⁻¹ K⁻¹ error +/- 0.5 eu
- 3) K_{eq} values are for 298K. *) REF (11)

TABLE TWO

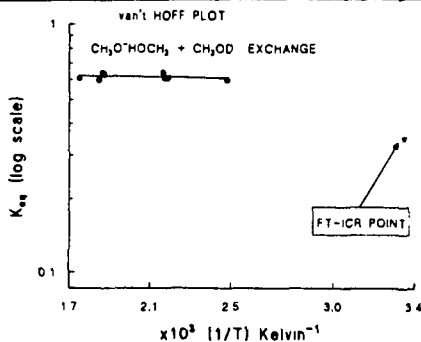
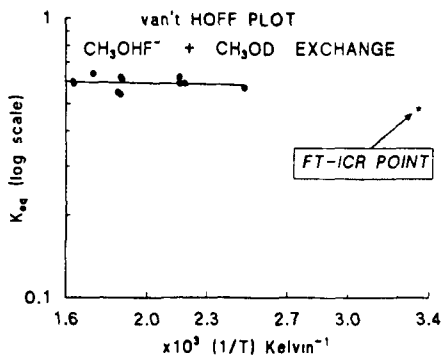
CLUSTERING EQUILIBRIA THERMOCHEMICAL DATA N-1, N-X* CLUSTERING ONTO ROH

ORDER ↓ REACTION	(0,1) ΔH ΔS	(1,2) ΔH ΔS	(2,3) ΔH ΔS	(3,4) ΔH ΔS
CH ₃ OH/F	30.6 23.4	18.8 22.7	18.0 32.0	12.1 21.0
C ₂ H ₅ OH/F	(A)	20.6 27.0	- -	- -
1-C ₄ H ₉ OH/F	- -	21.0 26.0	- -	- -
CH ₃ OH/Cl ⁻	17.4 24.4	- -	- -	- -
1-C ₄ H ₉ OH/Cl ⁻	18.3 23.4	- -	- -	- -
CH ₃ OH/ CH ₃ O ⁻	25.7 23.7	19.6 27.7	- -	- -

(ΔH - kcal mol⁻¹ ERROR +/- 0.5)

(ΔS - cal mol⁻¹ K⁻¹ ERROR +/- 2.0)

(A = (ΔG AT 640 K, -17.7)) (- = NOT MEASURED)



STRUCTURES AND ENERGETICS OF NO_3^- FORMED IN A GLOW DISCHARGE IONIZATION SOURCE

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We have recently coupled an atmospheric sampling glow discharge ionization (ASGDI) source¹ to an ion trap mass spectrometer (ITMSTM)². We have been using the MS/MS capabilities of the ITMS as well as kinetic measurements to characterize the ions and reactive neutrals that issue from the ASGDI source. During the course of this work we have obtained some unexpected results for the anions of m/z 62. We have interpreted these results based on the hypothesis that at least two structures of NO_3^- are formed in the ASGDI source and support this contention with theoretical calculations.

Figure 1a shows the MS/MS spectrum of the m/z 62 anion from normal background. Fragment ions are observed at m/z 32 (presumably O_2^-) and at m/z 46 (NO_2^-). The NO_2^- fragment is to be expected from NO_3^- with the D_{3h} structure (see Figure 2). However, an O_2^- fragment is unexpected. Figure 1b shows the MS/MS spectrum of m/z 62 when nitroglycerine was admitted into the ion source. Note that the intensity of the m/z 32 ion is unchanged while that of NO_2^- goes off scale.

We have interpreted the experimental results as being due to the formation of at least two structures of NO_3^- in the ASGDI source. Fehsenfeld et al.³ have previously hypothesized the existence of two stable structures of NO_3^- from kinetic data acquired in flowing afterglow studies. We have performed a series of calculations to elucidate other bound structures for the nitrate anion in addition to the common D_{3h} structure. Geometry optimization calculations were performed at the RHF level of theory using a 6-311+G(2d) basis set to account for the diffuse nature of anions. Subsequent single-point energy calculations including correlation effects were done at the MP2 level of theory using the same basis set. The computational results are shown in Figure 2.

In addition to the D_{3h} structure, we have located two linear structures with similar energies on the NO_3^- potential energy surface. Each linear structure can easily account for the formation of O_2^- from CID of m/z 62 formed by sampling ambient air. In each linear geometry the ON- O_2^- or NO- O_2^- bond is long and weak, especially for the NO- O_2^- species.

We have shown evidence that at least two forms of NO_3^- are formed in an ASGDI source. The form of NO_3^- obtained by sampling a typical explosive dissociates exclusively to NO_2^- , whereas at least some of the NO_3^- obtained by sampling ambient air dissociates predominantly to O_2^- . *ab initio* calculations reveal two bound linear forms of NO_3^- in addition to the common D_{3h} form. Either linear form can account for the formation of O_2^- upon CID of NO_3^- formed by sampling ambient air. However, because the alternate form of NO_3^- survives injection into the ion trap we can reasonably assume that the ON- O_2^- structure is more likely to be stored in the ion trap upon injection from the ASGDI source when sampling ambient air. The NO- O_2^- structure, due to its relatively weak O-O bond, is less likely to survive injection into the ion trap.

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Figure 1

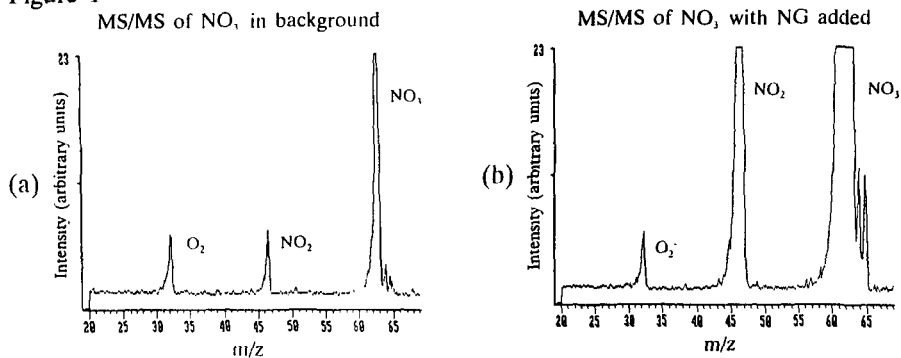
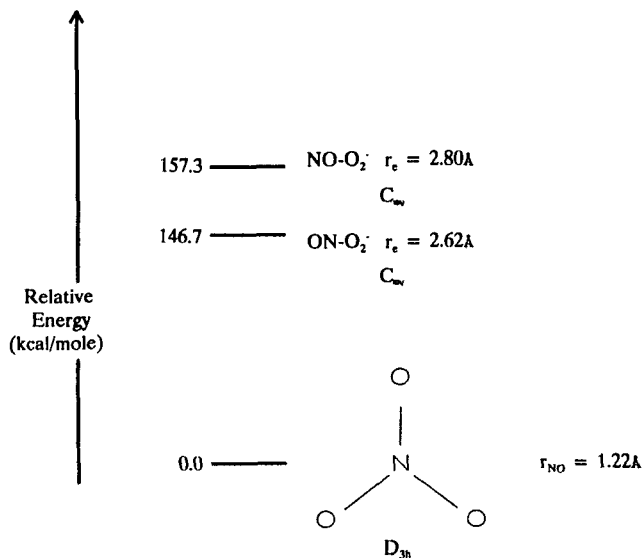


Figure 2



Research sponsored by U.S. Department of Energy, Office of Basic Energy Sciences
under contract AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

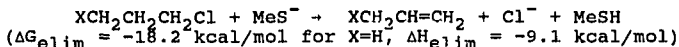
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RATE ENHANCEMENT OF β -ELIMINATION REACTIONS BY POLAR INTERACTIONS

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As part of comprehensive study of beta-elimination reactions in the gas phase, we report preliminary results on the nominal E2 process:



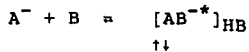
DePuy and coworkers have postulated that elimination upon alkoxide attack of ethers has some carbanionic character (ElcB mechanism), in spite of the very high energy alkyl carbanions involved. If substantial negative charge is developing at the beta-carbon in the transition state (ElcB-like), then placing an electron withdrawing substituent adjacent to that position should stabilize the transition state and accelerate the reaction. Such techniques have been utilized extensively in solution. The propyl chloride system was chosen because formation of the potential intermediate carbanion by MeS^- is endothermic by ca. 60 kcal/mol, yet the reaction is only slightly exothermic, to maximize substituent effects.

The work was done in an ICR spectrometer. Methanethiolate was generated by electron impact on MeSSMe . Chloride formed by dissociative attachment was ejected by double resonance prior to start of the kinetic measurements.

For substituents in $\text{XCH}_2\text{CH}_2\text{Cl}$ ranging from electron donating (-Et) to accepting ($-\text{CH}_2\text{CN}$), efficiencies from 4 to 25% were observed. If the observed rate constants (or efficiencies) are plotted vs. σ_I , there is a weak trend of increasing rate with electron accepting polar effect, with $\rho_I = 1.7$. This can be compared to $\rho_I = 17$ for the acidities of XCH_2OH , where a full negative charge is formed. The trend observed here correlates with a transition state possessing little carbanionic character. Even for $\text{X}=\text{Ph}$, no obvious carbanion character can be discerned; the point falls on the line. The formation of $(\text{PhCHCH}_2\text{Cl})^-$ is still too far uphill energetically. A base stronger than MeS^- or leaving group worse than Cl^- is needed to make the ElcB pathway accessible. That production of Cl^- in these reactions is the result of an elimination and not a substitution is supported by the following: (1) Olmstead and Brauman report a very low efficiency of 4.5% for $\text{MeS}^- + \text{MeCl} \rightarrow \text{MeS-Me} + \text{Cl}^-$. (2) We observe an even smaller efficiency in the case of $\text{X} = \text{Me}$. (3) We see no reaction of MeS^- with Et_3S nor with $(\text{CD}_3)_2\text{S}$.

Despite the apparent simplicity of this model system, there are competing reactions to consider. For example, Olmstead and Brauman observed production of HS^- from the reaction of MeS^- with MeSH , with 50% efficiency. We saw no ion gauge-independent production of HS^- using the substituted or parent propane thiols.

The rate constant for $\text{X} = -\text{CH}_2\text{OH}$ is ca. two times slower than expected from the σ_p correlation. The possibility of "non-productive" H-bonding, with a retardation of rate, is present here. Thus there is an alternate pathway "siphoning off" reactants otherwise going to observable products, as shown:

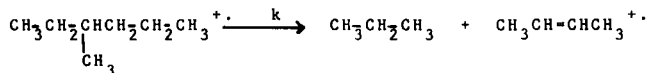
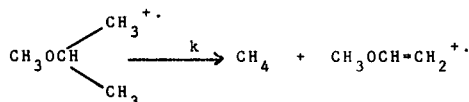
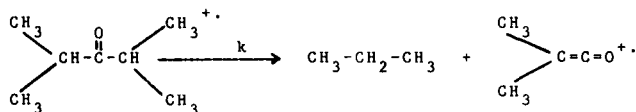
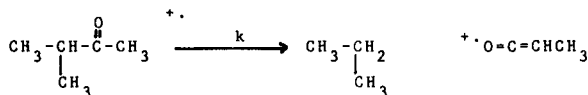


Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

AN FTMS STUDY OF ALKANE ELIMINATIONS FROM GAS PHASE IONS
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Unimolecular alkane eliminations from gas phase ions are considered to be ion-neutral complex-mediated [1]. We wished to determine rate constants for these decompositions. Recently Bensimon et al. [2] used FTMS to measure decay rates of some alkene ions in the $1 - 50 \text{ s}^{-1}$. Therefore we used FTMS to measure rate constants for alkane elimination. We also examined the threshold behavior of these processes using low energy collisionally activated dissociation.

REACTIONS STUDIED



EXPERIMENTAL

A Nicolet FTMS-1000 was used in all experiments. Parameters for determining rate constants for unimolecular fragmentations were electron energy = 15 eV, electron beam duration = 200-400 μs pressure = $1-6 \times 10^{-8}$ torr, magnetic field = 3 T, 64 K data points collected. For CAD experiments, electron energy = 10 eV, sample pressure = 1×10^{-7} torr, argon pressure = 5×10^{-6} torr, beam duration = 5 ms, 16K data points collected. RRKM rate constants were calculated with the program of Bunker and Hase [5].

Table 1. Measured and Calculated Rate Constants

Compounds	Measured rate ($s^{-1} \times 10^{-3}$)	RRKM rate*	
		-RH	-R
3-methylhexane	0.4	3.2×10^{10}	1.2×10^8
Isopropyl methyl ether	0.7	1.2×10^9	3.2×10^9
2-methyl-3-pentanone	0.3	2.8×10^7	1.1×10^7
2,4-dimethyl-3-pentanone	1.2	1.0×10^9	1.3×10^7

*Calculated at the critical energies.

RRKM rate constants for alkane eliminations are orders of magnitude higher than measured rates. Thus energy redistribution may not be rapid at low energies and/or in ion-neutral complexes. The breakdown curve for CAD experiments are similar to photoionization efficiency curves. The extrapolated breakdown curves for fragmentations of ionized acetone give thresholds of 0.4 ± 0.2 eV for methyl and methane losses. Correcting for thermal broadening ($0.6W_{0.5}(E_T)$) [4], the threshold becomes 0.6 eV, which is within experimental uncertainty of the 0.7 eV measured by photoionization [5].

CONCLUSIONS

1. The measured unimolecular rates for alkane eliminations are orders of magnitude lower than the RRKM predicted rates.
2. The thresholds for methane and methyl loss from ionized acetone are very similar and found to be 0.6 ± 0.2 eV, in reasonable agreement with photoionization results.

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Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

ASSIGNMENT OF ISOMERIC HYDROXYHYDANTOINS:
LINKED SCAN, TANDEM, AND HIGH RESOLUTION STUDIES

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The mass spectral fragmentation of hydroxyhydantoin isomers has been studied by a combination of high resolution, linked scan, and collisionally activated decomposition (CAD) experiments. This endeavor resulted in the structural assignment of four pairs of hydroxyhydantoin isomers. A key feature in differentiating 1-methyl-3-aryl-5-hydroxy-2,4-imidazolidinediones from 1-aryl-3-methyl-5-hydroxy-2,4-imidazolidinediones is that under electron ionization (EI) only the 1-methyl-3-aryl-5-hydroxy-2,4-imidazolidinediones yield an ion corresponding to $[\text{MeNCHOH}]^{+}$. The analogous $[\text{ArNCHOH}]^{+}$ ion (where Ar is the aryl group) was present in the EI spectra of both isomers.

Considerable research concerning the mass spectroscopic behavior of hydantoin isomers has been done.¹⁻³ There are no reports on the mass spectrometry of hydroxyhydantoin isomers, however, even though methods to synthesize this class of compounds are available.

Low resolution EI spectra were obtained on a Finnigan Model 1020 mass spectrometer using the solids probe. High resolution EI data were obtained on a VG Model 7070E-HF mass spectrometer. Metastable decomposition studies were also done on the VG Model 7070E-HF mass spectrometer using B/E linked scans. For comparison, collision activated dissociation experiments were done on a Finnigan MAT model ISO 70 Triple Quadrupole mass spectrometer using 0.7-1 mTorr Argon at -35eV collision energy.

Reaction of 1-methyl-3-aryl-ureas with glyoxylic acid (see Scheme) gives two isomeric products, 1-methyl-3-aryl-5-hydroxy-2,4-imidazolidinediones (1-Me-3-Ar) and 1-aryl-3-methyl-5-hydroxy-2,4-imidazolidinediones (1-Ar-3-Me). Relative intensities of ions found in the positive ion EI spectra of four isomeric pairs of hydroxyhydantoin isomers are summarized in the Table. The compositions of these ions were established by high resolution experiments.

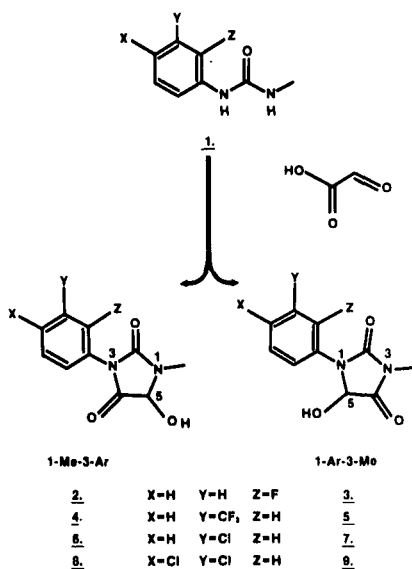
The EI spectra of isomeric pairs of compounds are very similar. Intensity differences can be seen. For example, the $\text{M}^{+}-\text{MeNCO}$, $[\text{ArCHNO}]^{+}$, and $[\text{ArCNO}]^{+}$ ions are consistently more abundant in the spectra of 1-Me-3-Ar isomers. Analogously, the $[\text{ArCH}_2\text{NO}]^{+}$, $[\text{ArNH}_2]^{+}$, and Ar^{+} ions are consistently more abundant in the spectra of 1-Ar-3-Me isomers.

The $[\text{MeCH}_2\text{NO}]^{+}$ ion is only seen in the spectra of 1-Me-3-Ar isomers. The assignment of hydroxyhydantoin isomers is based on this observation. The $[\text{ArCH}_2\text{NO}]^{+}$ ion was expected in the spectra of only the 1-Ar-3-Me isomer, however it was found in the EI spectra of all of the compounds examined. Linked scan and tandem experiments offer an explanation. The $[\text{ArCH}_2\text{NO}]^{+}$ ion from the 1-Me-3-Ar isomer was found to arise from the molecular ion by losses of carbon monoxide and methylisocyanate. Similarly the $[\text{ArCH}_2\text{NO}]^{+}$ ion from the 1-Ar-3-Me isomer arises from the molecular ion by losses of the same two neutral molecules. These analogous fragmentations suggest that the $\text{M}^{+}-\text{CO}$ ions from either isomer have the same structure. The fact that the metastable decomposition spectra of these ions are nearly identical supports this hypothesis. A possible structure for the $\text{M}^{+}-\text{CO}$ ion is a cyclic ion formed by loss of carbon 4 of the hydantoin ring as carbon monoxide.

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SUMMARY OF RELATIVE INTENSITIES OF SELECTED IONS

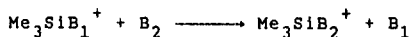
ION:	Compound:							
	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>
M^{+}	25	23	32	95	60	50	65	37
$M^{+}-CO$	25	-	8	-	10	-	-	-
$M^{+}-MeNCO$	8	-	5	3	15	-	25	-
$[ArC_2HO_2]^{+}$	-	5	-	15	-	8	-	5
$[ArCH_2NO]^{+}$	5	11	5	36	2	30	3	11
$[ArCHNO]^{+}$	36	3	53	4	42	7	97	3
$[ArCNO]^{+}$	100	30	100	50	100	70	100	70
$[ArCO]^{+}$	-	25	-	35	-	30	-	20
$[ArNH_2]^{+}$	18	100	10	100	20	100	20	100
Ar^{+}	5	25	15	30	15	30	10	25
$MeCH_2NO^{+}$	4	-	29	-	15	-	15	-
$MeCHNO^{+}$	13	5	22	15	30	36	41	30
$MeCNO^{+}$	5	4	3	11	3	9	3	10



THE TRIMETHYLSILYL IUM (Me_3Si^+) AFFINITY OF ALKYLAMINES

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The Me_3Si^+ affinities of monoalkylamines have been measured as an extension of previous studies of the association of Me_3Si^+ with oxygen and aromatic bases(1) and alkenes (2). A high pressure mass spectrometer with pulsed electron beam is used to obtain van't Hoff plots for the reaction



where B_1 and B_2 are amine or reference base.

Quantitative data for the binding energy (ΔH°) of Me_3Si^+ to ammonia, monoalkylamines and dimethylamine have been obtained by reference to previous absolute values for oxygen bases (1). Ethyl acetate and ethyl ether are the reference bases for ammonia. Ethyl benzoate is the reference base for the other amines.

The binding energy (ΔH°) increases with increasing polarizability of the alkyl group: $\text{NH}_3 < \text{MeNH}_2 < \text{EtNH}_2 < \text{BuNH}_2$. The butylamines however are not in the expected order (t-BuNH_2 i - BuNH_2 n - BuNH_2) which may be due to steric hindrance between the Me_3Si and the t-Bu- and $i\text{-Bu-}$ groups.

Accurate proton affinities of N-alkyl-trimethylsilylamines may be derived from the Me_3Si^+ affinities, provided that the enthalpies of formation of the N-alkyl trimethylsilylamines can be correctly estimated from the single literature value for $\Delta H_f^\circ(\text{Me}_3\text{SiN}(\text{Me})\text{H})$ (3). The values in Table 1 show that $\text{Me}_3\text{SiN}(\text{R})\text{H}$ has a slightly lower proton affinity than $\text{Me}_3\text{CN}(\text{R})\text{H}$ in agreement with theoretical predictions of the effect of replacing carbon by silicon (4).

Qualitative investigations of Me_3Si^+ transfer from ethyl benzoate to trialkylamines show that the Me_3Si^+ affinities of these amines are significantly greater than those of the monoalkylamines. However no oxygen base has been found with sufficiently high Me_3Si^+ affinity that it may be used as reference base for quantitative equilibrium transfer experiments with trialkylamines. In addition Me_3Si^+ transfer between trialkylamines is so slow that equilibrium is not attained during the available

ion residence times so that even the relative binding energies of these amines cannot be determined. This slow exchange is ascribed to steric hindrance. With the exception of dimethylamine, quantitative data has so far not been obtained for dialkylamines. Equilibrium was apparently obtained in Me_3Si^+ transfer experiments but the resulting van't Hoff plots are non-linear. The reason for this is not immediately obvious although in some, but not all, cases it can be ascribed to an ion induced isomerization of the amine.

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Table 1

Me_3Si^+ AFFINITIES OF AMINES AND DERIVED PROTON AFFINITIES FOR SILYLAMINES

AMINE	AFFINITY ^a		$\text{PA}(\text{Me}_3\text{Si}\cdot\text{amine-H})^a$	$\text{PA}(\text{Me}_3\text{C}\cdot\text{amine-H})^a$
	Me_3Si^+	H^+		
NH_3	45.7	204	221-227	221
CH_3NH_2	54.6	214	224	227
$\text{C}_2\text{H}_5\text{NH}_2$	56.6	217	227	230
$n\text{-C}_4\text{H}_9\text{NH}_2$	58.7	218	229	231
$i\text{-C}_4\text{H}_9\text{NH}_2$	57.1	221	227	233
$t\text{-C}_4\text{H}_9\text{NH}_2$	57.2	221	228	233
$(\text{CH}_3)_2\text{NH}$	59.2	221	223	232

^a units kcal mol^{-1}

Non-FT/ICR/MS: Bayesian Spectral Analysis for ICR Mass Spectroscopy

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Of the many methods for obtaining a discrete frequency-domain spectrum from a discrete time-domain signal, the (discrete) fast Fourier transform (FFT) is computationally fastest, but is not necessarily the "best".¹ For example, the "causality" assumption (namely, that only one spectrum fits the time-domain data) of the FFT method is no longer valid when noise is present. Moreover, truncation of the time-domain data can produce an FT spectrum with unwanted "Gibbs oscillations". Depending on the frequency separation between peaks, the time-domain signal-to-noise ratio, and number of frequency-domain peaks, non-FT methods can offer mass resolution and/or mass accuracy superior to that from FFT data reduction of the same time-domain discrete data. In this abstract we compare Bayesian and FFT analysis of experimental electron-ionization ICR time-domain discrete 16K signals for a mixture of N₂ and CO (Fig. 1) and for various xenon isotopes (Fig. 2).²

Bayesian spectral analysis iteratively determines the most probable spectrum that fits the time-domain data, conditional on any prior information ("prior") and on the likelihood function of the data.³ Figures 1B and 2B show the results obtained with a "prior" which assumes that the time-domain data (first 4096 points only) consist of a sum of exponentially damped sinusoids plus noise. Figures 1A and 2A show the corresponding magnitude-mode spectra computed by FFT from the full 16,384-point data sets. The Bayesian spectral peaks appear visually sharper, with higher signal-to-noise ratio, than those produced by FFT. A better comparison is provided in Tables 1 and 2, which list the ICR frequency differences for ions of various m/z at 3.016 tesla. The "true" frequency separations computed from the isotopic masses (minus the mass of an electron) relative to CO⁺ or ¹³²Xe⁺, respectively, are compared to those determined by Bayesian or FFT spectral analysis (in which the FFT peak frequencies are determined by 3-point parabolic fit to the three highest-magnitude points for each peak). From Tables 1 and 2, it is clear that Bayesian analysis gives a more accurate estimate of ICR frequency (and thus more accurate m/z) than does the discrete FT for signals that are close together in frequency (or m/z), even though the Bayesian analysis was based on only the first 1/4 of the time-domain data set subjected to FFT data reduction.

Experimental results based on a maximum-entropy "prior" have also been investigated. The entropy "prior" assumes no prior functional form for the signal, except that Gaussian-distributed random noise is present. Thus, the spectrum that has maximum entropy and still fits the time-domain data to within the standard deviation of the noise is found. We shall report elsewhere on our comparison of the two forms of Bayesian spectral analysis and the discrete FT.²

This work was supported by N.I.H. GM31683 and The Ohio State University.

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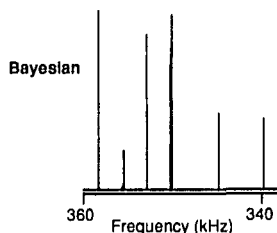
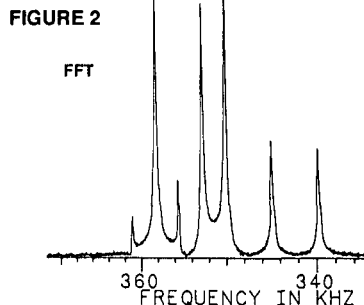
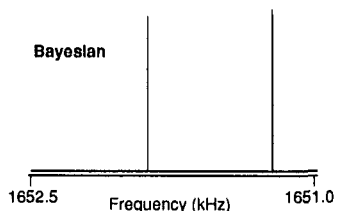
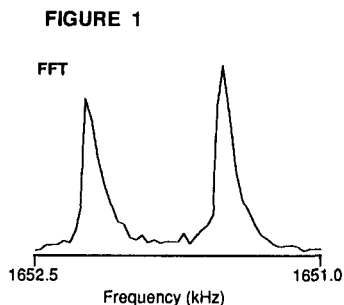


Table 1

Computed $\nu_{\text{CO}} - \nu_{\text{N}_2}$	Discrete FT $\nu_{\text{CO}} - \nu_{\text{N}_2}$	Bayesian $\nu_{\text{CO}} - \nu_{\text{N}_2}$
663.4	670.4 (+.00012 amu)	662.8 (-.000010 amu)

Table 2

Isotope	Computed $ \nu_{132\text{Xe}} - \nu_i $	Discrete FT $ \nu_{132\text{Xe}} - \nu_i $	Bayesian $ \nu_{132\text{Xe}} - \nu_i $
^{136}Xe	10342	10390 (+.0143 amu)	10368 (+.008 amu)
^{134}Xe	5248	5308 (+.018 amu)	5293 (+.013 amu)
^{132}Xe	0.0	0.0	0.0
^{131}Xe	2680	2678 (-.00060 amu)	2682 (+.00060 amu)
^{130}Xe	5408	5327 (-.024 amu)	5384 (-.0072 amu)
^{129}Xe	8170	8162 (+.0024 amu)	8169 (-.00030 amu)

MSCOINT: A COMPUTER PROGRAM DEVELOPED FOR THE INTERPRETATION OF COLLISIONALLY ACTIVATED MASS SPECTRA

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The program MSCOINT (Mass Spectrum Computerized Interpretation) is currently being developed in our laboratory with the aim to assist in the interpretation of mass spectra generated by high energy collision activation of organic molecules. The objective of the project is to produce a list of probable candidate-structures using mass spectral data such as the exact mass of the parent ion and the CAD spectrum. The program is structured in four parts that are related to the identification process:

- i) FOREMP: this is the first part of the program and its role is to generate empirical formulae, $C_xH_yO_nN_z$, from the exact mass of the parent ion.
- ii) ANALYS: this second part of the program decomposes all empirical formulae generated by FOREMP in linear combinations of predefined superatoms.
- iii) GENSTR: this is the part of the program that generates probable structures from the sets of superatoms that have been determined by ANALYS.
- iv) STAT: this last part of the program is responsible for determining and ranking the most probable candidates.

The first two parts of the program presently perform adequately. Using measures of the accurate mass of the parent ion and a set of constraints, the program generates a list of empirical formulae that correspond to the compound under study. Then, the second part of the program decomposes all the empirical formulae generated into sets of predefined superatoms using equation 1.

$$C_xH_yO_nN_z = \sum X_i S_i + \sum Y_j S_j \quad (1)$$

The sets of superatoms that constitute solutions to the equation are obtained by determining the frequencies of occurrence X_i and Y_j of the unsaturated and saturated superatoms i and j . Then the sets are used by the third part GENSTR to generate candidate-structures which will be ranked by STAT.

Several approaches can be considered in order to generate chemical structures from the sets of superatoms and there are also several ways to correlate the structures to the mass spectra and rank the most probable candidates. In initial trials that were conducted in order to verify the feasibility of using the program MSCOINT to assist in the interpretation of collisionally activated mass spectra, the sets of superatoms generated by ANALYS were arranged in structures using predetermined acyclic pseudo-alkane structures. This simple approach was found to be adequate to obtain all possible acyclic structures but it requires a data base of alkane structures to be constructed. The approach is very useful when a small number of superatoms are involved but as their number increases, the predetermined structures that have to be considered become very large and the size of the data base is enlarged considerably. For example, a data base of structures including up to 16 superatoms would require 18030 entries [1] which can create a problem. In context, it was deemed important to develop an algorithm that would generate only the structures that have a good correlation with the experimental spectrum. In this way, constraints to be defined by the user would be involved in the generation of the probable structures and their number would be decreased substantially by comparison to the former approach. This strategy has the added advantage that it does not require a permanent data base to be constructed.

The algorithm GENSTR, using the sets of superatoms provided by ANALYS generates exhaustive acyclic structures in a recursive fashion by linearization of structures of higher complexity. Since all double and triple bonds are initially included in the predefined superatoms, all the structures generated represent pseudo-alkanes in which carbon atoms are replaced by superatoms or clusters of superatoms. At each step of the construction process, the symmetry (equivalence of objects and positions) of the chain is verified in order to avoid redundancies. In order to show how the construction process operates, examples can be used by considering the

generation of simpler structures. The solutions (sets of superatoms) obtained from equation 1 are in the form of partition vectors PV as shown in equation 2 where {SP}, {SS}, {ST} and {SQ} are the lists of primary, secondary, tertiary and quaternary superatoms respectively. To each partition

$$PV = \{ \{SP\}, \{SS\}, \{ST\}, \{SQ\} \} \quad (2)$$

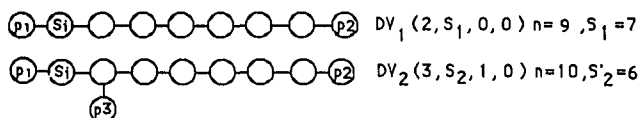
$$DV = (p, s, t, q) \quad (3)$$

vector is associated a distribution of superatoms DV where p, s, t and q are the cardinal numbers of {SP}, {SS}, {ST} and {SQ}. The total number of superatoms (n) is given by the sum of all types of superatoms as shown in equation 4 and the connectivity rule given in equation 5 must also be respected [2]. Thus the structures can be expressed as distribution vectors of p, s, t and q.

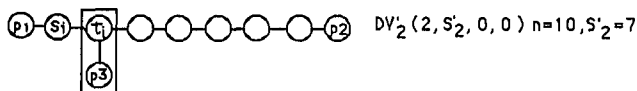
$$n = p + s + t + q \quad (4)$$

$$0 = t - p + 2q + 2 \quad (5)$$

For example, a distribution vector $DV_1 = (2, S_1, 0, 0)$ would represent linear structures with two terminal superatoms of valence one (p) and S_1 superatoms of valence two, and a distribution vector $DV_2 = (3, S_2, 1, 0)$ would represent branched structures with three terminal superatoms of valence one, S_2 superatoms of valence two and one superatom of valence three (t). The distribution vectors and the corresponding structures are shown below. For the construction of the structures



corresponding to the distribution vector DV_2 , a new distribution vector DV'_2 is considered in which the ensemble t_1-p_1 represents a cluster of superatoms as shown by the rectangle on the structure DV'_2 . It can be seen that the new structures represented by the distribution vector DV'_2 are now



conceptually equivalent to linear structures having seven divalent superatoms (S'_2) and two terminal superatoms (p_1, p_2) of valence one. Therefore it is possible to generate the appropriate structures using a given set of superatoms in a fashion totally analogous to the one used for the structures represented by DV_1 and it can be seen that any given distribution vector of higher order i.e. $DV_x = (p_x, s_x, t_x, q_x)$ can be reduced in a recursive manner to a vector $DV'_x = (2, S'_x, 0, 0)$ of lower order representing linear structures. In the example shown, clusters of superatoms corresponding to t_1-p_1 , t_2-p_2 and t_3-p_3 would be included in the structure generation process and tried in all the positions allowed within the constraints of non-redundancies and spectrum correlation requirements. For structures involving very high symmetries, the linearization process is not recommended because it produces redundant structures and in those cases, a different approach which is more suitable is used. Thus, the new GENSTR algorithm that has been developed and described allows more flexibility and has been incorporated in MSCOINT to replace the former approach.

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AN AUTOMATED CHEMICAL STRUCTURE ELUCIDATION SYSTEM (ACES) FOR MS/MS SPECTRA

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The goal of this work is the development of software tools for use in the construction of an "expert" structure elucidation system for MS/MS data. The software capable of producing and applying reliable substructure identification rules (MAPS: Method for Analyzing Patterns in Spectra) is crucial to the success of this system. The rules produced by a new generation method have greatly increased reliability *and* recall (i.e. 100% reliability and 100% recall for the "promazine" feature combination rule). This new method uses "feature combinations" rather than individual MS/MS spectral features as a basis for the rules. Collision gas pressure was also explored to determine the effect that different pressure regimes have on the content of the MAPS rules. Examination of MAPS rules derived from data at two different collision gas pressures reveals that the majority of the features for a given rule at one pressure are different than those for the same rule at a pressure three times higher (i.e. P1 vs. P2 as defined below).

A reference database which contains MS/MS spectra for 74 regulated drugs and 31 other standards was constructed. A total of 14,097 spectra were collected including 105 primary mass spectra, 6001 daughter spectra at P1 (0.4 mtorr Ar), 6001 daughter spectra at P2 (1.2 mtorr Ar), and 1990 daughter spectra at P3 (1.8 mtorr Ar). The compounds run include barbiturates, amphetamines, antipsychotics, morphine substitutes, stimulants, and phenols.

Substructure identification rules were generated using the MAPS software. The original method utilizes a uniqueness and correlation filter to determine the features included in a rule. The following rule was produced for the "promazine" substructure using U = 40% (feature uniqueness factor) and C = 50% (feature correlation factor):

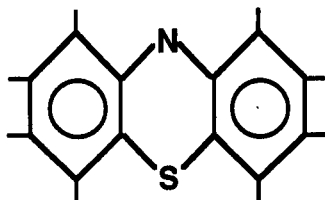
IF	"PD (58.0 30.0)	[40,62]	{F1}
and	"PD (58.0 43.0)	[53,62]	{F2}
and	"PD (70.0 27.0)	[42,85]	{F3}
and	"PD (70.0 44.0)	[50,69]	{F4}
and	"PD (84.0 42.0)	[41,54]	{F5}
and	"PD (99.0 70.0)	[70,54]	{F6}

THEN substructure "PROMAZINE" is present.

The recall estimate for a rule is a measure of the ability to predict the presence of a substructure. The reliability estimate for a rule is a measure of the accuracy of the predictions. These estimates are calculated by applying the rules against the spectra for each of the reference compounds. The following table shows the

recall and reliability estimates for the promazine rule at four different match factors.

MF (%)	RECALL (%)	RELIABILITY (%)
100	15 [2/13]	100
80	38 [5/13]	100
60	62 [8/13]	73
50	77 [10/13]	43



PROMAZINE SUBSTRUCTURE

The match factor specifies the percentage of the rule clauses that must be present in the MS/MS spectra of an unknown for the substructure to be identified as being present. Since it is necessary to maintain 100% reliability for the structure generation phase of the analysis, the 80% match factor is used for this rule (with a corresponding recall of 38%).

Another rule for the "promazine" substructure was generated using the feature combination method. This new method calculates a uniqueness and correlation value for each combination of features produced by a permutation of a given number of starting features. The rule produced for the "promazine" substructure is:

IF	F1 and F2 and F5	[100,38]
OR	F3 and F6	[100,54]
OR	F2 and F6	[100,31]
OR	F4 and F6	[100,38]

THEN substructure "PROMAZINE" is present.

The numbers in brackets are the uniqueness and correlation values for the combination. This combination rule has a total recall of 69% and a reliability of 100% when applied against the reference database. A third rule was generated using more features. This rule contained 27 feature combinations and had **100% recall**. Thus, the use of feature combinations greatly enhances the recall and reliability of the MAPS rules.

MASSPEC: A GRAPHICS BASED DATA SYSTEM
FOR CORRELATING A MASS SPECTRUM WITH A PROPOSED STRUCTURE

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A graphics-based user friendly tool was developed to aid in the analysis of a mass spectrum when a proposed molecular or fragment ion structure is provided. The program correlates the observed ion masses with the fragment masses of the proposed structure. The program is written in VAX FORTRAN and ReGIS graphics.

INPUT DATA AND INPUT COMMANDS

Three inputs to the program are required: 1- Exact or nominal ion masses observed in the mass spectrum (NOLMAS), 2- The proposed structure described as a set of bonded superatoms, and, 3- A set of non-bonded superatoms to describe possible rearrangements, adduct ions and neutral/radical losses. (Superatoms are groups of atoms which do not readily fragment.)

The system allows the chemist to draw the proposed chemical structure on the terminal screen by positioning the superatom locations by use of a mouse or cursor keys. Bonded superatoms are entered by use of the ADD command which also requests a label and mass for the superatom. The BOND command draws bonds between the bonded superatoms of any desired bond order. Non-bonded superatoms are entered by use of the FREE command which also requests a label, mass and minimum and maximum numbers allowed for the non-bonded superatoms. Additional commands, HIGHLIGHT, REDRAW, CONNECT, LABEL, MOVE and ERASE, manipulate the drawn superatoms. The drawn superatoms can be SAVED and retrieved (GET) from the computer memory. The MACCS .MOL file structure is used for representing the chemical structures.

ALGORITHM

The masses of all possible combinations of bonded superatoms (MM1) and non-bonded superatoms (MM2) are computed. The method used to produce the combinations of connected superatoms is referred to as the "Bond Removal Method" [1]. The mass difference between the observed ion mass (NOLMAS) and masses MM1 is searched in the table of masses MM2. All successful searches are possible solutions. All possible solutions having bond order sums of the cleaved bonds (NBROKE) less than the desired upper limit (Kmax) and satisfying fragmentation rules [1] are the final substructure solutions. (See the accompanying MASSPEC Flow Diagram.) The EXECUTE command implements the above computations.

ANALYSIS OF FINAL SUBSTRUCTURE SOLUTIONS

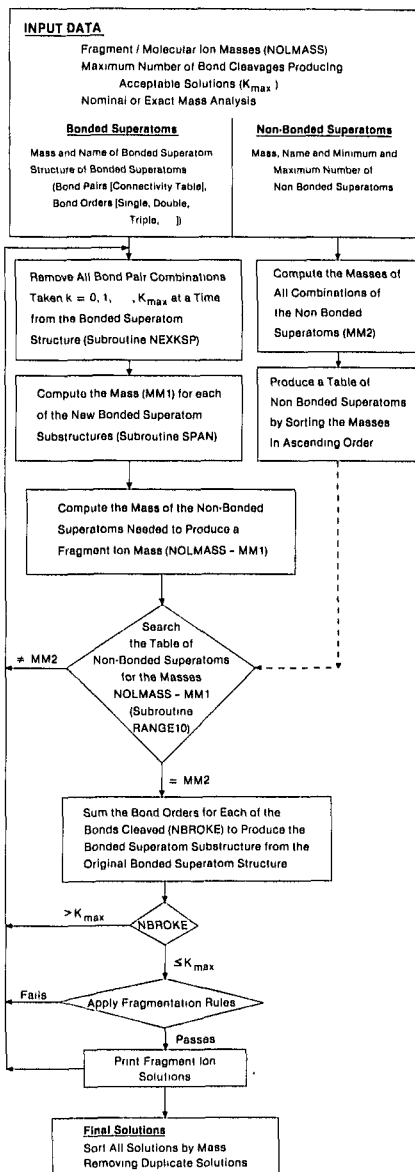
The final substructure solutions are accessed using the OUTPUT command. The accompanying Figure illustrates the typical graphics of the terminal screen for the final substructure solutions. The lower portion of the Figure illustrates the table of solutions consisting of the computed masses with corresponding bonded and non-bonded superatoms, bond cleavages and mass error for high resolution data. This table can be scrolled UP, DOWN and by MASS. A desired entry in the table can be displayed in the upper right portion of the screen. Superatoms present in the proposed fragment ion structure are enclosed in rectangles, those absent are not enclosed. The upper left portion of the screen contains the fragment ion mass and the sum of the bond orders of the cleaved bonds and information about the non-bonded superatoms for the proposed structure.

The full structure in the Figure corresponds to the m/z 211 fragment ion produced in the thermospray mass spectrum of the cephalosporin antibiotic cefixime [2]. The fragment ion masses were those observed in the TSP/MS/MS spectrum of the m/z 211 ion. The observed spectrum and its interpretation obtained using the MASSPEC program are illustrated.

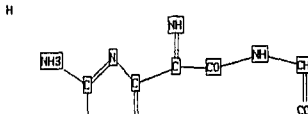
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[2] M.M. Siegel, Anal. Chem. 59, 989 (1987).

MASSPEC FLOW DIAGRAM



Mass 183.0900 # 2.00
Free RCOE Mass Number
1.0000 0

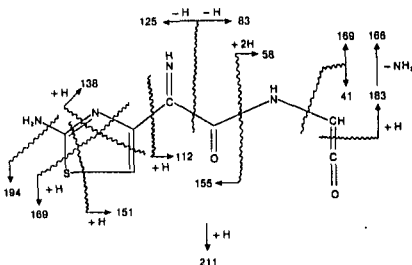
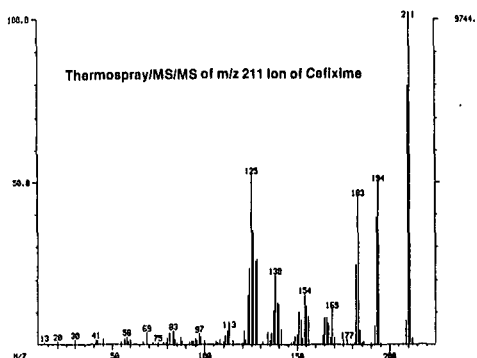


NAME CEFIXIME KETENE

Element FRAGMENT IDH

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NH3 C S CH C N C NH CO NH CO H
mass rate 17 12 32 13 14 12 15 28 15 13 28 1
183.0900 3.00 + + S CH C N C NH CO NH CO H
+ 183.0900 2.00 NH C S CH C N C NH CO NH CO H
183.0900 3.00 + + S CH C N C NH CO NH CO H
193.0900 1.00 + C S CH C N C NH CO NH CO H
UN UP, DN Down, R nearest mass, Ccr>display, S Save, C Clear, Q Quit, X Exit
  
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MACPROMASS - A COMPUTER AID FOR THE ANALYSIS OF MASS SPECTRAL DATA OF PEPTIDES AND PROTEINS

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A computer program called MacProMass has been written which is designed to assist in analyzing FAB mass spectral data of peptides and proteins. Written in QuickBasic, the program will run on any MacIntosh with 1 Mb of memory. Full advantage is taken of the MacIntosh user interface. All functions of the program are readily accessible through pull down menus, and many of the commands have keyboard equivalents. Protein sequence data entry can be done through the keyboard or reading a file from the hard disk. MacProMass is designed to read any text file; so sequences down loaded from a protein data base are readily accommodated. Once entered, sequences are easily edited using standard MacIntosh editing commands. The six letters of the alphabet not permanently assigned to the common amino acid residues can be programed to code for any residue selected from a list of unusual amino acids or amino acid derivatives. Similarly, lists exist for selecting N-terminal and C-terminal substituents. The user can make his own entries to any of these lists. Displayed on the main screen (Fig. 1) along with the sequence for all, or a selected portion of the sequence are: amino acid composition; elemental composition; average and monoisotopic mass values for the protonated molecular ion; Bull and Breese free energy values; and the Browne, Bennett and Solomon HPLC index.

In connection with the main display are a full complement of search routines including: searching for a given sequence, searching for and replacing specific amino acid residues, searching for a mass corresponding to a protonated molecular ion of a protein fragment, and a search from a list of protonated molecular ions.

The main feature of MacProMass is series of subroutines that calculate the expected fragments produced when a protein is chemically or enzymatically degraded. The program supports directly the enzymes trypsin, chymotrypsin, Staph. Aureous, and Asp-N in addition to cleavage with cyanogen bromide. A theoretical enzyme called Superzyme allows the user to program any endopeptidase activity. The program calculates values for each peptide fragment for the mass of the protonated molecular ion, the free energy, and the HPLC index. The list of peptide fragments can be sorted using any of these values. Additionally, a routine is included which will systematically consider all of the single amino acid substitutions which will

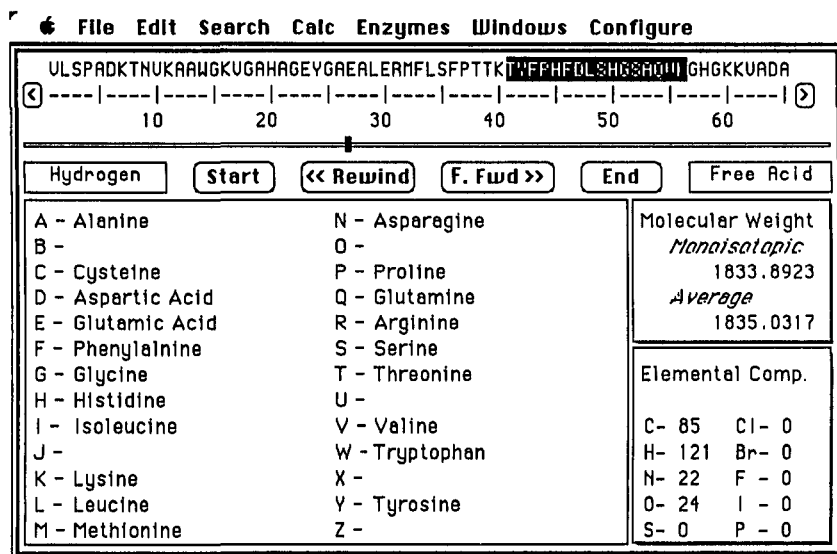
account for the mass difference between an observed mass in an enzyme digest mixture and those calculated based on the expected sequence. This is a very useful feature when searching for variants of a known protein.

Included in the program is the capacity to calculate predicted mass spectral fragments for a peptide or protein fragment. This facilitates the assignment of fragment ions in FAB mass spectra. Values in the table of fragments can be searched and marked to show correspondence with spectral data.

A subroutine has been included for plotting hydrophobicity values for sequence data. Presently, the program supports the scales of Hopp and Woods; Kyte and Doolittle; and Engleman, Steitz and Goldman. The hydrophobicity plots can be displayed on the screen, sent to either a Laserwriter or Imagewriter printer, or copied to the clipboard for export to other Macintosh applications.

Copies of MacProMass and an instruction manual are available from the authors.

Fig. 1



IMPROVED RETRIEVAL OF HIGH RESOLUTION UNKNOWN MASS SPECTRA
WITH EXACT MASS-PROBABILITY BASED MATCHING

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The Exact Mass-Probability Based Matching (EM-PBM) algorithm is now fully operational. This evolution of PBM uses elemental composition information contained in high resolution unknown mass spectra to aid in their retrieval and identification. During the EM-PBM matching process, unit resolution reference spectra from the Wiley/NBS Registry of Mass Spectral Data are compared to the unknown for possible matches. Against unit resolution unknown spectra, it has been shown that approximately half of incorrect retrievals have also incorrect chemical formulas,¹ and would be expected to benefit from knowledge of elemental composition.

PBM's probability base stems from the calculation of a confidence (K) value which reflects the probability that a peak matching in the unknown and reference spectrum occurs by chance. EM-PBM modifies the K value of a match to include the composition uniqueness (U_x value):

$$U_x = -\log_2(P_c)$$

where P_c is the probability of the composition occurring randomly in the reference library.

Because the Wiley/NBS library contains unit resolution electron ionization mass spectra, the probability of elemental compositions was calculated by a computer program that assigned compositions based on logical neutral losses from the molecular ion. The validity of this assignment process is shown by comparison of the results to the probabilities determined to mass 150 in Mass Spectral Correlations.²

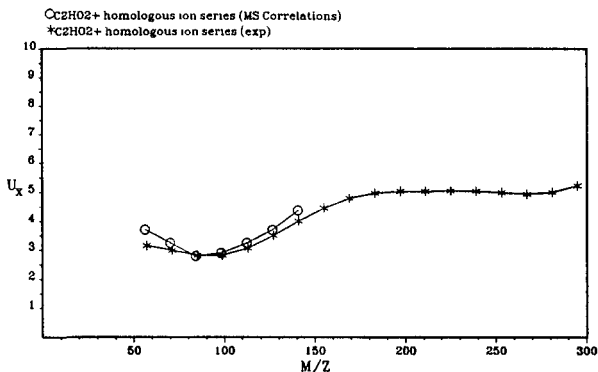


Figure 1. Computed and known uniqueness values.

EM-PBM uses a two part strategy to improve the estimation of peak comparisons between reference and unknown. First, peak matches are nullified if the peaks have different compositions. Since the reference spectra are unit resolution, this determination is made only on the reference molecular ion, and small neutral losses. Other unknown peaks however, must have compositions compatible with that of the reference molecule. Second, reference and unknown peaks with matching compositions have a K value augmented by a Ux value.

On a set of 385 unknown spectra selected from the Wiley/NBS reference library, EM-PBM gains an additional 14% in correctly retrieved spectra. Duplicate spectra are retrieved at an average predicted reliability increase of 10%. Figure 2 shows a change in reliability versus recall comparing EM-PBM with PBM. Recall is defined as the percentage of all correct reference spectra retrieved, and reliability is the percentage of retrieved spectra that are correct. The data were calculated from 90 values of from 99 to 10 percent predicted reliability. An improvement in retrieval performance is indicated by a shift toward higher reliability at a given recall value.

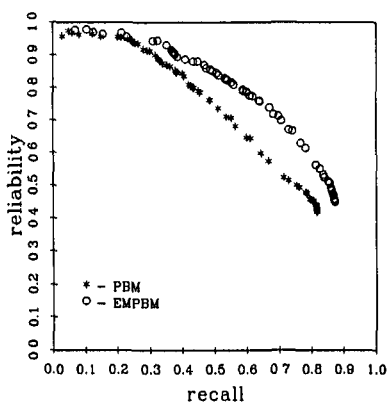


Figure 2. Reliability vs. Recall for PBM and EM-PBM

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QUALITATIVE EFFECTS OF ION ABUNDANCE TUNING CRITERIA
ON MODERN QUADRUPOLE MASS SPECTROMETER SYSTEMS,
RESULTS FROM AN INTERLABORATORY STUDY

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Work over the last few years (1, 2, 3, 4 and 5), and the evolution of instrumentation over the last 15 years (including data systems, mass spectral libraries, and software), have prompted a study to examine, in part, the qualitative effects of ion abundance tuning on modern quadrupole mass spectrometer (QMS) systems. We have reviewed computer ranking and matching information obtained from 7 different laboratories. Each laboratory acquired data under five different tune regimes over at least 5 concentration ranges for at least 82 semi volatile priority pollutants and pesticides using different QMS systems. In this paper we present selected results from this effort.

Review of the computer ranking and matching results (approximately 40,000 individual determinations) shows that exceeding the 1975 DFTPP criteria has little, if any, effect on the ability of modern QMS systems to identify targeted analytes. The primary 1975 assertion (that a QMS tuned to the 1975 DFTPP criteria "will, in general, generate mass spectra of organic compounds which are very similar to spectra generated by other types of mass spectrometers") implied that data outside of the published ion abundance boundary would not match library spectra. This study does not support this utilization of these criteria. To illustrate, we calculated the recognition rate for computer matching results for the complete study (see Table 1). In a number of instances, "out of tune" results were found to be numerically higher or equivalent to "in tune" results. The statistical significance of this observation is a moot point, as from a global perspective, most analytes were identified most of the time, almost irrespective of whether, for example, m/z 198, 442, or "low" m/z rearrangement ions were the base peak. While the specific results for each system need to be considered, it was, not surprisingly, observed that when ion current, as indicated by the electron impact mass spectrum of DFTPP was suppressed at high mass, certain analytes, such as the chlorinated pesticides, were not efficiently identified. Concurrently, when ion current was suppressed at low mass, significant non linearity was observed for many targeted analytes. Consequently, new criteria, if they are developed, need to consider the lower levels of ion abundance windows carefully from a qualitative analysis perspective.

However, because of the results of this study, we have been led to question at a fundamental level the utility of the DFTPP ion abundance criteria as an aid for the qualitative identification of both targeted and non targeted analytes, as there seems to be little correlation between compound qualitative identification and the ion abundance criteria tested in this study. This was noted except at tune extremes, where "low mass" or "high mass" sensitivity was greatly suppressed. This global observation is probably due to a number of factors, including: (1) the great difference in spectral characteristics of the targeted analytes, as compared to the ion abundance reference compound; (2) the evolution of library search routines (some of which utilize a library relative retention time weighting factor); (3) hardware evolution; and other factors. Whatever the driving force or forces, the primary observation of our work to date is that the old DFTPP ion abundance criteria have little meaning in regards to the ability of modern QMS systems to identify targeted analytes.

Table 1.
Semivolatile Analyte Recognition Rate
(N = 82 Analytes)

<u>Tune</u>	<u>Laboratory</u>						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
A, Traditional	96	98	95	97	95	98	96
B, 442 base peak	96	98	95	96	97	98	94
C, per B low m/z suppressed	96	97	94	97	96	98	93
D, 51 base peak	96	98	93	97	94	98	93
E, per D high m/z suppressed	96	98	88	97	92	98	88

References:

1. A. D. Sauter, et al., "Development of a Proposed National Reference Set of Electron Impact GC/MS Relative Response Factors for the U.S. Environmental Protection Agency's Contract Laboratory Program," Proceedings of the 36th ASMS Conference on Mass Spectrometry and Allied Topics, June 5-10, 1988, p. 1423-1424.
2. R. K. Mitchum, et al., "Ion Abundance Criteria for Gas Chromatographic/Mass Spectrometric Environmental Analysis," Anal. Chem. vol. 71, no. 2, p. 434-439 (1988).
3. Y. Tondeur, et al., "Ion Chemistry of a Gas Chromatographic/Mass Spectrometric Ion Abundance Calibrant," BEMS, p. 429-439, 1988.
4. J. Fisk, Proceedings from GC/MS Tuning Conference, Alexandria, Virginia, April, 1987.
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REPORTING ANALYTICAL RESULTS WITH PC SOFTWARE

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PROBLEM

Comprehensive reports of analytical studies are difficult to produce. The task may entail consolidating large amounts of information from many different types of instruments to produce a series of data tables. The problem is further complicated when graphical data from multiple instruments must be compared. Output from different data systems are usually combined by much trial and error graphics enlargement and reduction to produce a comparably scaled image. The manual operations involved are both time-consuming and error-prone.

APPROACH

The problems may be minimized by consolidating the raw data files from multiple instruments into a single data format and creating standard intermediate files for use in personal computer software. In this study, we used the Hewlett-Packard RTE Mass Spectrometer Data System as the point of central storage and data processing.

The RTE data system can accept raw data files from a number of sources. GC/MS and LC/MS data files can be transferred from both the HP Pascal and the Unix ChemStation series of data systems. Gas and liquid chromatographs attached to the HP Laboratory Automation Systems and the HP 3392 integrator may also be used.

Once the files are on the MS computer, the user can convert it for use with the applications software to display, print, or plot the data. The Aquarius target compound software can also be used to quantitate GC and LC data as easily as standard GC/MS data.

The standard software was used to produce a number of different standard output files. Some of these files are used directly (optical character recognition and barcode), while others are intermediate files for PC applications. We have produced simple ASCII files, tagged files, field delimited files, character delimited files, reduced results files, and HPGL files. In all of these cases, the files may be produced automatically through a single command line entered manually or as part of a sequence.

RESULTS

We will show representative reports created with various common personal computer software from intermediate RTE files. These applications include word processors, spreadsheets, desktop publishing software, and others. By directly creating intermediate files on the RTE data system for these applications, the likelihood of transcription error is reduced and the speed of report production is increased. In all cases, the intermediate files are accepted directly without additional formatting.

CONCLUSIONS

GC, LC, and MS raw data can be consolidated on the RTE MS Data System for processing.

Standard intermediate data files can be created automatically via procedure files.

These intermediate files can be used directly in PC applications, without additional operations.

Data Transcription from Hewlett Packard GCMS Workstation
to IBM Personal Computer (or compatible)
Gardner, J.M., Quick, A.N. and Fennessey, P.V., University of Colorado
Health Sciences Center, Mass Spectrometry Facility, 4200 E. 9th Ave.,
C232, Denver, Colorado 80262

Data manipulation and transfer can become time consuming and labor intensive in a laboratory where a large volume of samples are processed daily. Our laboratory has two Hewlett Packard Mass Selective Detectors, each fitted with an auto injector, which combine to process approximately 24,000 samples annually. The lack of commercially available software requires that the data be manually transcribed if personal computer based spreadsheets and databases are utilized. To process data on the H.P. workstations (models 216 and 300) all data acquisition must stop while data is being processed. The data storage space of the H.P. workstation (20 megabytes on the model 216) limits the operation of the mass selective detector to one or two days of automated data acquisition. This limited space impedes long term storage and archiving of data files. Our inability to electronically transfer and store data prohibited the rapid processing, analysis and reporting of these large volumes of data.

We initially tested commercially available software from a number of different suppliers. The programs were found to be completely unsatisfactory. They were found to be user unfriendly and destructive to our operating system. As a result, we have designed, built and tested an interface package called 'Siphon' using "off the shelf" components.

Siphon software transfers, stores and archives H.P. data files. It is a user-friendly, menu-driven and extensively tested program that ensures that the data will be transferred rapidly and processed accurately. Once the data has been transferred to the PC, your chemstation is free to acquire more data. Siphon provides an exact copy of the original data file containing: sample name, bottle number, retention time, peak areas, peak heights, library matches, bar code, time, and date. This file can be stored on hard disk, floppy disk, hard cartridge, or magnetic tape. This allows easy access of the file information and the archiving of data files. Siphon's parsing option allows the user to extract the pertinent information from the original data file, which can reduce the file size up to 90%. This type of processing can be customized for each user. Siphon generates two file formats: a quick reference file and spreadsheet importable file. The quick reference file is used for checking the data for anomalies and precision. The spreadsheet import file is readable by Lotus, Supercalc, databases and other programs that import using the same file formats.

The program is controlled on the H.P. side by a series of soft keys and on the PC side by a series of menus. The Siphon transfer softkeys are inserted in the H.P. software and can be stored for future use. This softkey execution of the transfer reduces the problems associated with add-on software because the program becomes an integrated part of the H.P. software. Siphon is user-friendly, in that, the menu's instructions are clearly described and easily executed. Siphon resides on the PC's hard disk saving disk space on the H.P. and allowing for easy access of the program.

Siphon's hardware and software requirements:

- H.P. 200 or 300 series workstation
- IBM or IBM compatible personal computer
- 232 Siphon custom cable
- Siphon Transfer Software
- Tablers - H.P. Freeware program

Siphon requires H.P. software's version to be from 1.1 to 3.1, software version 3.2 will not work until H.P. provides a new version of Tablers. The model 216 requires a custom cable because of the centronix pin configuration and will not work with a standard null-modem cable.

This high performance, user friendly, fully automated program enables the efficient processing of vast amounts of data. Our laboratory has benefited by the use of Siphon in that our transcriptions errors have been significantly reduced, while increasing our productivity.

MASS SPECTROMETRY DATA CENTRE

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The Mass Spectrometry Data Centre (MSDC), Royal Society of Chemistry (RSC), UK offers a unique information service to the mass spectrometric community. At present this service is 3-fold: production of the *Mass Spectrometry Bulletin* (MSB), a monthly current awareness journal; production of the *Eight Peak Index of Mass Spectra*, the largest printed index of mass spectral data in the world; collection and validation of mass spectral data for input to the NIST/EPA/MSDC Mass Spectral Database. For the MSDC to continue to make its important contribution to mass spectrometry, it is imperative for existing or potential users to contribute their own ideas and suggestions about the services. To do this, however, users must first be aware of the MSDC's current activities.

The MSDC was established in 1966 at the Atomic Weapons Research Establishment, Aldermaston and was transferred to the Chemical Society (now the RSC) in 1978. The Centre employs 4 professional and 3 clerical staff and is supported by other departments of the RSC. The technical activities of the Centre are overseen by a Technical Advisory Committee composed of renowned mass spectrometrists representing industrial, academic and government interests.

The majority of staff time and effort is spent producing the *Mass Spectrometry Bulletin*. Coverage of the literature to find papers for inclusion in the MSB is comprehensive: more than 900 primary journals are covered, along with relevant conference proceedings, books, technical reports and patents. Coverage of the subject matter is comprehensive too: instrument design and techniques, isotopic analysis, chemical analysis, organic chemistry, atomic and molecular processes, surface phenomena and solid state studies, thermodynamics and reaction kinetics. From each selected article the title, authors and publication details are abstracted. In addition, each article is analysed with respect to mass spectrometric content, and index terms and a mass spectrometric content/data indicator are assigned. The abstracted information is checked thoroughly for accuracy and consistency by means of an extensive proofreading and cross-checking system, thus ensuring a high quality product. Most articles appear in the MSB within two months of receipt of the original document at the MSDC. With an annual input of approximately 10,000 items, the MSB contains more than 3 times as many references as CA Selects: Mass Spectrometry. This, along with its unique series of indexes (subject, compounds classification, elements, general, author), makes the MSB a most important method by which mass spectrometrists can keep up-to-date with the current literature. The MSB is available in printed and PC versions and online via ESA-IRS.

The *Eight Peak Index of Mass Spectra* is an excellent aid for identifying unknown compounds. It is also a convenient single point of entry to the various full mass spectral collections and to mass spectral data appearing in the scientific literature. The spectra, collected from a variety of sources, are put through an extensive checking process to ensure the high quality and consistency of the data. Individual spectra are checked and corrected; they are then compared with one another and selected (or not) for inclusion in the Index. Of an original 100,000 spectra, 66,720 spectra, covering 52,332 compounds, were published in the 3rd edition. Data in the Index are sorted in three different ways for ease of use:

- Volume 1: by molecular weight and formula
- Volume 2: by molecular weight and m/z
- Volume 3: by m/z of most abundant ion

The third aspect of the MSDC's activities is the collection and validation of mass spectral data: the MSDC collects and prepares data for inclusion in the NIST/EPA/MSDC Mass Spectral Database. A variety of checks, including checks on chemical name and formula, molecular weight and the mass spectral data, are carried out by the MSDC. The data are then sent to the original contributor for further verification. In 1988 the MSDC expanded its activities in this area by joining the National Institute of Standards and Technology in the marketing and distribution of the PC Version of the NIST/EPA/MSDC Mass Spectral Database.

The MSDC provides an information service for you, the mass spectrometrists. It is from your comments and suggestions that we hope to provide you with an even better service with new products and enhancements to existing services.

QUANTITATION IN DATA EDITOR USING HP 3.2 CHEMSTATION SOFTWARE

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The ability to read/write user defined files in the 3.2 Revision of HP ChemStation Software allows the user to carry out quantitative analysis within the Data Editor program. This ability saves significant time in the processing of data and allows a completely customized output from the system. Previous revisions of ChemStation software required the quantitation be accomplished in the Report program or be manually calculated. The addition of this new capability saves time by not having to load the Report program with each sample which must be quantitated. This loading process typically takes 15 seconds to accomplish. This is significant in a process that takes a total of just over one minute, thus giving a time savings of approximately 25%.

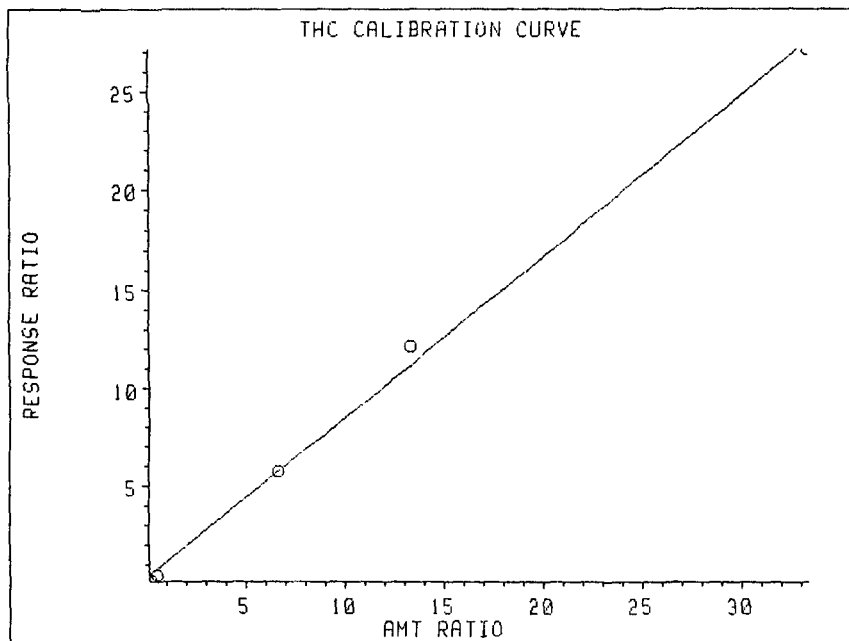
Both single and multipoint calibration curves may be generated. Linear, log, power, exponent and second order curve fits can be applied to multipoint calibration curves. Preparation of a single point calibration curve is the simplest procedure. The area (or height) of the internal standard and the area (or height) of the compound in the calibration standard is written to a user defined file during the data analysis of the standard. During the data analysis of an unknown sample, the user defined file is read to allow comparison of the unknown to the calibration standard. The equation used is as follows:

$$[Dy] = \frac{\text{Area } Dy}{\text{Area } Ds} \times \frac{\text{Area } ISs}{\text{Area } ISy} \times [Ds]$$

Multipoint calibration requires the storing of area (or height) data from a number of calibration standards. This can be accomplished in several ways, one of which is using the file append option for a user created file if the data are written after each standard is injected. Another option is to inject all of the standards followed by the data analysis of each, one after the other, writing the data from each to the file then closing it. Once all data are stored in this file, the calibration curve parameters are calculated using the CREATE function to generate the dataset (user object). The CURVEFIT command can then be used to compute a number of statistical functions. These include the slope and intercept as well as the correlation coefficient for the fit of the data to the line. This results in a calibration curve which can then be used to quantitate unknown samples. The number of calibration points used in the curve is virtually unlimited. The data may also be appended to develop an average of data over a number of calibration curves.

The capability also exists to plot the calibration curve as is seen in the detailed internal standard report. This is easily accomplished by defining the title, X and Y axis labels as a part of the CREATE function. Once defined, the curve can be drawn to any window or redrawn to a plotter. An example of a five point linear calibration curve created in the Data Editor is shown in Figure 1. The STATPARM function can be used to return the slope and intercept. These two values can then be stored in a data file and used with unknown samples using the equation for a straight line. The fit of the data points can be demonstrated by computing the correlation coefficient. This value is returned with the CORRCOEFF function after the CURVEFIT function is executed.

Time can be saved in the analysis of every sample due to the simplicity of the quantitation within the Data Editor. More advantageous is the ability of the quantitation report to look exactly like the user wants rather than settling for the standard format provided by the vendor.



$$\text{Response Ratio} = 8.15332\text{E-}001 (\text{Amt Ratio}) + 3.39847\text{E-}001$$
$$r^2 = 9.98767\text{E-}001$$

FIGURE 1

Reference:
HP 59970 MS ChemStation Commands, Hewlett Packard Publication No. 59970-90035, (1988).

REAL-TIME REACTION MONITORING BY CONTINUOUS INTRODUCTION ION SPRAY TANDEM MASS SPECTROMETRY

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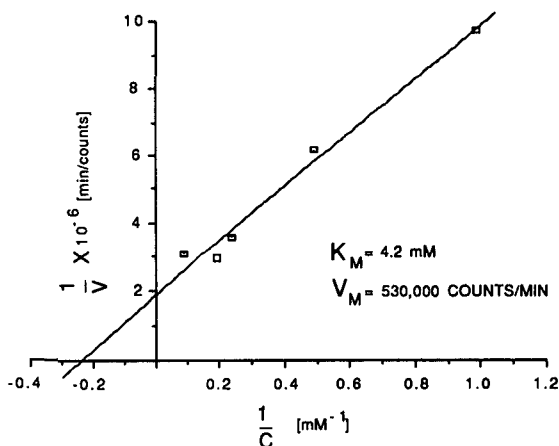
The study of reaction mechanisms and kinetics is fundamentally important for characterizing synthetic pathways of chemical reactions and for understanding biochemical processes occurring in life sciences. Nearly all known analytical techniques have been used to follow reaction mechanisms and kinetics. The objectives and challenges imposed by the complexity and speed of the reaction under investigation require instrumentation possessing the necessary selectivity and sensitivity to follow the reaction to completion.

The high degree of specificity of mass spectrometry for the identification of molecular species in mixtures, and its excellent sensitivity are attractive for studying reaction mechanisms and kinetics. Mass spectrometry and in particular Fourier transform mass spectrometry have been the most powerful techniques for monitoring reaction mechanisms of gas phase chemistry. The assumption that prior vaporization is a prerequisite of ionization prevented mass spectrometry from playing an important role for the real-time investigation of liquid phase reactions. It has not been until the advent of soft ionization techniques that allows direct sampling of liquids such as FAB, Thermospray, and electrospray that the mass spectrometer has been able to monitor reactions occurring in solution.

For these reasons we have developed a continuous introduction/ion spray inlet with mass spectrometry for real-time monitoring of reactions in solution. The purpose of the study was not to show highly sophisticated reaction studies, but to introduce electrospray ionization at atmospheric pressure as a tool for the continuous monitoring of condensed phase reactions by mass spectrometry. The potential of this new analytical approach was demonstrated using simple, well understood model systems.

A temperature-controlled reaction vessel was closely coupled to the ion spray LC/MS interface on a atmospheric pressure ionization mass spectrometer and used to study mechanistic and kinetic aspects of reactions taking place in solution. Free access to the reaction vessel and temperature control as well as minimal sample consumption make continuous introduction ion spray mass spectrometry a useful approach for real-time measurement of reactions. The reaction medium, reactants, and products are

transferred to the ion source of the mass spectrometer in less than a second. The half-life for the solvolysis of methandrostenolone sulfate to epimethandrostenolone in aqueous medium was determined to be 4.5 min. The Michaelis-Menten constant was determined to be 4.2 mM for the enzymatic hydrolysis of O-nitrophenyl- β -galactopyranoside by lactase to galatose and O-nitrophenylate. The enzymatic hydrolysis of dynorphin 1-8 by chymotrypsin and aminopeptidase and the reduction of the disulfide bridge in oxytocin with β -mercaptoethanol were observed on-line to emphasize the technique's capability for peptide sequencing and structural elucidation.



Lineweaver-Burke plot for the enzymatic hydrolysis of O-nitrophenyl- β -galactopyranoside by lactase to galactose and O-nitrophenylate. Conditions: 1, 2, 4, 5, and 10 mM solutions of OPNG prepared in 5-mM ammonium acetate; 2 drops of lactase was diluted with 20-mL of water after which a 100 μL aliquot was added to a 1-mL volume of the OPNG solution in the reaction vessel.

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DETERMINATION OF CHARGE STATE OF IONS IN THERMOSPRAY MASS SPECTROMETRY

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Palo Alto, California 94304

Protonated molecular species $(M+H)^+$, multiply charged ions $(M+nH)^{n+}$, and cluster ions $(nM+H)^+$ are often observed in mass spectra obtained by thermospray ionization (TSP). To obtain unambiguous molecular weight information, it is necessary to properly assign the number of charges that are present on an ion. Differentiation of multiply charged species in spectra obtained on single stage quadrupole instruments is not straightforward (1-3), especially when data are obtained and displayed in centroid mode. Therefore, we have developed a simple procedure for identifying the charge state of ions by analysis of the peak profile of their isotopic clusters. Experimental results and theoretical calculations are presented to verify this procedure. The applicability of this technique is illustrated by the characterization of a peptide of MW 5090 daltons using TSP-MS on a quadrupole instrument.

TSP mass spectra were obtained on a Finnigan TSQ-70 mass spectrometer. Typical operating conditions were: jet, 210-240 °C; vaporizer, 92-95 °C; repeller, 75-135 V; flow rate 1.2 ml/min. The mobile phase for loop injections was 30% acetonitrile in water with 0.1% trifluoroacetic acid. Centroid spectra were obtained by scanning the mass range of interest in two seconds. Profile data were obtained by scanning a twenty m/z units window centered around the ions of interest; scan time was 0.2 second.

Figures 1 and 2 show the full scan, centroided TSP mass spectra of two peptides, Met-Arg-Phe-Ala (MRFA, MW 523.3 daltons) and Syntex compound RS-26306 ($C_{40}H_{113}N_{14}O_{12}Cl$, MW 1568.8 daltons), respectively. If the most intense ions detected in the two spectra were singly charged $(M+H)^+$, the MW of MRFA and RS-26306 would be interpreted as 523 and 785, respectively. Alternatively, if those ions were doubly charged species $(M+2H)^{2+}$, the MW of the two peptide would be assigned as 1046 and 1568. Thus, to unequivocally determine the MW of these two peptides, unambiguous assignment of the charge state of the ions detected is necessary.

By monitoring the profiles of the ions detected, we observed that isotopic ions of a multiply charged species, which would appear at fractional m/z values, are not resolved when the analyzer is operated at unit mass resolution. This observation allowed us to determine the charge state of the ions present in the spectra shown in Figures 1 and 2. Figures 3 and 4 show the profiles of the most intense ions in Figures 1 and 2, respectively. Figure 3 clearly shows that all the isotopic ions are resolved; therefore, the ion of m/z 524 is the $(M+H)^+$ of MRFA. Figure 4 shows the profile of the isotopic cluster of the ion at m/z 785.5 in Figure 2. The various isotopic ions are not resolved. Experimental conditions were such that unit mass resolution was achieved in this mass region, and was verified by obtaining isotope profile data on singly charged ions in this m/z region. Therefore, the ion at m/z 785.5 can be assigned as $(M+2H)^{2+}$ and the MW of RS-26306 is determined to be 1569 daltons.

To confirm that the profile observed in Figure 4 is due to a doubly charged species, we modified the computer program "THEOMS" (4) to generate theoretical isotopic profiles of multiply charged species from a given elemental composition and instrument resolution. The simple modification involves the use of m/z values of the elements in place of isotopic masses for calculations. Figure 5 shows the theoretical profile of the $(M+2H)^{2+}$ ions of RS-26306 at a resolution of 750. The widths at half height of the experimental and theoretical profiles are both 1.9 m/z units. Theoretical calculation, therefore, confirms the assignment of the profile shown in Figure 4 as a doubly charged ion cluster.

As we extended these calculations, we observed that the width at half height of a multiply charged ion cluster decreases as the number of charges increases. This observation has facilitated the identification of doubly and triply charged ions of a peptide whose MW (5090 daltons) is above the mass range of the instrument used (m/z 10-4000).

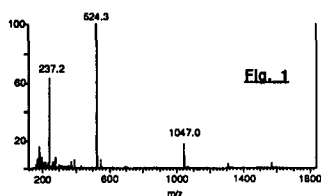
Figure 6 shows the centroid TSP mass spectrum of a peptide consisting of amino acid residues 1 to 44 of bovine growth hormone releasing factor (bGRF). The average MW of the peptide is 5089.9 daltons. The ions at m/z 2546 and 1698 can be assigned as the $(M+2H)^{2+}$ and $(M+3H)^{3+}$ of bGRF, respectively. However, from the data shown in Figure 6, one could not be sure that these ions were generated by bGRF or by extraneous species in the sample. The profiles of these two ion clusters are shown in Figures 7a and 7b. The width at half height of the doubly and triply charged ion clusters are 2.7 and 2.1 m/z units, respectively. The relative width of the triply charged species with respect to the doubly charged ions is therefore 0.78. For comparison, the theoretical profiles of the two ions are shown in Figures 8a and 8b. The theoretical widths at half height for the $(M+2H)^{2+}$ and $(M+3H)^{3+}$ are 2.2 and 1.7 m/z units, respectively. These profiles were generated using a resolution of 1700 for the triply charged ions and a resolution of 2500 for the doubly charged ions. The resolutions chosen were the ideal conditions where the quadrupole instrument would have unit mass resolution at the m/z region where the two ions were detected. The experimental resolutions were lower, however, which resulted in larger widths at half height of the ions detected. Nevertheless, the width of the triply charged ions relative to the doubly charged cluster, measured from the calculated profiles, is 0.76. The relative widths, observed experimentally and calculated theoretically, differ by less than 3%. These data support the identification of the two ions and therefore confirm the MW of the peptide as 5090 daltons.

In conclusion, we propose the use of this "Isotope Profile Monitoring" technique to acquire data in conjunction with ionization techniques where multiply charged species can be generated. In addition to the ability to determine the charge state of ions detected, this technique (similar to selected ion monitoring) provides improved signal-to-noise ratio over conventional full scan data acquisition, since more time is spent monitoring the ions of interest and signal averaging is readily carried out.

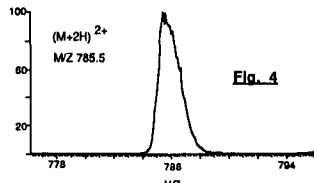
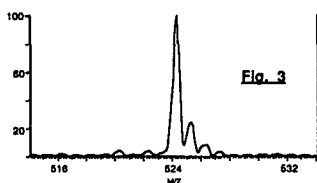
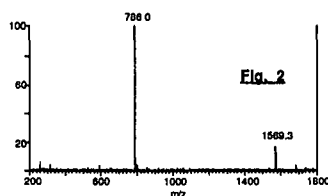
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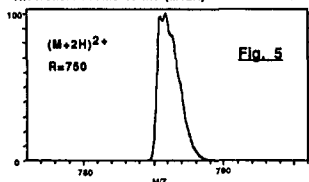
MOLECULAR WEIGHT = 523 or 1046 ?



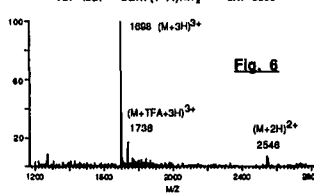
MOLECULAR WEIGHT = 785 OR 1568 ?



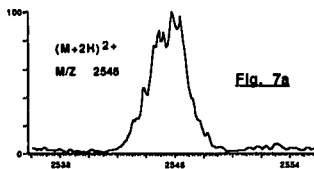
Theoretical Profile of the $(M+2H)^{2+}$ of RS-26306



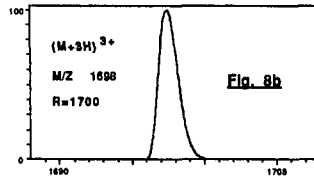
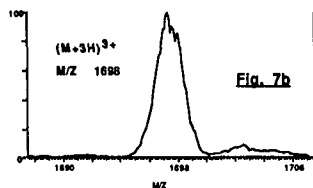
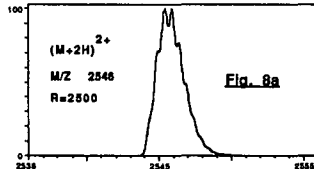
TBP MS: bQRF(1-44)NH₂ MW 5090



Experimental Profiles of the
Molecular Ions of bQRF



Theoretical Profiles of the Protonated
Molecular Ions of bQRF



**Thermospray LC/MS With a Quadrupole Ion Trap and
Recent Applications of Ion Injection**

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As both a reactor for gas phase ion/molecule reactions and as a sensitive mass analyzer, the quadrupole ion trap mass spectrometer has proven to be a versatile tool for the qualitative and quantitative analysis of volatile organic compounds. The sensitivity of ion traps and the ability to efficiently dissociate ions in tandem experiments, has increased the value of these instruments. Recent developments in ion trap technology have allowed injection of externally formed ions into an ion trap for reaction and mass analysis. (1) Ions generated via electron-impact ionization or desorption ionization have been successfully trapped.

Cs⁺ desorption ionization offers a versatile method for the analysis of biological compounds. Figure 1 illustrates the instrumentation. A Cs⁺ ion gun is used to ionize either solid samples or samples dissolved in a liquid matrix. The pulsed extractor lens allows Cs⁺ projectile ions to desorb sample from a probe as a pulsed ion beam. Figure 2 shows a spectrum of 200 pmol of a peptide (in 1:1 thioglycerol:glycerol) with the sequence: Lys-Pro-Ala-Glu-Phe-(4-NO₂)Phe-Arg-Leu. Both fragment ions and matrix ions are observed in the spectrum obtained by using axial modulation. (2) An alternative method of ion injection is illustrated in Figure 3. Ions of a selected mass are introduced into the hybrid instrument of a BET (BE/ion Trap) configuration. (3) Figure 4 illustrates the FAB mass spectrum of leucine-enkephalin injected with 50 eV energy into the ion trap. Lower injection energies yield less fragmentation of the peptide.

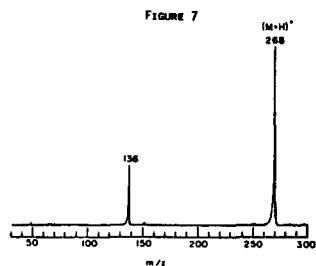
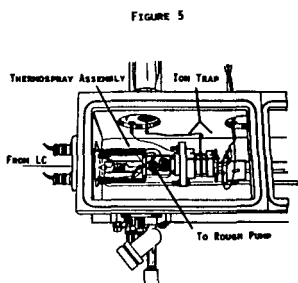
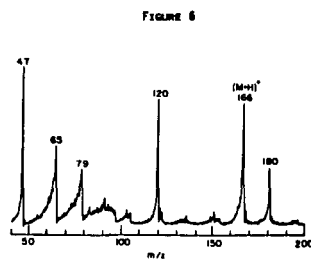
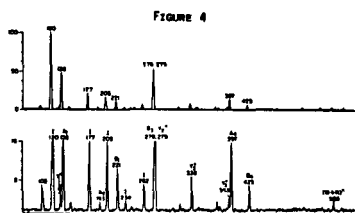
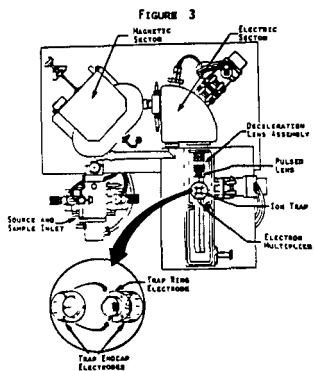
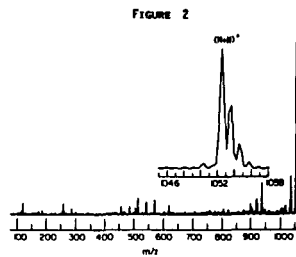
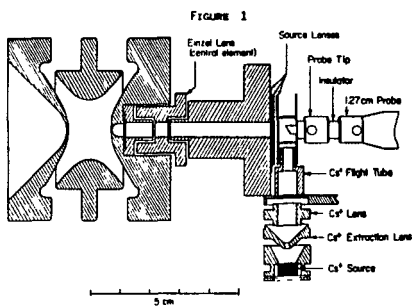
Yet another application of ion injection into an ion trap is the interfacing of a thermospray (TSP) source. The weaknesses of TSP, such as relatively poor sensitivity for some compounds, poor quantitative capabilities and a strong dependence on experimental parameters should be alleviated by accumulating ions in a trap over a period of time. The instrument, shown in figure 5, utilizes a large vacuum cradle to allow alignment of the trap with a Finnigan TSP source. Two turbomolecular pumps, 550 L/sec and 170 L/sec, are used to keep an operating pressure of 2×10^{-4} torr at an effluent rate of 0.5 ml/min. The vaporized effluent is differentially pumped using a cold trap and a roughing pump. The ions are generated by discharge ionization and are injected into the ion trap using a gated lensing system. Figure 6 illustrates a TSP mass spectrum of 100 ppm phenylalanine dissolved in 80:20 methanol:water. Solvent cluster ions are observed at masses below 100 Da. An I/M product, methylated phenylalanine, is observed at m/z 180; however, at lower injection times, the relative abundance of the I/M product is decreased. Figure 7 is a second example of a TSP generated mass spectrum. Adenosine, again dissolved in 80:20 methanol:water, shows only slight fragmentation.

One problem with ion traps is the limited number of ions which can be trapped without loss in sensitivity and resolution. As observed in Figures 6 and 7, injection of solvent ions causes "space charging" of the trap, thereby decreasing the sensitivity of the system. Methods to eliminate space charging due to the solvent are required. Resonant ejection of solvent ions is possible by applying a supplementary AC voltage to the endcap electrodes during injection in resonance with the solvent ions.

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(2) R.E. Kaiser, Jr., J.N. Louris, J.W. Amy, and R.G. Cooks, Rapid Commun. in Mass Spect., in press.

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MASS SPECTROMETRIC INSIGHTS INTO BIOLOGICAL REDOX REACTIONS

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The on-line combination of enzymes and electrochemistry can provide valuable insight into the metabolic and degradation reactions. The sensitivity of mass spectrometry and the time resolution of an on-line system allows the detection of intermediates and products formed during enzymatic and electrochemical (EC) redox reactions. Since metabolism and degradation processes often involve redox reactions, electrochemical methods can provide insight into these processes. The electrochemical and enzymatic oxidation pathways of a model compound, uric acid, were determined by on-line thermospray/tandem mass spectrometry (TSP/MS/MS). In addition, the electrochemical oxidation pathway of 6-thiopurine, a potent antineoplastic agent, was also investigated.

The products and intermediates formed as a result of electrooxidation of uric acid were monitored as the electrode potential was varied. As can be seen in Figure 1, electrooxidation of uric acid generates a highly reactive diimine which can undergo a hydration reaction to form the imine alcohol (MW 184) or an amination reaction to form the imine amine (MW 183). Tandem mass spectrometry was used to characterize these intermediates and provides convincing evidence that the primary intermediate produced during both the enzymatic and electrochemical oxidation of uric acid has a quinonoid diimine structure.

By coupling an enzyme column on-line with TSP/MS, products and intermediates formed as a result of the enzymatic oxidation of can be monitored as the enzyme-substrate reaction time is varied. In the enzymatic oxidation of uric acid with peroxidase and H_2O_2 , the same intermediates and products were observed as in the electrochemical oxidation. The TSP/MS information obtained using an on-line high pressure enzyme reactor indicates that the electrochemical and horseradish peroxidase catalyzed oxidations of uric acid proceed by similar, if not identical, chemical mechanisms. Figure 2 illustrates the negative ion mass spectra of the electrochemical and enzymatic oxidation intermediates and products obtained when the uric acid substrate residence time in the reactor is ca. 1 min. Comparison of the mass spectra obtained from the peroxidase-catalyzed and the electrochemical oxidation of uric acid shows that the intermediates and products formed in the enzymatic oxidation are the same as those formed in the electrochemical oxidation. Most importantly, the results show that the on-line combination of electrochemistry with thermospray/tandem mass spectrometry provides otherwise difficult to obtain information about redox and associated chemical reactions of biological molecules such as the structure of reaction intermediates and products, as well as providing insight into reaction pathways.

The compound 6-thiopurine (6-TP) is known to be extensively metabolized along several routes such as the transformation to its nucleotide and nucleoside. However, Hyslop and Jardine have recently described a previously unknown oxidation pathway of 6-TP which involves activation by cytochrome P-450 with subsequent binding to protein via mixed disulfide bonds (Figure 3).

Using EC/LC on-line with UV-VIS spectrophotometry and mass spectrometry, we have determined that at potentials $> +0.50$ V vs Pd the thiol group of 6-TP can be oxidized to purine-6-sulfinic acid (RSO_2H), purine-6-sulfinamide (RSO_2NH_2), and purine-6-sulfonic acid (RSO_3H). Figure 4 illustrates the on-line EC/LC chromatogram of the oxidation products of 6-TP at $+0.90$ V with UV-VIS detection. Since solution-phase reactions are responsible for producing both RSO_2NH_2 and ROH (hypoxanthine) which form only during electrooxidation of 6-TP at potentials $> +0.50$ V, these products must be the result of either hydrolysis or amination reactions with a very reactive intermediate such as a sulfinic acid ($RSOH$). Since RSO_2H has been identified, it is reasonable to assume that once $RSOH$ is formed via a direct two electron transfer reaction, it could be easily oxidized at these potentials to RSO_2H . Although this reaction is a net four-electron oxidation process, only a direct two-electron transfer mechanism is required to form the primary electrochemical intermediate, $RSOH$ (Figure 5). The fact that disulfide production still occurs at potentials $> +0.50$ V supports this theory of a competing two-electron reaction mechanism at higher potentials.

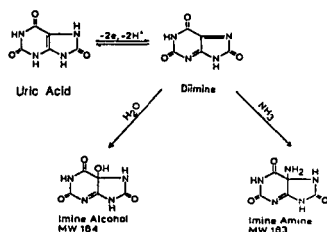


Figure 1.

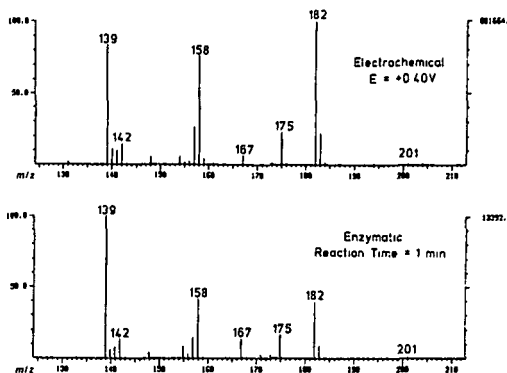
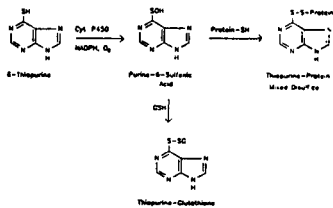


Figure 2.



Hyslop, R.H., Jordan J. J. Pharmacol. Exp. Ther. 1981 225, 421

Figure 3.

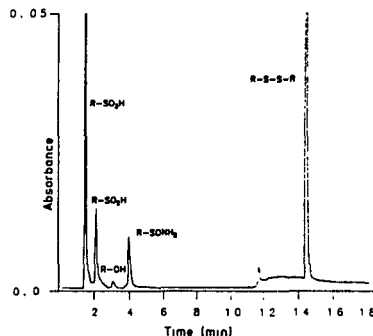


Figure 4.

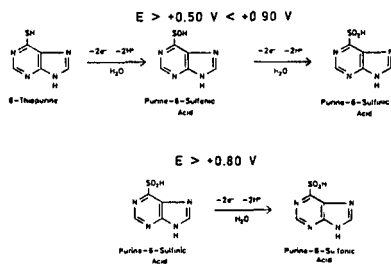


Figure 5.

MASS-FLOW ENHANCEMENT APPROACHES IN LC/MS

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The coupling of liquid chromatography and mass spectrometry (LC/MS) has been effectuated by various interface approaches and has proven to be a powerful tool in the analytical laboratory.

The characteristics of the various interfaces are quite different with respect to the possibility of applying conventional HPLC systems. However, since the mass spectrometer is a mass-flow sensitive detector all approaches can benefit from mass-flow enhancement techniques. The general formula for the mass-flow is given by :

$$dm/dt = C(t) F$$

where $C(t)$ is the concentration profile of the analyte after the chromatography, and F is the flow-rate of the mobile phase.

In principal, two approaches are possible in mass-flow enhancement, i.e. concentration enhancement by peak compression (A) and flow-rate programming (B).

A. Peak compression by Phase-System Switching.

"Phase-System Switching" (PSS) is based on a simplified column switching system allowing trapping, concentration and flow-rate adaption on the second column. Besides the compatibility improvement of LC/MS by PSS it was found that peak compression can be obtained. These effects were observed in (a.o.) the analysis of metoprolol enantiomers in plasma using Thermospray and for dextromethorphan detection with continuous-flow FAB.

In the case of metoprolol the chromatographic separation was optimized using an al-acid-glycoprotein chiral stationary phase and a 20 mmol/l phosphate buffer, while after trapping and peak compression 20% methanol in 50 mmol/l ammonium acetate was used for the thermospray-MS/MS experiments. For each enantiomer a detection limit of 37 pmol could easily be obtained.

B. Mass-flow programming.

An other approach in mass-flow programming can be realized by increasing the mass flow after chromatography. This can be achieved in various ways, of which belt speed programming using a moving belt interface is an example. Another approach, applicable with miniaturized chromatographic columns is the following: The system comprises of a trapping loop after the miniaturized chromatographic

system operated at flow-rates of about 10 $\mu\text{l}/\text{min}$. After trapping the peak(s) of interest in the loop the introduction of the analyte(s) is performed by flushing the loop with a flow rate of 1.5 ml/min into a Thermospray source. The experimental setup and the results for the betablocker metoprolol are given in Figure 1.

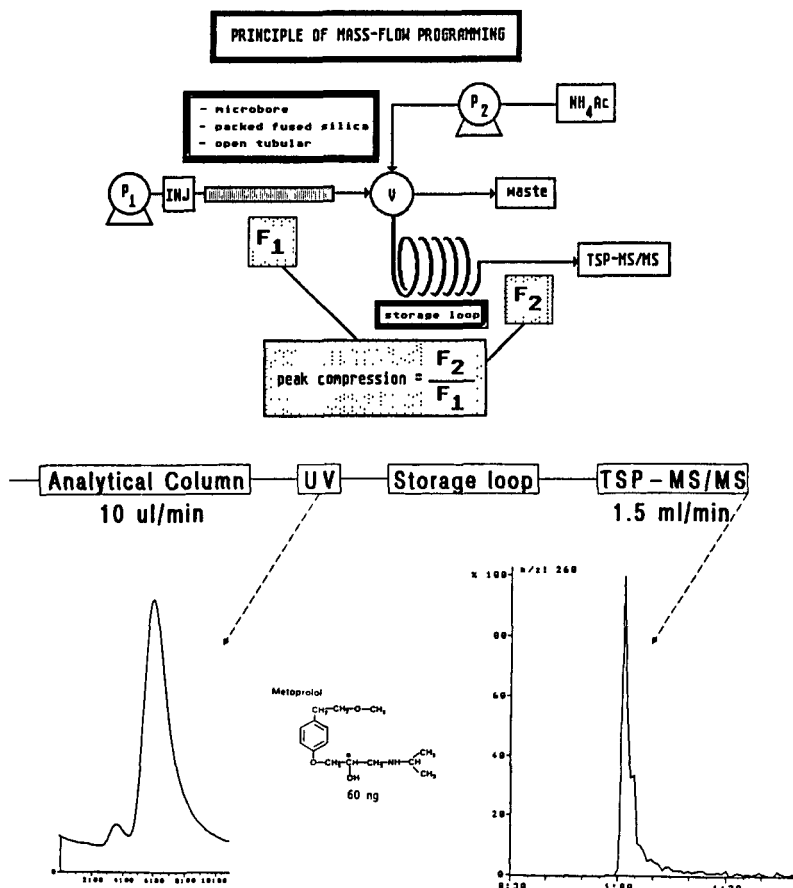


Figure 1. Experimental setup for mass-flow programming

MASS SPECTROMETRIC DETECTION FOR HIGH PERFORMANCE ALKALINE ANION EXCHANGE LIQUID CHROMATOGRAPHY OF CARBOHYDRATES

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High performance alkaline anion exchange liquid chromatography (HPAELC) provides efficient separation of saccharide positional isomers and epimers which is not always possible with more conventional normal or reverse phase HPLC techniques. Unfortunately HPAELC presents special challenges for its direct interfacing to mass spectrometry, primarily removal of non-volatile mobile phase salts. We are currently evaluating several interfaces to combine HPAELC with MS for carbohydrate analyses. One of the most challenging is the direct coupling of HPAELC to thermospray MS (TSP MS). We have successfully constructed an on-line interface between HPAELC and TSP MS. Using a dialysis membrane, or suppressor, between the HPAELC column outlet and the TSP MS inlet permits removal of non-volatile components (i.e. NaOH) from the chromatographic mobile phase after the separation is performed and prior to entrance into the TSP MS source. Thus the chromatograph and mass spectrometer may be operated somewhat independently of each other. Our work has shown nearly 100% removal of Na⁺ from the mobile phase by the suppressor. Figure 1 illustrates the on-line total ion chromatogram for the isocratic separation of N-acetylglucosamine (GlcNAc), N-acetylmannosamine (ManNAc) and N-acetylglucosamine (LacNAc) obtained using the integrated HPAELC/TSP MS system. A polymeric anion exchange column and a 10 mM NaOH mobile phase were used to achieve the separation.

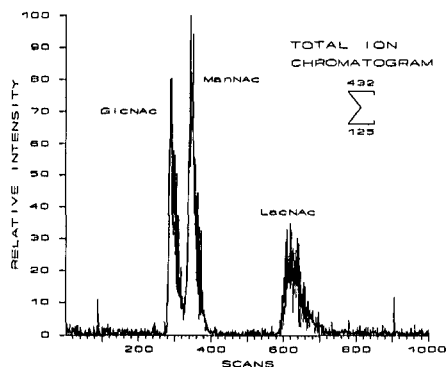


Figure 1. HPAELC/TSP MS total ion chromatogram of isocratically eluted N-acetylated sugars GlcNAc, ManNAc, and LacNAc.

Conventional direct injection (i.e. no column) TSP mass spectra of various N-acetylated sugars were shown to be similar to those obtained with the integrated HPAELC/TSP MS system. Figure 2 compares the TSP mass spectrum of GlcNAc obtained by direct injection to that obtained on-line by isocratic elution through the HPAELC/TSP MS system. The spectra are very similar, with the protonated molecular ion appearing at m/z 222.

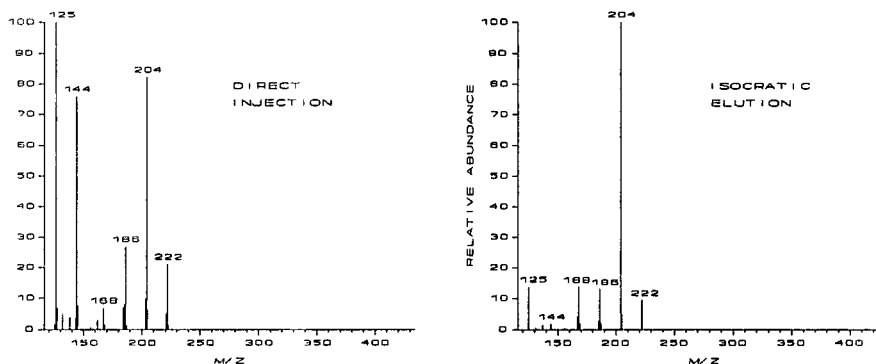


Figure 2. Comparison of GlcNAc TSP mass spectra obtained by direct injection and isocratic elution from the interfaced HPAELC/TSP MS system.

In addition to isocratic chromatographic separations the HPAELC/TSP MS system is also capable of operating in the gradient elution mode, thus reducing the time required for the chromatographic separation to be completed. The total ion chromatogram for a sodium hydroxide gradient separation of GlcNAc, ManNAc, and LacNAc is shown in Figure 3.

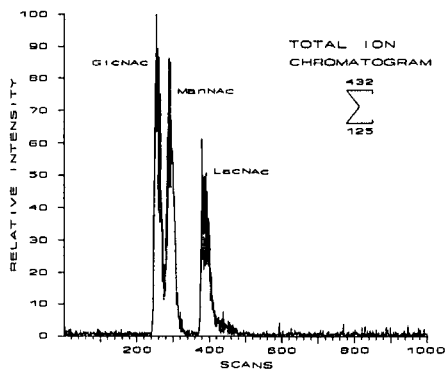


Figure 3. HPAELC/TSP MS total ion current chromatogram for a sodium hydroxide gradient separation of GlcNAc, ManNAc, and LacNAc.

The on-line gradient mass spectra are similar to those obtained with isocratic elution, but with an increase in intensity due to higher analyte concentration in the chromatographic bands due to peak compression caused by the gradient profile. Sensitivity studies were conducted using a mixture of the three N-acetylated sugars listed above. The results demonstrated the ability of the interfaced HPAELC/TSP MS system to provide minimum detection limits in the microgram range.

DEVELOPMENTS IN SUPERCRITICAL FLUID CHROMATOGRAPHY MASS SPECTROMETRY

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Introduction

Supercritical fluid chromatography (SFC) and its on-line coupling with mass spectrometry (SFC/MS) is now a well-established analytical tool in various application areas, e.g. polymer- and petrochemistry. In the last two years significant progress has been made in the field of bioanalytical applications of SFC and SFC/MS [1]. In bioanalysis several problems are encountered, e.g. the limited sample loadability of the capillary SFC columns, the detrimental effects of the free silanol groups in the analysis of polar compounds with packed column SFC, and the relatively (compared to LC) small injection volumes. Furthermore, the injection of aqueous solutions in SFC is prohibited. Other polar solvents may upon injection dramatically influence the retention characteristics of the SFC system, which will especially be a problem in the analysis of pretreated real samples.

Experimental

Packed column SFC and SFC/MS experimental were performed with a laboratory built SFC system, described in detail elsewhere. A Finnigan MAT HSEQ-30 hybrid mass spectrometer equipped with a moving belt interface, or a Finnigan MAT TSQ-70 mass spectrometer equipped with a thermospray interface were used.

Results and discussion

In order to enable the direct analysis of aqueous samples from biological origin (and in environmental analysis as well), a valve switching approach was developed, which is based on the pre-column technology designed for LC [2]. The analytical procedure, called 'phase-system switching' consists of the following three steps, schematically depicted in figure 1: First, the aqueous sample is injected onto a short pre-column, either directly or in a stream of water. Next, the pre-column is washed to remove water soluble constituents and dried with preheated gas to remove the excess of water. Finally, the pre-column is switched in a stream of a supercritical fluid, which desorbs the analytes of interest, transfers them to an analytical packed SFC column, where separation takes place. Detection is performed with either UV or MS. This approach has been applied in the analysis of diuron in plasma using a moving belt interface [3], and of the cytostatic drug mitomycin C (MW 334), using a thermospray interface (see figure 2).

The phase-system switching approach in SFC and SFC/MS has several interesting features. Not only a change in the phase-system from aqueous solutions to a supercritical mobile phase is accomplished, also preconcentration and on-line sample pretreatment, both based on the solid-phase isolation technique used, are achieved.

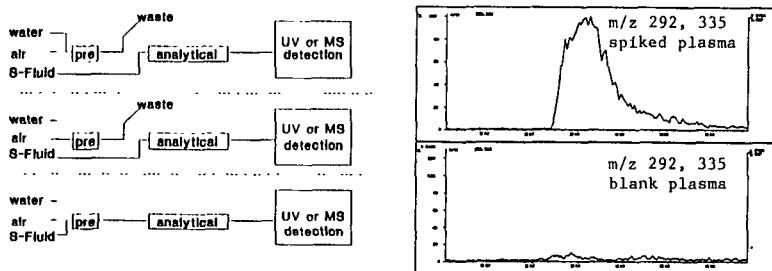


Figure 1: Schematics PSS approach. Figure 2: PSS-SFC/MS of Mitomycin C in plasma

Packed column SFC/MS can be performed with either a moving belt interface giving EI or CI spectra, or a thermospray interface, in which the vaporizer capillary is used as the back-pressure restriction. Applying the thermospray ion source in the discharge-on mode it is possible to alter the ionization conditions by changing the potential at the repeller electrode, which is positioned opposite to the sample cone in our Finnigan MAT thermospray source. At low repeller potential and a mobile phase containing 2-15% of methanol as an organic modifier proton transfer CI spectra are observed, resulting in hardly any fragmentation, while at high repeller potentials charge exchange type of spectra are obtained, showing considerable fragmentation. By choosing an appropriate repeller potential it is possible to get either a strong protonated molecule for quantitation or fragmentation for identification. These effects are clearly demonstrated in figure 3 by the spectra of caffeine, obtained at two different repeller potentials.

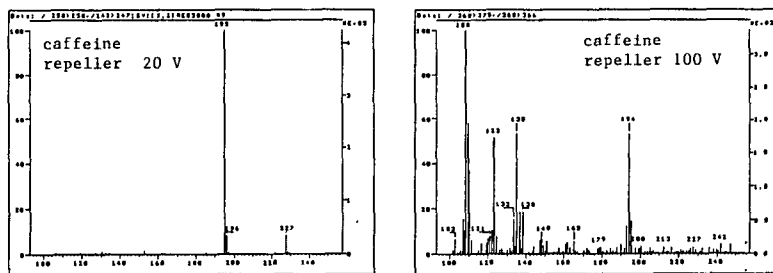


Figure 3: Thermospray SFC/MS spectra of caffeine at two repeller potentials

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OPTIMIZATION AND APPLICATION OF PARTICLEBEAM HPLC/MS
FOR COMPOUNDS OF PHARMACEUTICAL INTEREST

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HPLC/MS plays an important role in pharmaceutical research. The particlebeam (PB) HPLC/MS interface allows the acquisition of EI and CI spectra for structural elucidation, making particlebeam HPLC/MS technique very powerful for identification of unknowns. PB sensitivity, as with most HPLC/MS techniques, is dependent on the HPLC and interface operating parameters. This presentation evaluates various particlebeam interface conditions, HPLC conditions and MS conditions to determine their effect on sample sensitivity. Furthermore, EI and CI spectra generated by particlebeam were compared to thermospray to help determine the role each interface plays in pharmaceutical analysis.

The main variables in the particlebeam interface are the nebulizer position, desolvation temperature, and nebulizer helium flow rate. The nebulizer position is a critical parameter in the interface that requires adjustment to achieve optimum sensitivity. The particlebeam interface uses a coaxial nebulizer where a 100 μ M fused silica capillary can be moved towards or away from the nebulizer orifice in a helium stream to optimize aerosol formation. The optimization of the nebulizer position using a methanol HPLC mobile phase showed two maxima for spectinomycin response. The major maximum was at the position where the capillary tip was retracted 0.52 mm from the nebulizer tip. This position provides an aerosol formation that results in the best mass transport for the chosen solvent/solute system under the existing desolvation conditions. This position can vary for various HPLC solvents, but was close to the optimum distance ($\pm 10\%$) for most compounds tested. Varying the desolvation temperature between 30-70°C did not change the total ion current by more than 15% when using a methanol mobile phase (Figure 3). Operating with high percentages of aqueous solvent or at flow rates above 0.6 mL/min required the desolvation temperature to be increased to maintain sensitivity. Higher desolvation temperatures are needed at higher flow rates or with solvents with large ΔH_{vap} to replace heat lost by adiabatic expansion (evaporation).

The helium flow rate into the nebulizer affects sensitivity and the position the fused silica capillary in the nebulizer. For a fixed capillary position in the nebulizer, changes in the helium flow rate can change signal level. Comparable signals (within 20%) could be achieved at other helium flow rates if the capillary position in the nebulizer was reoptimized. The best overall sensitivity was achieved using higher helium flow rates with the fused silica tube in the nebulizer optimized for the helium flow employed.

HPLC conditions including solvent choice, buffer choice and flow rate play an important role in particlebeam operation. The particlebeam interface sensitivity varied with different reversed-phase HPLC solvents. Particlebeam sensitivity was optimal when using a high percentage of organic in the mobile phase. Sensitivity decreased as the percentage of water increased, indicating particlebeam sensitivity would vary across a HPLC gradient. It is important to note that the optimal position of the fused silica tube in the nebulizer varied with the HPLC solvents chosen. The change in solvent from methanol to water increases the surface tension/density ratio resulting in the formation of larger droplets. The desolvation of these larger droplets becomes more difficult due to size and the greater heat capacity of water over methanol. It is interesting to note that the order in sensitivity (methanol>acetonitrile>isopropanol>water) is inversely related to the heat capacity of the solvents.

The use of buffers in the particlebeam interface was not required, but volatile buffers can be employed to achieve a desired separation. Three volatile buffers, including ammonium acetate, acetic acid, and ammonium hydroxide, did not play a role in particlebeam sensitivity.

HPLC flow rate is another variable in particlebeam performance. The interface operates best at low flow rates 0.2-0.6 mL/min when using low boiling point solvents such as methanol. Increasing the aqueous content of the solvent further restricted the upper HPLC flow rate even when the desolvation temperature was raised to 70°C.

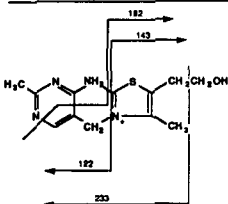
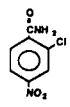
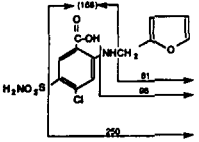
The source temperatures played a role in the signal response observed in particlebeam MS. Since the particlebeam interface currently relies on gas phase ionization techniques,

it is not surprising that the best responses were obtained at high source temperatures when particularly the lack of volatility for many pharmaceuticals is considered. However, it is not always possible to analyze a compound at the highest temperatures due to thermal decomposition. For these reasons spectra are usually acquired at source temperatures between 200-300°C.

The analysis of a variety of compounds (including β -lactams, cephalosporins, tetracyclines, methylene blue, furosemide, spectinomycin, cytidine, 2-chloro-4-nitrobenzamide, and thiamine) was possible using the particlebeam interface. The success of particlebeam MS under EI conditions relates to compound stability and volatility. Samples that are thermally unstable or nonvolatile would be best analyzed under particlebeam CI conditions or thermospray HPLC/MS. Thermospray/MS proved to be the best method for the analysis of the β -lactams, cephalosporins, and cytidine. The thermospray spectra exhibited $[M+H]^+$ ions as well as structurally important fragments. The analysis of more stable or volatile compounds (thiamine, methylene blue, 2-chloro-4-nitrobenzamide, spectinomycin and furosemide) proved inadequate by thermospray since only $[M+H]^+$ ions and one or two simple fragment ions were detected, insufficient for validation (Table I).

This research was supported by the Health and Human Services of the Food and Drug Administration under Cooperative Agreement No. FD-U-000244-01.

Table I. Tabular Listing of Particlebeam EI, Methane CI and Thermospray Spectra for Several Compounds

Thiamine 264		EI			CI			Thermospray		
		m/z	% RI	Identity	m/z	% RI	Identity	m/z	% RI	Identity
		85	32	IU	123	43	(3)+H	182	47	(3)
		112	100	[m/z 143-CH ₂ OH] ⁺	126	69	[m/z 144-H ₂ O] ⁺	265	100	[M+H] ⁺
		122	36	(3)	144	100	(3)+H			
		143	27	(3)	172	22	IU			
		233	8	(3)	247	7	[M+H-H ₂ O] ⁺			
		264	5	[M] ⁺	265	59	[M+H] ⁺			
Compound MW		EI			CI			Thermospray		
		m/z	% RI	Identity	m/z	% RI	Identity	m/z	% RI	Identity
2-Chloro-4-nitrobenzamide 200 		75	55	[110-Cl] ⁺	201	100	[M+H] ⁺	(2) 199	28	[M-H] ⁻
		110	21	[M-NO ₂ -CONH ₂] ⁺				259	100	[M+OAc] ⁻
		126	25	[M-NO ₂ -CO] ⁺						
		138	39	[M-NO ₂ -O] ⁺						
		154	22	[M-NO ₂] ⁺						
		184	100	[M-O] ⁺						
		200	51	[M] ⁺						
Furosemide 330		EI			CI			Thermospray		
		m/z	% RI	Identity	m/z	% RI	Identity	m/z	% RI	Identity
		81	100	(3)	168	90	(3)-H	(2) 249	18	[M+H-HNO ₃] ⁻
		96	6	(3)	250	91	(3)	329	100	[M-H] ⁻
		330	11	[M] ⁺	286	18	[M-CO ₂] ⁻			
					294	100	[M-HCl] ⁻			
					330	88	[M] ⁻			

IU = Identity Unknown

- (1) Negative ion methane CI spectra
- (2) Negative ion thermospray spectra
- (3) Identity shown on structure

INTERMEDIATE TERM STABILITY OF ION INTENSITIES AND
ELECTRON IMPACT MASS SPECTRA WITH PARTICLE BEAM
LIQUID CHROMATOGRAPHY MASS SPECTROMETRY SYSTEMS

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With the development of several types of particle beam interfaces for high performance liquid chromatography/mass spectrometry (HPLC/MS) systems, a new approach has emerged for the determination of compounds (nonvolatiles) not amenable to capillary column gas chromatography mass spectrometry (GC/MS). Two critical issues are the stabilities of integrated ion intensities over a minimum of an eight-hour period, and the relative stabilities and reproducibilities of the electron impact (EI) mass spectra over a similar time period. Ion intensity stability over a minimum of an eight-hour period is required so that concentration calibrations maintain validity for a reasonable number of samples. Similarly, EI spectra must be relatively stable to permit automatic peak identifications via reverse search algorithms and spectra from reference data bases.

Stabilities of signal intensities and EI spectra over two-day periods were studied for sixteen compounds shown below that are not amenable to GC/MS (nonvolatiles).

Compound Name	MW	Compound Class	Capillary Col. GC Status
aldicarb sulfone	222	carbamate (S)	no peaks
carbaryl	201	carbamate	decomp.
carbofuran	221	carbamate	decomp.
methiocarb	225	carbamate (S)	decomp.
methomyl (Lannate)	162	carbamate (S)	no peaks
mexacarbamate (Zectran)	222	carbamate	decomp.
o-chlorophenyl thiourea	186	N-substituted thiourea	decomp.
diuron	232	N-substituted urea (Cl)	no peaks
ethylene thiourea	102	N-substituted thiourea	no problem
linuron (Lorox)	248	N-substituted urea (Cl)	decomp.
N-(1-naphthyl)thiourea	202	N-substituted thiourea	no peaks
siduron	232	N-substituted urea	no peaks
benzidine	184	aromatic diamine	marginal
3,3'-dimethoxybenzidine	244	aromatic diamine	marginal
3,3'-dichlorobenzidine	252	aromatic diamine	marginal
rotenone	394	natural product	no peaks
DFTPP	442	the greatest	perfect

Experiments were conducted with two commercial particle beam HPLC/MS systems: (1) a Hewlett-Packard (HP) system consisting of an HP model 1090 HPLC, an HP particle beam interface, and an HP model 5988 mass spectrometer; and, (2) a system consisting of a Waters PB 600 MS HPLC, an Extrel Thermabeam interface, and an Extrel ELQ 400-1 mass spectrometer.

Other equipment and operating conditions were the same on both systems. All HPLC injections were made with autoinjectors. The HPLC column was a Waters Bondapak 30 cm x 2 mm (id) packed with 10 micron silica particles with octadecyldimethylsilyl (C-18) groups chemically bonded to the silica surface. Supelco LC-18 or DuPont C-18 guard columns were used to protect the analytical column. The mobile phase was a mixture of acetonitrile and water containing 0.01 M ammonium acetate. The solvent composition was held for one minute at 30% acetonitrile, then programmed to 100% acetonitrile in 29 minutes. Injection size was 5 μ L and the flow rate 0.4 mL/minute. The ion source temperature was 250 $^{\circ}$ C, and the electron energy 70 eV. The mass spectrometers were repetitively scanned from 62-450 amu at 1.5 seconds/scan which gave at least 10 scans per HPLC peak.

All mass spectrometric data acquired was from injections of the 16 compound mixture into the HPLC analytical column, and injections without the analytical column were used only for preliminary tuning of the instrument systems.

Compound Name	System A		System B	
	Rel. Resp. (RSD)		Rel. Resp. (RSD)	
aldicarb sulfone	0.12	(14)	0.15	(7.0)
carbaryl	0.19	(8.8)	0.18	(14)
carbofuran	0.06	(6.4)	0.07	(18)
methiocarb	0.11	(8.5)	0.08	(17)
methomyl (Lannate)	0.03	(11)	0.04	(15)
mexacarbate (Zectran)	0.04	(14)	0.07	(23)
o-chlorophenyl thiourea	0.23	(10)	0.30	(12)
diuron	1.00	(12)	1.00	(26)
ethylene thiourea	0.37	(8.2)	0.59	(17)
linuron (Lorox)	0.02	(21)	0.02	(22)
N-(1-naphthyl)thiourea	0.11	(11)	0.31	(8.6)
siduron	0.86	(8.3)	0.60	(26)
benzidine	0.22	(7.4)	0.53	(10)
3,3'-dimethoxybenzidine	0.31	(8.1)	0.51	(18)
3,3'-dichlorobenzidine	0.39	(7.0)	0.47	(23)
rotenone	0.09	(9.4)	0.10	(15)

The relative responses shown above are for the integrated abundances of the quantitation ions over the HPLC peak. The quantitation ions were the base peaks in the EI spectra above mass 62. The relative standard deviations (RSD) were calculated from the means of the responses for 10-14 injections into the HPLC column over two working days. The amounts of each compound injected were 0.385-1.53 μ g.

Incorporation of MS/MS Detection for the Characterization of Peptide Digests
by Continuous Flow FAB LC/MS

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The use of continuous flow FAB (CF-FAB) as an interface for capillary HPLC has made possible the analysis of peptide mixtures at picomole levels. As utilization of this technology grows, there is a need to investigate the advantages of MS/MS detection. Traditionally, MS/MS has been used to increase either the selectivity or structural content of MS analyses. The latter application would be useful for confirming assignments given to peptides based on molecular weight; particularly when post-translational modifications are present or if non-specific enzymes are used. Ultimately, LC-MS/MS should be investigated as a means for increasing the efficiency of peptide sequencing by tandem MS.

As a starting point for these investigations, trypsin and chymotrypsin digests of glucagon (290 pmol) were analyzed by LC-MS/MS using capillary CF-FAB. In each case, daughter ion spectra were recorded for the major hydrolysis products observed in the LC/MS profiles. Although the entire sequence for glucagon was not verified, sequence ions which supported the proposed assignments were present in the daughter spectra of each digestion product examined. In the example involving chymotrypsin, evidence for trypsin cleavage of chymotryptic digestion products was obtained using MS/MS.

The system developed for capillary LC/MS incorporated the following features: gradient elution, on-line UV detection, and coaxial post-column matrix addition. Separation was performed using a (25cm x 0.320mm i.d.) fused silica column, slurry-packed in-house with 5 μ m Spherisorb ODS particles, at a flow rate of 2 μ L/min. The matrix-containing solution (ACN:H₂O:glycerol:thioglycerol, 4:2:1:1) was added post-column at a flow rate of 1 μ L/min. The entire eluent (3 μ L/min) was transferred to a VG ZAB2-SE double focusing mass spectrometer using the probe supplied by the manufacturer. Daughter ion spectra were recorded using B/E linked scanning at a parent ion attenuation to 30% with helium.

Figure 1 displays the UV and total ion profiles from the LC/MS analysis of 290 pmol glucagon (trypsin digest). A linear gradient from 5 to 60% ACN in water (0.1% TFA) was performed over 30 min. The data indicate a total analysis time of less than 40 min. and that the chromatographic integrity was preserved by the CF-FAB interface. The sequence of glucagon, indicating the observed sites of hydrolysis, is shown in Figure 2. Despite the relative simplicity of this mixture, only 3 of the 4 tryptic peptides were detected by direct FAB analysis. The profiles in Figure 1 indicate that all 4 tryptic peptides were separated and detected. In addition, evidence for a minor chymotrypsin cleavage product: T_{3c} (MH⁺ 1162) was also obtained from the analysis.

In a subsequent analysis, daughter ion spectra were acquired for each of the 4 tryptic fragments identified by LC/MS. Figure 3 shows a single daughter ion scan recorded for tryptic fragment T₃ (MH⁺ 1352). Nearly complete sequence information was obtained (290 pmol injected). These results indicate the ability to acquire satisfactory daughter spectra from subnanomole quantities of peptides in mixtures using capillary CF-FAB and encourage further work in this area.

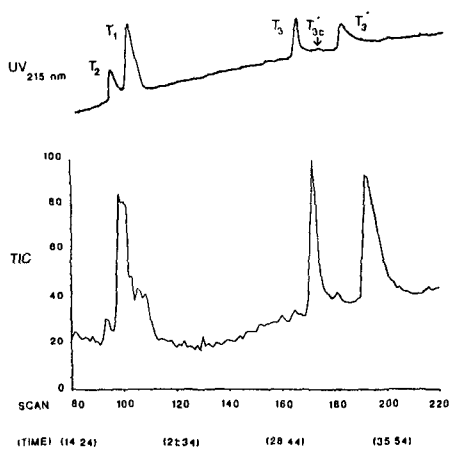


Fig. 1

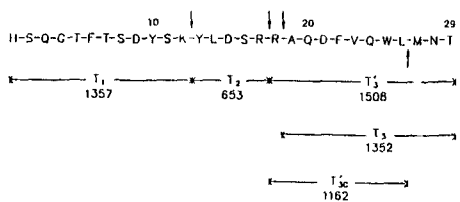


Fig. 2

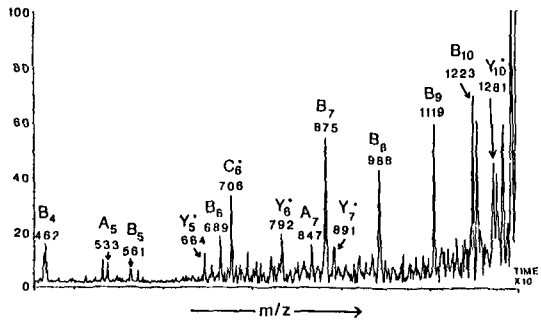
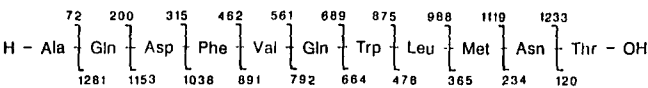


Fig. 3

**TANDEM MASS SPECTROMETRY FOR THE ANALYSIS OF BIOMOLECULES
VIA COAXIAL CONTINUOUS FLOW-FAST ATOM BOMBARDMENT
AND CAPILLARY ZONE ELECTROPHORESIS**

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We have recently reported the design of a coaxial continuous flow-fast atom bombardment interface in which the analytes and the FAB matrix are independently delivered to the probe tip (1,2). Briefly, the coaxial CF-FAB interface consists of a fused silica column (typically 10 μm id) which is surrounded by a second fused silica column (typically 150 μm id) in which the FAB matrix is introduced. As a result, there is no mixing of the matrix with the analytes until both have reached the FAB probe tip. This coaxial continuous flow design is advantageous since the chromatography is not affected by the matrix. In addition, the ability to independently control both the OTLC flow rate and the matrix flow rate and composition is desirable. We have also adapted this coaxial design for use as a coaxial capillary zone electrophoresis/FAB interface (3).

In this paper, we demonstrate the ability to obtain on-the-fly (i.e. chromatographic real time) MS/MS spectra of biomolecules as they elute from the capillary column. MS/MS spectra of the $(M + H)^+$ ion of the tripeptide Met-Leu-Phe were acquired on-the-fly from a 220 pg injection (Fig. 1) as well as a 22 pg injection. The mass spectrometer was scanned at 3 sec/dec resulting in a total acquisition time of 2 seconds. The $(M + H)^+$ ion for m/z 410 fragments into the common backbone cleavages of A_2 , B_2 , and Y_2 as shown.

This coaxial CF-FAB interface is equipped with an injection system such that a continuous flux of analyte solution can be introduced into the mass spectrometer over extended periods of time (i.e. hours) as well as over short time periods (i.e. seconds). The MS/MS spectra of a variety of compounds, including peptides, sugars, phospholipids, fatty acids, and steroids, have been obtained using the coaxial CF-FAB interface to deliver a constant flux of analyte. This constant flux of analyte can be advantageous for the acquisition of collisionally activated decomposition spectra that may be otherwise cumbersome (e.g., MS/MS/MS spectra). The MS/MS/MS spectrum of the tetrapeptide, Phe-Gly-Leu-Met-NH₂, is shown in Fig. 2 and is representative of 500 ng of material. Upon CA, the $(M + H)^+$ of m/z 466 fragments to give the ion of m/z 318 which corresponds to the loss of HMetNH₂. In the MS/MS/MS experiment, the ion of m/z 318 fragments to give the loss of CO and the Leu residue (fragment B_2). The daughter ion of m/z 119 (fragment A_1) corresponds to the loss of CO from the Phe residue.

Using a packed microcapillary (50 μm id packed with 10 μm particles), the separation of a mixture of Met-enkephalin (2 picomoles) and Met-Leu-Phe (3 picomoles) was obtained. As the analytes were eluting from the column, their MS/MS spectra were acquired (Fig. 3) while scanning the mass spectrometer at 5 seconds/decade. Fragmentations resulting from typical backbone cleavages were observed for both of the analytes studied.

The coupling of capillary zone electrophoresis with mass spectrometry has recently become an area of increased interest. The primary advantage of capillary zone electrophoresis over liquid chromatography is the much superior separation efficiencies which can be obtained with CZE. Using the coaxial continuous flow-FAB interface, we have developed an on-line coaxial continuous-flow capillary-zone electrophoresis/FAB interface. The design is such that active electrophoretic transport of the analytes directly to the FAB probe tip occurs. Since a transfer line from the end of the CZE capillary to the probe tip is obviated, no zone broadening that would otherwise occur is observed. The electrophoretic separation of mixtures of peptides has been obtained. For example, a mixture of proctolin and Met-

Leu-Phe was obtained and as the Met-Leu-Phe was eluting from the CZE column, the $(M + H)^+$ ion was collisionally activated. The MS/MS spectrum which was acquired is shown in Fig. 4 and corresponds to 130 femtomoles of the tripeptide.

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Figure 1

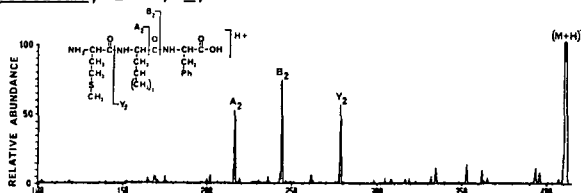


Figure 2

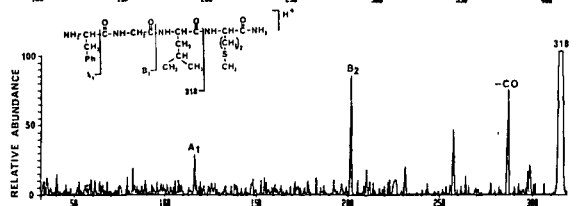


Figure 3

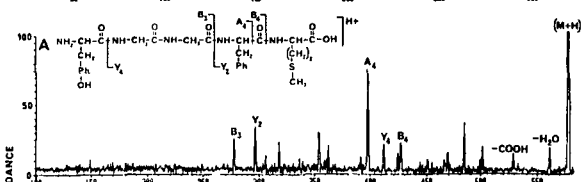
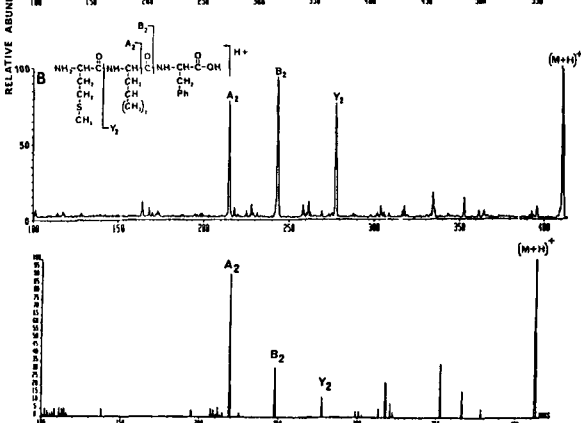


Figure 4

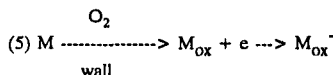
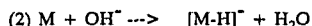


SURFACE-CATALYZED REACTIONS OF AROMATIC AMINES
WITH OXYGEN AND NITROGEN IN NICI MASS SPECTROMETRY*

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In previous studies it was found that the O_2/N_2 negative ion chemical ionization (NICI) mass spectra of polycyclic aromatic hydrocarbons (PAH) and methyl-substituted PAH showed unusual ions that could not be explained by simple electron-molecule (1) or ion-molecule (2-4) reactions. These unusual ions dominate the O_2/N_2 NICI mass spectra when the ion source temperature is elevated to 300°C, are observed under positive CI conditions, and exhibit characteristic tailing ion chromatograms when gas chromatography is used for sample introduction. Recent studies [Org. Mass Spectrom. 24, 94-104 (1989)] indicate that these ions result from surface-catalyzed oxidation reactions followed by electron-capture ionization (reaction 5).



Under similar conditions, unusual ions appearing at $[M+11]^{\bullet -}$ and $[M+14]^{\bullet -}$ were observed in the spectra of amino-substituted aromatics such as 1- and 2-aminonaphthalene and 1- and 2-aminoanthracene. These ions were also enhanced when the ion source temperature is elevated, were observed under positive CI conditions, and exhibited tailing ion chromatograms, which suggested that they were also formed by reaction (5). Studies to ascertain the identity of these ions were carried out using Fourier transform mass spectrometry (FTMS). The $[M+11]^{\bullet -}$, $[M+14]^{\bullet -}$, and an additional ion at $[M+13]^{\bullet -}$ were observed when the FTMS cell temperature was elevated to approximately 200°C. Exact mass measurements indicated that the ion at $[M+14]^{\bullet -}$ corresponds to $[M+O-2H]^{\bullet -}$, while the ions at $[M+11]^{\bullet -}$ and $[M+13]^{\bullet -}$ correspond to $[M+N-3H]^{\bullet -}$ and $[M+N-H]^{\bullet -}$, respectively.

The ion at $[M+14]^{\bullet -}$ was further studied using a quadrupole instrument equipped with a chemical ionization source. Both O_2 and $^{18}O_2$ were added to the N_2 reagent gas. The $[M+14]^{\bullet -}$ ion was significantly enhanced by the addition of O_2 , and an ion at $[M+16]^{\bullet -}$ was observed upon addition of $^{18}O_2$. These observations support the $[M+O-2H]^{\bullet -}$ assignment made using FTMS. Additional studies using aniline- ^{15}N and aniline-2,3,4,5,6- d_5 indicated that the $[M+O-2H]^{\bullet -}$ ion results from oxygen addition at the amino nitrogen to produce a nitroso group.

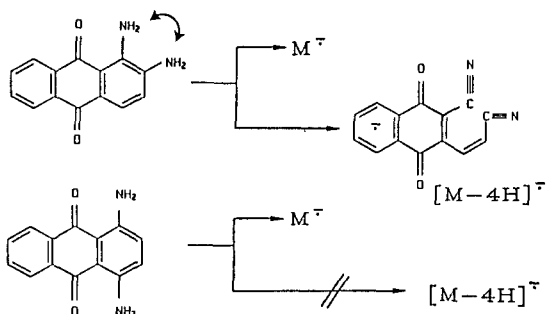
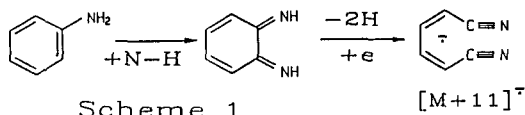
The ions at $[M+11]^{\bullet -}$ and $[M+13]^{\bullet -}$ were also studied to determine the source of added nitrogen. On the quadrupole instrument the $[M+11]^{\bullet -}$ ion was much more intense than the $[M+13]^{\bullet -}$ ion. It was found that the intensity of the $[M+11]^{\bullet -}$ ion increased as a function of % N_2 when argon was used as the buffer gas. These results, in conjunction with experiments using aniline- ^{15}N indicated that the nitrogen reagent gas, not the amino group, was the source of nitrogen in the $[M+N-3H]^{\bullet -}$ ions. Measurements of the spectra of aniline- ^{15}N and aniline-2,3,4,5,6- d_5 indicated the nitrogen addition occurred in conjunction with loss of the two amine hydrogens and one ring hydrogen.

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Examination of the spectra of various methyl-substituted anilines indicated the the $[M+N-3H]^+$ ion was not observed when two methyl groups were substituted ortho to the amine group. This result suggested that a site ortho to the amine group was needed for nitrogen addition. The resulting $[M+N-3H]^+$ product may correspond to a ring-opened dicyano compound (see Scheme 1). This hypothesis is based upon the documented lead tetraacetate oxidation of ortho-substituted diaminoaromatics. For example, the spectra of 1,2-diaminoanthraquinone shows an $[M-4H]^+$ ion, which is not observed in the spectrum of the corresponding 1,4-isomer (see Scheme 2). The $[M-4H]^+$ ion is also observed in the spectra 2,3- and 3,4-diaminotoluene (ortho-substituted diamines), but not in the spectra of the 2,4- or 2,6-diamino isomers (meta-substituted diamines).

In conclusion, these experiments indicate that the O_2/N_2 NICI mass spectra of aromatic amines show unusual ions that result from surface-catalyzed oxidation reactions with both oxygen and nitrogen prior to ionization. The products of these reactions are enhanced by elevated ion source temperatures, show tailing ion chromatograms, and are observed under positive chemical ionization conditions. Certain ions can be used to differentiate diamino and methyl-substituted aminoaromatics.



Scheme 2

IONIZATION EFFICIENCY IN AN ELECTROSPRAY SOURCE

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In evaluating the analytical sensitivity of a mass spectrometer system one would like to know the overall efficiency with which analyte molecules entering the system actually contribute to the output signal. In other words, what fraction of those molecules end up as ions at the detector. That overall efficiency is the produce of two major components: the fraction of analyte molecules that become transformed into ions in vacuo and the fraction of those ions entering the vacuum system that finally reach the detector. We report here on the first of these components, the effectiveness with which an Electrospray (ES) ion source transforms injected analyte molecules into ions in vacuo.

Figure 1 shows the essential features of the apparatus. The ES source has been previously described. (1) It produces a dispersion of ions in bath gas at atmospheric pressure. Some of that ion-containing bath gas enters an orifice or a glass capillary tube and emerges as a supersonic free jet expanding into a vacuum system. The glass capillary tube was used this work because it provides design flexibility and decreased exposure to the hazards of high voltage. The total ion current leaving the capillary was measured by intercepting the entire free jet flow with a Faraday cup connected to ground through a picoammeter. Because we were interested in how the ionization efficiency depended on analyte molecular weight measurements were made with a series of poly ethyleneglycols (PEGs) whose average molecular weights ranged from 200 to 17,500. Samples comprised about 11 μ L of a solution of PEG at concentrations of 0.1 or 0.05 μ g/ μ L in 50-50 methanol-water. They were injected into a steady 3.5 μ L/min flow of the same solvent entering the ES source.

The gross current I_g due to PEG ions reaching the Faraday cup was taken as the the peak value of the free jet current reached after sample injection. MS studies had shown that the presence of PEG suppresses the formation of ions from smaller solvent and solute molecules so there was no need to subtract a "background" current that was measured when only solvent was flowing. It seems highly unlikely that all the PEG molecules in a charged droplet are desorbed as molecular ions. Therefore, each droplet must leave behind some residue that includes any undesorbed PEG as well as any charge left on the droplet after individual ions stop desorbing. It is important to determine the contribution of these charged residues and subtract it from the total ion current I_g in order to arrive at In the net current due to ions that are charged individual molecules. We determined that contribution by applying a retarding potential V_r to the ions en route to the Faraday cup.

In principle it is possible to determine this fraction from an I_g - V_r curve. Alternatively, because the charged residues have lower mobility than the individual molecular ions it is possible to adjust the countercurrent bath gas flow rate so as to prevent those residues from entering the capillary. We found that the latter approach gave more definitive and reproducible results. Clearly, because the distribution of fields and flows in the

source chamber are not well defined and uniform, a bath gas flow rate sufficient to sweep away the residues will also prevent some of the single molecule ions from entering the capillary. Thus, the ionization efficiencies that we determine are conservative in the sense that they do not include all the single molecular ions actually produced that could have entered the vacuum system if the bath gas flow rate had been lower.

Figure 2 shows curves of I_g vs V_r when the solute ions were Tetrabutylammonium ($M = 243$) and Tetraheptylammonium ($M = 441$). The inflection points on the curves indicated by the arrows occur as they should at a value of V_r equal to the kinetic energy of a singly charged solute ion accelerated in the free jet expansion to the actual velocity of the bath gas. Because of distortions by stray fields, velocity lags, and "contact potentials" due to differences in the work function of the metalized exit face of the capillary and the skimmer-grid structure used as a Faraday cup, such agreement cannot always be expected. Figure 3 displays I_g - V_r curves for two PEG samples. The scale in the upper right hand corner shows the abscissa position for the kinetic energy that ions with indicated m/z values would have at the actual velocity of the bath gas.

Figure 4 shows the values of I_n for solutions at two concentrations of PEG covering the range of molecular weights M for which we have samples. Flow rates were 3.5 $\mu\text{L}/\text{min}$ in both cases. There is clearly a definite increase in current as M increases. To be remembered is that increasing M corresponds to a decreasing number flux of PEG molecules. We know from our earlier study of PEG how the number of charges per ion increases with increasing M . (2) The effects of these factors on I_n are calculable so we can determine the effect of M on "charge yield" in coulombs/ μg , a frequently used index of ionization efficiency. Figure 5 shows this dependence. It is also possible to determine the number of ions in the current flux so we can arrive at a value for "ion yield," the fraction of injected analyte molecules that enter the vacuum system as ions. The dependence of this "ion yield" on M is shown in Figure 6. It is clear that both of these measures of efficiency increase substantially with increasing M . We do not yet understand the reasons for this welcome trend but it does not seem to be shared by most other ionization techniques. Also of note is that since this work was done some source changes have resulted in a 100 fold gain in ion current.

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SPECULATION ON THE MECHANISMS(S) OF ELECTROSPRAY IONIZATION

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As originally reported by Fenn and coworkers,¹ proteins can be effectively addressed by electrospray ionization (ESI), yielding a distribution of charge states. Reasonable mass spectra can be obtained with picomole quantities of protein and the molecular weight of unknown substances can be accurately calculated based upon any two peaks (assuming one knows that the peaks are related). Given typical distributions, such as shown in Figure 1 for a mixture of horse heart cytochrome c and tuna cytochrome c, the calculation is straightforward and the accuracy of the determination (generally 0.005 to 0.05% depending upon sample purity and S/N) is enhanced by use of several peaks. Simple mixtures may be readily addressed and the MW of each component determined.

Figure 2 shows a plot of the highest charge state detected vs. the number of basic amino acids for a representative set of proteins, showing a crude linear relationship. We have as yet seen no charge state distributions for large proteins for which a maximum intensity is indicated above m/z 1700, suggesting that electrostatic restrictions may exist on the desorption rates for higher m/z ions. The maximum charge state for proteins is accurately predicted by the number of readily protonated sites, as shown by the good linear correlation observed up to about 25,000 dalton between the maximum number of charges and the number of basic amino acid residues (e.g., arginine, lysine, histidine, etc.). For example, angiotensin I (MW 1296) contains 1 arginine and 2 histidine residues in its sequence (along with an N-terminal amino group), consistent with the $(M + 4H)^{4+}$ ion being the maximum charged species observed experimentally. The $(M + 5H)^{5+}$ and $(M + 6H)^{6+}$ are the most abundant and most highly charged species, observed for melittin from bee venom, a peptide for which protonation sites include the 5 basic amino acids and the terminal NH_2 group.³

Dithiothreitol (DTT) is a reagent which reduces disulfide bonds between cysteine residues has been found to have a significant effect on the maximum charge state observed. The effect is illustrated in Figure 3 for lysozyme and in Figure 4 for bovine albumin. The substantial increase in maximum charge state apparently results from the greater accessibility of the basic amino acid residue charge sites in the electrospray droplets, and greater extension (i.e., loss of tertiary structure) of the gas phase ion. As shown in Figure 2, bovine albumin treated with DTT more closely approaches the maximum charge state expected.

Acknowledgment

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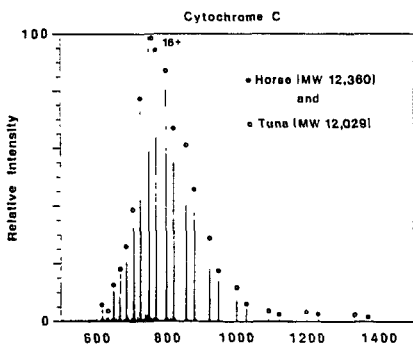


Figure 1

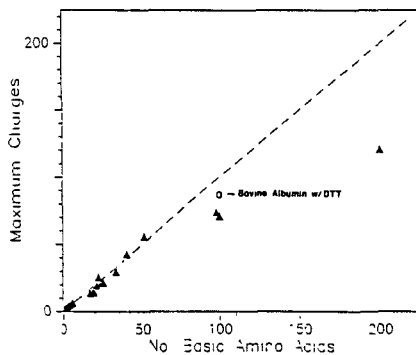


Figure 2

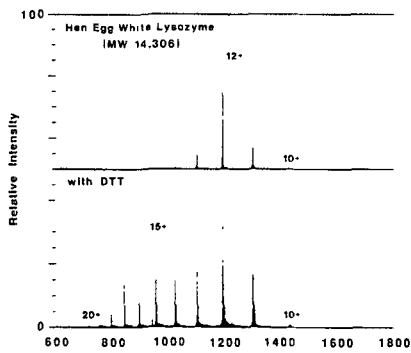


Figure 3

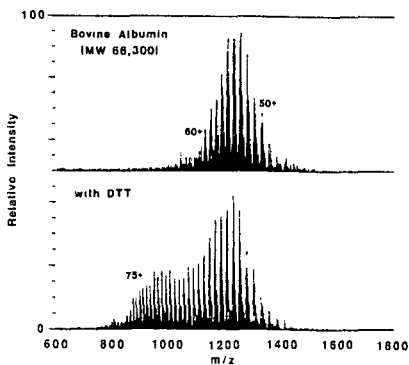


Figure 4

VISCOSITY EFFECTS IN FAST ATOM BOMBARDMENT MASS SPECTRA

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Fast atom bombardment (FAB) spectra are known to be strongly dependent on matrix temperature, T_b . At low temperature, the spectra tend to degrade to a series of non-descript peaks. The reason for this strong effect has been unclear, however. In a previous study¹, we suggested that it was not the temperature per se, but rather the viscosity, that affect the FAB spectra. Here, we report investigations into the effect of viscosity on FAB spectra of some simple systems.

As the glycerol matrix is cooled from +25°C to -30°C, the viscosity increases from 10^3 to 10^6 centiPoise (cP). In order to be able to distinguish temperature effects from viscosity effects, it is necessary to be able to change the viscosity at constant temperature. This can be done by mixing in up to 60 w% (c_a) glucose into the glycerol. This is nearly ideal since glucose is chemically similar to glycerol and also, because of its low gas-phase basicity, almost "transparent" in the spectra. In this study, the viscosity has been varied in two ways, by varying the temperature (T_b) and by varying the glucose concentration (c_a). Figure 1 compares the results for glycerol. The two graphs are seen to be quite similar. The major glycerol ions decrease strongly in both cases and the noise ion intensity increases. Also, there is a gradual decrease in glycerol clustering and an increase in fragmentation. Figure 2 shows the result for a 10 mol% solution of diethanolamine (DEA) in glycerol. Again, the T_b and the c_a studies are quite similar. In addition to the features in Fig. 1, a rapid decrease for the analyte ions is also seen. The trends seen in Figs 1 and 2 were seen in all investigated systems. The figures also show that severe spectral degradation sets in at a much lower viscosity, when DEA is added, 3,000 vs 50,000 cP. This additional degradation is due to increases for noise ions like $(M+14)H^+$ which must be products of radiation chemistry.

Despite the significant similarities between the T_b and the c_a experiments, there are also differences. As an example, Fig. 3 shows the analyte to matrix ion ratios for the T_b and the c_a studies of a 1.7 mol% solution of adenosine in glycerol. The rapid increase observed in the c_a experiments can be understood as a result of changes in the rates of the various transport processes at the liquid/vacuum interface. Evaporation of the matrix tends to increase the surface concentration of an involatile analyte. However, this tendency is opposed by diffusion and sputtering. In the T_b experiment both evaporation and diffusion is slower at high viscosity. In contrast, in the c_a experiment, diffusion is slower but the effect on evaporation is small. The result is increased analyte surface enrichment as reflected in Fig. 3. This figure also shows an increase in the analyte to matrix ion ratio at the lowest temperature. This effect was observed for most other systems. The tentative interpretation is that this reflects that a direct desorption, precursor ion formation mechanism is becoming more efficient at very high viscosity.

It is concluded that temperature per se has a small effect on FAB spectra and that the viscosity is a more important parameter. The viscosity effects in turn are due both to changes in desorption mechanism and to changes in the transport processes and to how these effect surface concentrations and radiation chemistry.

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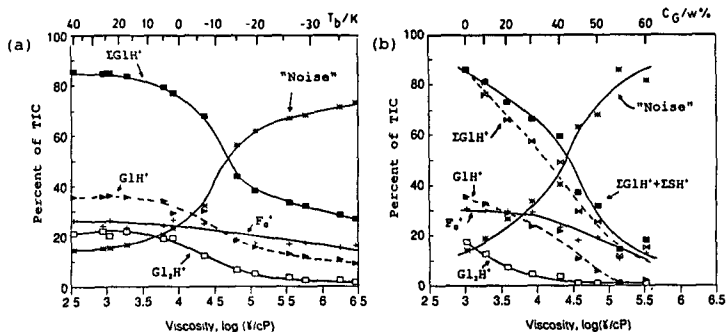


Figure 1. Change of intensities of major glycerol (and glucose) ions with matrix viscosity in FAB spectra; a) T_b -study; b) C_2 study (see text); Gl =glycerol; F_2 =major glycerol fragments; S =glucose; $EG1H^+$ includes all major glycerol ions.

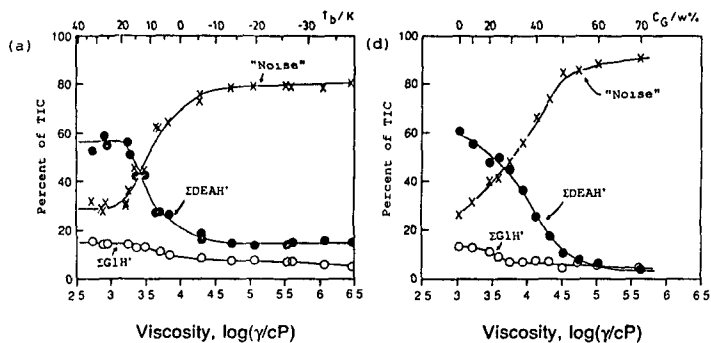
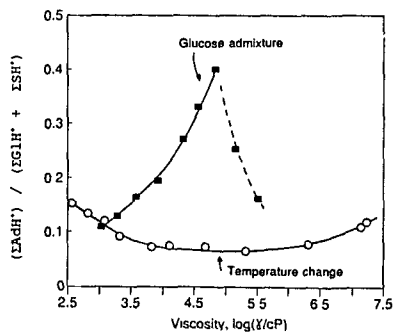


Figure 2. As Figure 1, but 10 mol% DEA added to the glycerol; $EG1H^+$ and $IDEAH^+$ include all the major ions of respective compounds.

Figure 3. Ratio of major adenosine ions to major glycerol and glucose ions in FAB spectra of 1.7 mol% adenosine solution in glycerol; (O) T_b study; (■) C_2 study.



ON THE FORMATION AND DISSOCIATION DYNAMICS OF CLUSTER IONS IN FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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Fast atom bombardment has been, for several years, used widely as a desorption-ionization technique for the mass spectral analysis of polar and high molecular weight compounds. The technique is appealing because it is experimentally easy to use and can contribute useful information in a wide number of analytical applications such as peptide identification and sequencing. However, the mechanisms responsible for the ionization and generation of the mass spectra are still not fully understood and are presently the topic of several investigations¹⁻³. The matrix plays an important role in fast atom bombardment mass spectrometry and recently studies have been focussed on the function of the liquid matrix in the ionization-fragmentation processes. The function of the matrix is not only to dissolve the analyte and allow the bombarded surface to be renewed, but it also has a determining role in defining the types of ions that will be observed and their intensities. When polar matrices such as glycerol, thioglycerol or diethanolamine are used, several types of cluster ions are observed in the mass spectrum. For example, when glycerol is used for the analysis of peptides, several types of cluster ions are observed and they are of the type G_nH^+ , $G_nA_mH^+$ and A_mH^+ where G and A represent glycerol and the analyte respectively.

Since cluster ions are believed to play an important role in the generation of the FAB spectra, studies have been undertaken in order to identify important experimental factors that influence the dynamics of formation and dissociation of these clusters in the hopes that valuable information leading to a better understanding of the generation of FAB mass spectra could be obtained. In initial studies to determine whether FAB desorption-ionization was mainly a solution or a gas phase process, comparison was made between normal FAB using glycerol as the liquid matrix and CI using glycerol as the reagent gas. The results of those experiments revealed that even if some aspects of the spectra were similar, dissimilarities related to fragmentation and presence of higher cluster ions existed². Similar results were obtained by other groups who could not generate cluster ions G_nH^+ with more than two molecules of glycerol under conditions of chemical ionization³. Following these experiments, a series of solution parameters were varied in FAB experiments in order to investigate their effects on the formation and dissociation of the different types of clusters observed. MS/MS experiments demonstrated that the higher clusters, whether they be of the type G_nH^+ or $G_nA_mH^+$, dissociated unimolecularly by loss of one molecule of glycerol giving fragments of the type $G_{n-1}H^+$ or $G_{n-1}A_mH^+$. At higher internal energies, such as those that can be achieved by collision activation, the clusters dissociate by loss of one or several molecules of G or A to give lower mass clusters or fragments corresponding to those observed in conventional FAB spectra thus indicating that the FAB spectrum could be generated and explained by the decomposition of these clusters. Results of experiments related to the formation of the positively and negatively charged clusters indicated that solution parameters such as pH of the matrix and concentration of the analyte had an influence on the intensities and internal energies of homogeneous (G_nH^+ , A_mH^+) and inhomogeneous $G_nA_mH^+$ clusters. Figure 1 shows the important effects of pH and analyte concentration on the intensities of G_nH^+ clusters. Figures 2A and 2B show the influence of analyte concentration on the variations of the intensities of both positively and negatively charged clusters of the type $G_n\pm H^\pm$, $A_m\pm H^\pm$ and $G_nA_m\pm H^\pm$. The effect of concentration on the internal energies of mixed clusters of the type $G_nA_m\pm H^\pm$ has also been investigated and the results obtained for the negatively charged clusters $G_nA_m-H^-$ are given in figure 3. It is found that the internal energies of both positively and negatively charged clusters increase with the concentration of the analyte.

Thus the type and intensities of the clusters that are formed are intimately related to the composition and conditions of the liquid matrix as it has been reported in experiments on mixtures of glycerol and diethanolamine¹. However, even if hypotheses have been suggested, it is still difficult to explain all the experimental evidence with regards to the factors affecting events in the gas phase and in solution. A series of experiments have been designed so that some of these factors can be isolated. This can be done by examining the incorporation of ¹³C glycerol, in

the vapor phase of a tight source, to clusters generated by FAB using ^{12}C glycerol as a liquid matrix.

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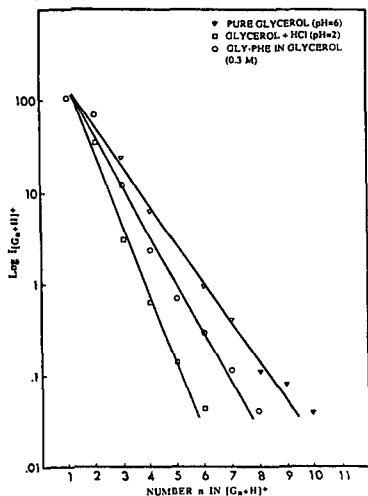


Figure 1.

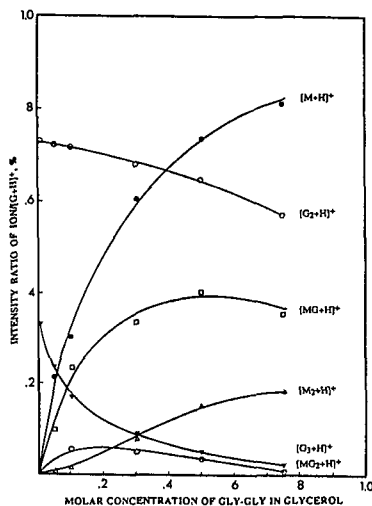


Figure 2A.

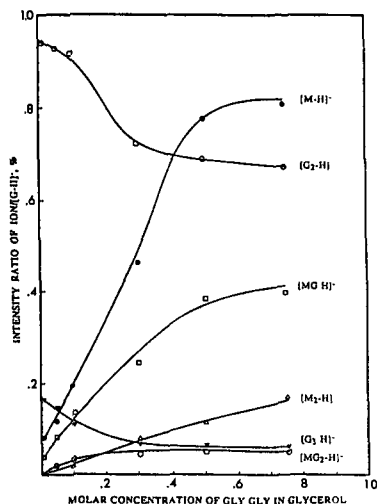


Figure 2B.

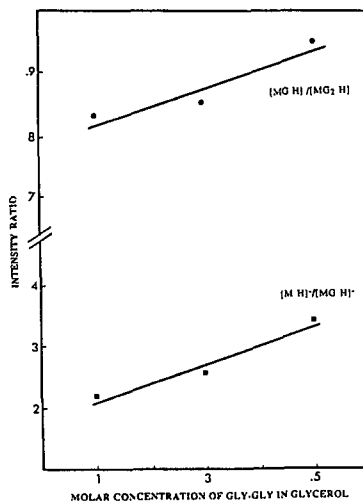


Figure 3.

Solute Introduction and Liquid SIMS

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Theories proposed to explain the enhanced emission of solute secondary ions from glycerol solutions may be divided into two main areas. The first group concerns the overall mechanism by which secondary ions are emitted. A second group of theories concerns the effects that surface activity, diffusion, radiation damage, etc., have on the intensity of characteristic secondary ions emitted. Within the second group, it is often impossible to rectify evidence supporting one rationalization, such as the effect of surface activity without the implication that another conceivable factor, such as the low rate of diffusion, is an unimportant factor. In this report, we compare LSIMS spectra obtained from a series of alkylamines introduced onto the surface of a glycerol sample with spectra obtained from solutions obtained from the corresponding alkylammonium halides. When the amines are admitted as a gas, it can be demonstrated that diffusion does not play a role in the intensity of characteristic secondary amine ions, so that the role of surface activity can be examined cleanly.

For the present work, surface activity and Henry's Law Constants for C_4 - C_{10} alkylamines in glycerol solutions were measured. When introduced via the gas phase, the intensity of amine-characteristic secondary ions was found to vary with pressure and as a linear function of the inverse Henry's Law constant. For intensity to be a linear function of surface concentration when amines are introduced via the gas phase, the intensity would have to be both a linear function of the surface activity and an inverse function of the Henry's Law constant. Consequently, the results indicated the origin of secondary ions was not the surface monolayer, but rather some layer beneath it. This result is in direct conflict with conclusions of others. Furthermore, for the corresponding spectra of the amine HCl solutions, there was a direct correspondence between the intensity of secondary protonated amine ions and their respective surface activities.

The conflict was resolved by the curious correlation between the surface activity and Henry's Law Constants for the amine/glycerol systems studied. Figure 1 shows that when the log of each set of constants is plotted as a function of alkyl chain length, the plots are parallel. This means that any function that correlates with Henry's Law Constant also correlates with surface activity. A plot of sensitivity ($d[M+H]/dP$) vs. surface activity is shown in Figure 2. Since it is observed that intensity correlates with pressure, correlation with surface activity rather than Henry's Law requires that the surface precursor to the detected amine-characteristic ion be other than free base amine, and is most likely adsorbed protonated amine. If the precursors to detected secondary amine ions were adsorbed free base amine, then secondary ion intensity would correlate with the quotient of surface activity and Henry's Law Constant. The correlation of secondary ion intensity with pressure must arise because the pressure determines the rate at which amine molecules strike the surface, are adsorbed, and then protonated. Reactions 1-3 describe the mechanism and are consistent with results:



Reaction (3) indicates that adsorbed protonated amine, $AmH^+(a)$, concentration is in equilibrium or near equilibrium with dissolved protonated amine, $AmH^+(l)$ that is diffusing into the liquid sample from the surface. Equations (1)-

(3) indicate the surface concentration of protonated amine is determined by the surface activity of the protonated amine, and the partial pressure of amine. This is because surface activity determines the rate at which the protonated amine leaves the surface, and amine pressure determines the rate that adsorbed protonated amine can be formed. Under the steady-stage conditions of the experiments, the two rates must be equal. Since neither of these rates is a function of Henry's Law, no correlation between sensitivity and Henry's Law Constants would be expected.

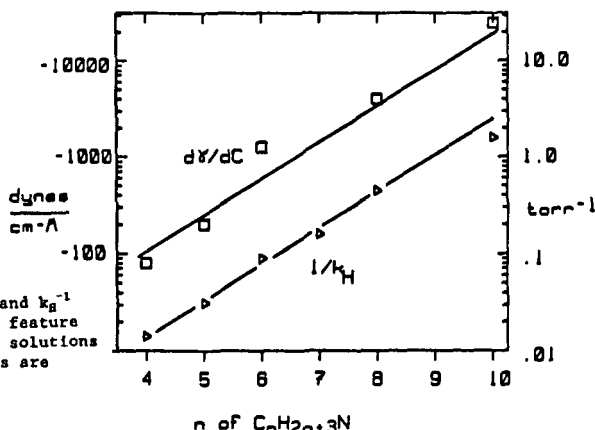


Fig. 1. Surface activity and k_H^{-1} vs. alkyl chain length. A feature peculiar to amine/glycerol solutions is that these two functions are linearly related.

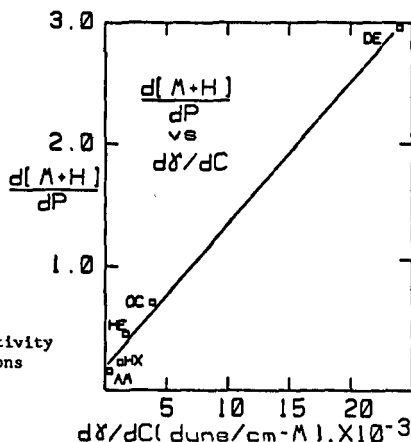


Fig. 2. Correlation between sensitivity and surface activity. Secondary ions are emitted from the surface.

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NEGATIVE MOLECULAR IONS FROM SAMPLES ADSORBED ON NITROCELLULOSE FOR keV AND MeV PARTICLE BOMBARDMENT

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In recent comparisons between keV and MeV particle bombardment mass spectrometry, the strong similarity between positive ion spectra of organic molecules (mostly peptides) from insulating surfaces has been emphasized [1,2,3], although the yield for MeV bombardment is higher especially for large molecules.

For peptides adsorbed to nitrocellulose, the yield of negative molecular ions ejected by MeV bombardment is about 5 to 10 times lower than that for positive molecular ions. We were therefore surprised to observe that for keV bombardment, the signal-to-noise ratio for negative molecular ions of insulin (MW 5733) is comparable to that of positive molecular ions as shown in fig. 1. Similar results have been obtained for glucagon (fig. 2) and renin substrate (MW 1760). We have also observed a small 1^- peak for phospholipase A_2 (MW 13980) and a broad peak that may correspond to 2^- (fig. 3).

The two measurements in fig. 1 were made by reversing the polarity of the target from +12 kV to -12 kV so the energy of the incident Cs^+ ions emitted at 22 kV is very different in the two cases: 10 keV for positive acceleration and 34 keV for negative acceleration. For these rather different energies, the absolute yield for insulin is approximately the same. Since the fission fragment yield drops by a factor of 5, changing target polarity (and thereby increasing the incident energy of keV Cs^+ ions) increases the ratio of keV to MeV yield by a factor of 5 under the present conditions. Much or all of this increase is probably due to the increase in incident energy.

The results have implications on the mechanism of energy loss associated with the desorption of large molecules by keV bombardment. From our previous comparison with positive molecular ions [3] the absolute yield of insulin molecular ions from nitrocellulose is about 20 times higher for fission fragment bombardment than for 18 keV Cs^+ bombardment. For negative molecular ions of insulin, the present results therefore indicate the yield is only a few times higher for MeV bombardment than for 34 keV Cs^+ bombardment. The desorption of molecular ions by MeV bombardment is clearly associated with the electronic stopping power, i.e. inelastic collisions with the target electrons. For ^{252}Cf fission fragments the electronic stopping is about 500 eV/Å. However, Cs^+ ions in the 10 keV range lose about 100 eV/Å by collisions with the target nuclei (nuclear stopping) but only about 10 eV by electronic stopping. Given the strong (approximately quadratic) dependence of yield on energy loss for MeV bombardment, it seems unlikely that the observed yield for negative molecular ions of insulin with keV bombardment is associated with electronic stopping.

The results also have some practical value: For a positive primary ion beam the energy at the target is higher for negative acceleration than for positive by twice the acceleration voltage. Furthermore, the primary ion energy does not limit the negative acceleration potential because the acceleration field does not retard the primary ions. Fig. 4 shows the spectrum of insulin taken with -15 kV acceleration instead of -12 kV of fig. 1. The improved detection efficiency of higher velocity ions and the increased incident energy gives a higher yield and better signal-to-noise ratio. In addition to molecular ions, the negative spectrum of insulin also shows much more intense peaks for the A and B chain fragments than does the positive ion spectrum.

In summary, negative ion spectra from nitrocellulose targets are practical for keV bombardment (in contrast to MeV bombardment) and in some cases give information complementary to the positive spectra. The high yield of negative molecular ions suggests that for keV bombardment, nuclear stopping is responsible for desorption.

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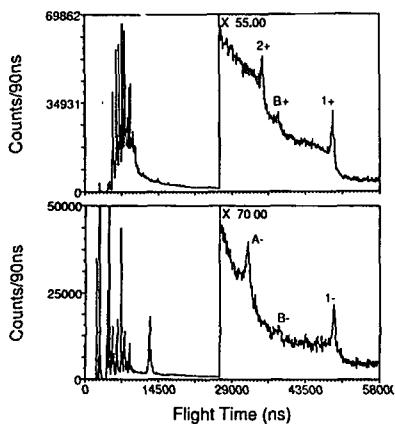


Fig. 1: Positive and negative spectra of insulin obtained with 10 keV and 34 keV Cs^+ bombardment. The secondary acceleration was 12 kV.

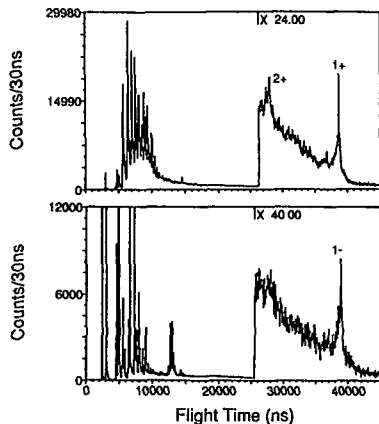


Fig. 2: Positive and negative spectra of glucagon (MW 3483).

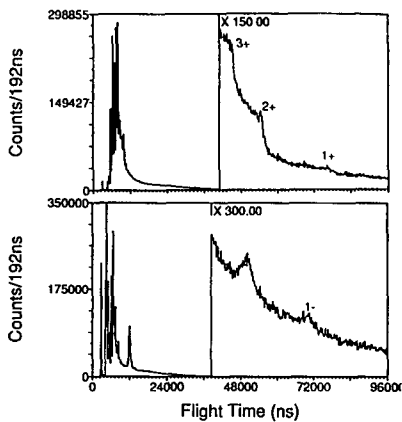


Fig. 3: Positive and negative spectra of phospholipase A_2 (MW 12980).

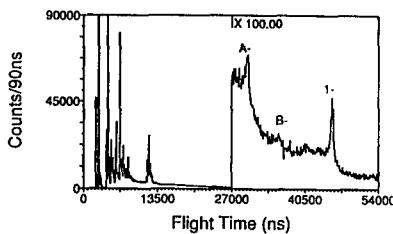


Fig. 4: Negative spectrum of insulin with -15 kV acceleration, and 37 keV incident Cs^+ ions.

ELUCIDATION OF ELECTRONIC AND VIBRATIONAL ENERGY LEVELS OF MOLECULAR IONS BY HIGH RESOLUTION TRANSLATIONAL ENERGY SPECTROSCOPY:

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The electronic and vibrational states of small molecular ions has been investigated at very high energy resolution, by means of Translational Energy Spectroscopy (TES). A high resolution spectrometer, of novel design, has been used which is capable of resolving energies ≤ 0.1 eV for collisions of ≤ 8 keV ions with collision gases. The technique is primarily suited to the detection of quasibound (metastable) states. It has similarities with photoelectron spectroscopy except that applications can now be made to ionic species which do not have stable neutral counterparts and also to multiply-charged ions.

This presentation concentrates on the selected dications CO^{2+} , NO^{2+} and N_2^{2+} which will illustrate the utility of the technique. The high-resolution translational energy spectrum of 6 keV CO^{2+} scattered off Ne and the energy change spectrum of single-electron capture by the same ion in collision with a Ne target, the latter is shown in Figure 1. Both the spectra and the dependence of the observed reaction channels on the ionising electron energy clearly show that only ground-state $\text{CO}^{2+}({}^3\Pi)$ and possibly $\text{CO}^{2+}({}^1\Sigma^+)$ ions live long enough (~ 1 μs) to reach the target cell. The excited state at 1.7 eV above the $\text{CO}^{2+}({}^3\Pi)$ ground state reported recently was not observed. The double ionisation energy of CO was measured to be 41.76 ± 0.1 eV.

Transitions among the three lowest $\text{X}^2\Sigma^+$, $\text{A}^2\Pi$ and $\text{B}^2\Sigma^+$ bound states of NO^{2+} are investigated by means of high resolution (0.08 - 0.35 eV FWHM) translational energy spectroscopy (TES). As well as electronic transitions between the ground and the two lowest electronic excited states, the spectra clearly show two distinct groups of vibrational transitions having average vibrational spacings of 0.22 eV and 0.17 eV respectively, shown in Figure 2. The two groups of vibrational levels are assigned to transitions involving $\text{NO}^{2+}(\text{X}^2\Sigma^+, 7_{\text{v}} \rightarrow 3 \leftrightarrow \text{v}=0)$ and $(\text{A}^2\Pi, 6_{\text{v}} \rightarrow 3 \leftrightarrow \text{v}=0)$. The measured vibrational spacings are in accord with the values of 0.158 eV and 0.242 eV predicted by the latest *ab initio* calculations of Cooper.

The high-resolution translational energy spectrum of 6 keV ${}^{29}\text{N}_2^{2+}$ scattered off He and energy change spectra of one-electron capture by the same ion in collision with He and Ne atomic targets are shown in Figure 3. The electron capture spectra recorded at different ionising electron energy indicate the presence of two metastable excited states at energies of 1.2 and 1.5 eV above the $\text{N}_2^{2+}(\text{X}^3\Sigma_g^-)$ ground state. Based on the latest *ab initio* configuration interaction quantum chemical calculations the two states are assigned to $\text{N}_2^{2+}(\text{C}^1\Delta_g, \text{b}^1\Pi_u)$ and $(\text{C}^3\Sigma_g^+)$ respectively.

Finally, energy loss spectra have recently been obtained for the excitation of ion beams, for example, of noble gases, of with surfaces. A channel plate array was employed and driven into the ion beam. Although the technique requires further refinement it shows great promise in enabling processes requiring high excitation energies and also allows measurement of excitation and de-excitation without the presence of the precursor ion beam, which is blocked off.

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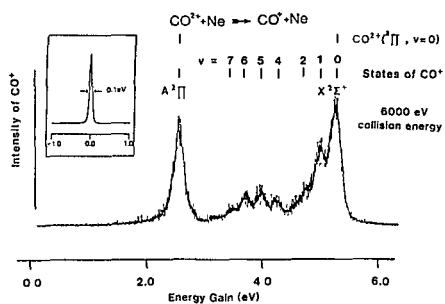


Figure 1

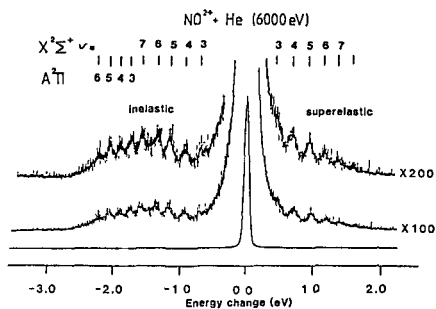


Figure 2

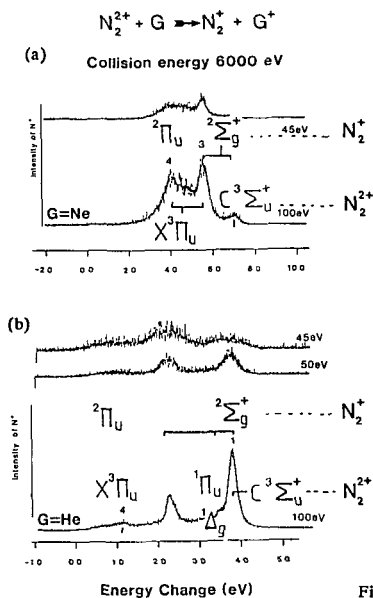
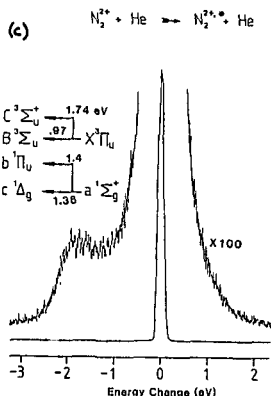


Figure 3



- Figure 1 Energy change spectrum for CO^+ ions produced by 6 keV collisions of CO_2^+ ($^3\Pi, v=0$) with Neon atoms.
- Figure 2 Translational energy spectrum of 6 keV NO_2^+ in collision with He.
- Figure 3 Energy change spectrum of 6 keV N_2^+ formed from single-electron capture with (a) He and (b) Ne. Translational energy spectrum of 6 keV N_2^+ in collision with He(c).

Structure Elucidation of Distonic Radical Ions
by Low-Energy Collision-Induced Dissociation

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The properties of distonic ions have been the focus of intensive research since it was discovered that these unusual ions are often kinetically as well as thermodynamically more stable than their conventional counterparts.¹ The present study of these ions has a two-fold goal: (i) to better understand the differences between two different activation methods used to study ion structures in quadrupole mass spectrometry and (ii) to provide further insight into the gas-phase chemistry of some interesting ionic species. We are especially interested in the stability of the isomeric compounds under multiple collision conditions, experimental conditions that are commonly used in quadrupole instruments and that are known to cause rapid isomerization of many ions prior to dissociation. We examined the dissociation behavior of several distonic radical ions and their conventional counterparts in a triple quadrupole mass spectrometer (Finnigan TSQ70) and in a Paul-type quadrupole ion trap (prototype Finnigan ITMS). Ions investigated include $[\cdot\text{CH}_2(\text{CH}_2)_n\text{OH}_2^+]$ vs. $[\text{CH}_3(\text{CH}_2)_n\text{OH}]^{+\cdot}$ ($n=0-2$) and the pyridine isomers, ionized 4-methylpyridine $[\text{CH}_3\text{C}_5\text{H}_4\text{N}]^{+\cdot}$ vs. $[\cdot\text{CH}_2\text{C}_5\text{H}_4\text{NH}^+]$. The ions of the $\text{C}_n\text{H}_{2n+2}\text{O}^{+\cdot}$ series have previously been studied by keV CID, metastable ion dissociation, NRMS, and ion/molecule reactions. The pyridine isomers have not been studied previously; the distonic isomer is representative of a series of ions produced by alkyl radical losses from compounds of the general type $^+\text{XCH}_2(\text{CH}_2)_n\text{CH}_3$. The ions of this series are of interest as possible intermediates and products of remote-site (charge-remote) fragmentation.²

The results indicate that low-energy (eV) collision-induced dissociation (CID), under single or multiple collision conditions in a triple-quadrupole instrument, can be used to distinguish the above pairs of isomers. For example, ionized ethanol $[\text{CH}_3\text{CH}_2\text{OH}]^{+\cdot}$ fragments by loss of methyl radical to produce protonated formaldehyde while the distonic isomer, $[\cdot\text{CH}_2\text{CH}_2\text{OH}_2^+]$, produced by electron ionization of 1,3-butanediol, fragments predominantly by loss of H_2O . Results obtained on the triple-quadrupole instrument are summarized in Table 1 and illustrated in Figure 1. For the $\text{C}_n\text{H}_{2n+2}\text{O}^{+\cdot}$ isomers, the quadrupole ion trap CID spectra were indicative of distinct structures only for the isomers $[\text{CH}_3\text{CH}_2\text{OH}]^{+\cdot}$ vs. $[\cdot\text{CH}_2\text{CH}_2\text{OH}_2^+]$. This may be explained by the fact that no products which required deposition of more than 1.3 eV were observed in the ion trap CID spectra; the light helium target used for collisional activation in the ion trap makes fragmentation pathways with high internal energy requirements inaccessible. The results from the two different types of instruments suggest that the isomerization barrier between the ions of the pair $[\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}]^{+\cdot}$ vs. $[\cdot\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}_2^+]$ is lower than the barriers associated with the shorter chain homologues ($n=0$ or 1), which is consistent with results recently obtained for the nitrogen analogues. The data obtained, in conjunction with thermochemistry from the literature, suggest isomerization barriers of > 29 eV ($n=1$) and approximately 12 eV ($n=2$) for the conversion $[\cdot\text{CH}_2(\text{CH}_2)_n\text{OH}_2^+] \rightarrow [\text{CH}_3(\text{CH}_2)_n\text{OH}]^{+\cdot}$ (Figures 2 and 3). These values are consistent with previous estimates.

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Table 1. Low-energy (eV) collision-induced dissociation products characteristic of distonic radical ions and their conventional counterparts (QQQ, multiple collisions w/ Ar).

ION	DISSOCIATION
CH_3OH^+	-OH
$\cdot\text{CH}_2\text{OH}_2^+$	$-\text{H}_2\text{O}$
$\text{CH}_3\text{CH}_2\text{OH}^+$	$-\text{CH}_3, -\text{H}$
$\cdot\text{CH}_2\text{CH}_2\text{OH}_2^+$	$-\text{H}_2\text{O}$
$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}^+$	$-\text{C}_2\text{H}_5, -\text{H}_2\text{O}$
$\cdot\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}_2^+$	$-\text{H}_2\text{O}$
$^+\text{HN}(\text{C}_6\text{H}_5)\text{CH}_2\cdot$	$-\text{CH}_4$
$^+\text{N}(\text{C}_6\text{H}_5)\text{CH}_3$	$-\text{CH}_3$

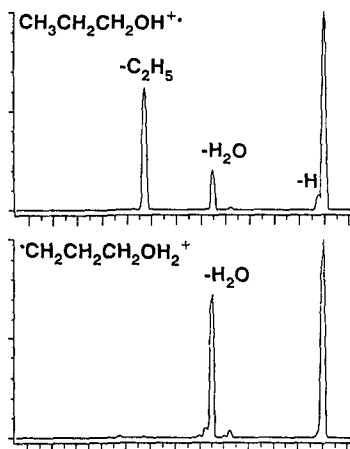


Figure 1. Low-energy collision-induced dissociation spectra of ionized propanol and its γ -distonic isomer (QQQ, 10 eV collisions, 0.3 mtorr Ar).

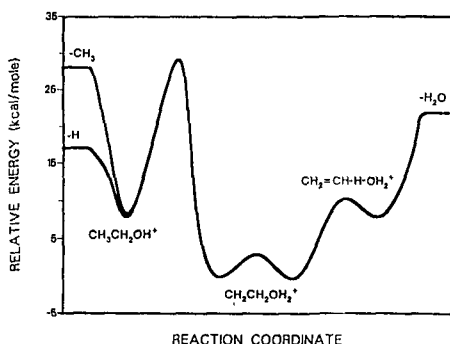


Figure 2. Potential energy diagram for ionized ethanol and its β -distonic isomer.

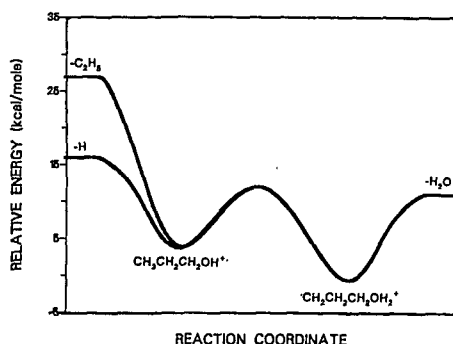


Figure 3. Potential energy diagram for ionized propanol and its γ -distonic isomer.

TANDEM MASS SPECTROMETRY OF LARGE BIOMOLECULES:
WHAT IMPEDES ITS USE FOR STRUCTURAL CHARACTERIZATION.

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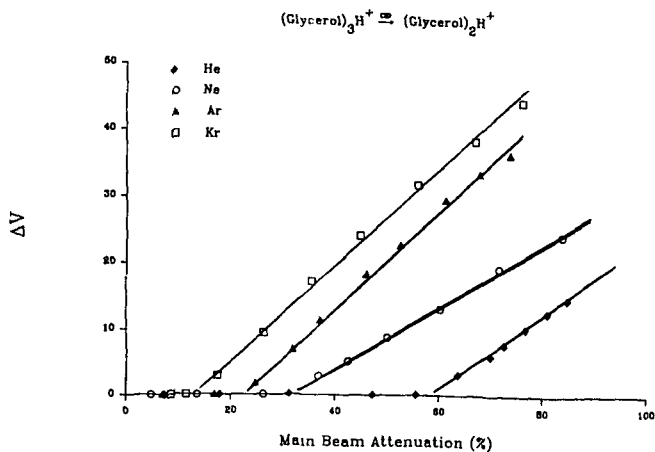
Tandem mass spectrometry is used in a number of laboratories for the structural characterization of complex organic and inorganic molecules. The degree of success of this work varies widely and is strongly dependent upon the type of molecule examined. As a general rule, tandem mass spectrometry studies of peptides and related molecules are the most successful, but even for this class of molecules the studies are limited to molecular weights of approximately 1500 daltons. Although one can find exceptions to the general statement, there are few cases where MS-MS spectra of peptides above m/z 1500 contain analytically useful data. It is also quite surprising that MS-MS data for peptides obtained on keV energy sector instruments is comparable to that obtained on triple quadrupole instruments. In fact, it can be argued that the low energy triple quadrupole data is superior (in terms of the signal-to-noise ratio and, presumably, the fragment ion yield) to that obtained for keV energy collisions with high performance sector instruments. *The questions that I wish to consider in this paper are directed toward understanding what physical processes limit the use of tandem mass spectrometry to analytical problems of biomolecules.*

Collision-induced dissociation is the most commonly used method for acquiring MS-MS spectra, but it is now well known that the efficiency for dissociating the $[M+H]^+$ ions of large molecules decreases as the size of the molecule increases. Although Derrick and co-workers have shown that a large fraction of the translational energy of the incident ion is lost upon collision with the neutral target,¹ work from this laboratory shows that only a small fraction of the energy loss value actually appears as internal energy of the ion.² In fact, studies performed by ion cyclotron resonance suggest that the cross-section for endothermic charge transfer reactions between the incident ion and the neutral target is much greater (by at least two orders of magnitude) than collisional activation of the incident ion.³ On the basis of earlier work,⁴ the greater cross-section for charge exchange (electronic excitation) (at keV energy collisions) is not at all surprising! *What then is the mechanism for keV energy collisional activation?* Undoubtedly, keV energy collisional activation involves vibrational excitation of the incident ion, and the vibrational excitation occurs by multiple collisions between the incident ion and the target neutral. Experiments performed in this laboratory on energy loss accompanying collisional activation and obtained by using a biased activation cell⁵ show clearly that large energy loss values are only obtained at high collision gas pressures, and above the threshold pressure where large energy loss values are observed the energy loss increases linearly with pressure (see Figure 1). Clearly, these data are consistent with collisional activation occurring by a multi-collision process.

Studies on the mechanism of charge-inversion CID also are consistent with the proposed involvement of multi-collision in CID of polyatomic species. The energy loss values accompanying CID of either positive or negatively charged metal ions, e.g., $M(CO)_y^{+/-}$ ($y=1-5$), are constant for all product ions. Conversely, the energy loss values for charge-inversion increase as the m/z ratio of the product ion decreases. In addition, the abundance of the low m/z product ions increases as the pressure of the target gas is increased (pressure measurements were correlated with %attenuation of the incident ion beam).

Another factor which greatly limits the development of tandem mass spectrometry is the current trend in instruments. Mass spectrometry is a complex field and the trend toward larger, more complex and costly instruments only serves to make the use of the methods more difficult for the biochemist in need of analytical information. There are less costly high performance instruments (with greater mass resolution, sensitivity, and experimental versatility) available than the highly complex four-sector instruments that are currently in vogue. Time-of-flight mass spectrometers are capable of performance which rival the most sophisticated sector instruments, especially at high mass. A separate paper from this laboratory describes a new instrument under development which has a high resolution sector for MS-I and a high performance TOF as MS-II. Instruments of this type will become more common over the next few years and will ultimately be the instruments which solve the structural characterization problems provided by the biotechnology scientific community. These types of instruments will make tandem mass spectrometry the long promised analytical method.

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THE PHOTODISSOCIATION AND METASTABLE DECOMPOSITION OF FAB PRODUCED IONS IN AN EXTERNAL SOURCE-FOURIER TRANSFORM MASS SPECTROMETER

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The external-ion source, quadrupole-FTMS(1) is ideal for studying the internal energy and fragmentation mechanism of FAB produced ions. In this report, laser photodissociation (PD)(2) is used to probe the structure of FAB produced ions and evidence for metastable decomposition and their rates are provided.

With small tetrapeptides, fragment ions produced by both FAB and PD (single pulse, 193 nm, 20 mJ) are derived from similar if not identical dissociations. For example, fragments from the tetrapeptide methionyl-arginyl-phenylalanyl-alanine (MH^+ , m/z 524) correspond to cleavages primarily along the peptide bonds in both processes. By far, the most abundant fragment ions correspond to the protonation of the terminal methionyl and the basic arginyl amino acids suggesting that, as with FAB, photofragmentation is governed by simple thermodynamic considerations. In general, photodissociation provides a greater abundance of low mass ions. The only evidence for photo specific processes is from the presence of m/z 120 corresponding to phenylalanyl immonium ion. Although present in the FAB spectrum, this ion is strongly enhanced in the PD spectra of all phenylalanine-containing peptides. The phenyl group is a stronger chromophore than the carbonyl group. Ejection pulses preceding each laser shot eliminates uninteresting ions. Thus even low intensity signals can be confidently assigned in the photodissociation spectrum.

The effectiveness of laser PD becomes more evident when used to determine the structures of FAB fragment ions. With the FTMS spectrometer, it produces more fragmentation and is more convenient to use than collisionally induced dissociation. For example, the FAB spectrum of the cyclic, symmetrical decapeptide, gramicidin S (MH^+ , m/z 1142) contains a half-fragment (m/z 571) which can be one or some combination of five isobaric pentapeptide species. Isolation of m/z 571 by selective ion ejection and subsequent photodissociation (Figure 1) yields granddaughter ions corresponding to sequential losses of amino acids, as well as all combinations of dipeptide, tripeptide, and tetrapeptide ion fragments. Analysis of the granddaughter ions prove unequivocally that the structure is that of a single species, corresponding to 1.

By using short injection pulses (5 msec) and short detect delay (1 msec to 100 msec) the metastable decomposition of the FAB produced ions are observed. Figure 2 shows an exponential decay for the relative intensity of the molecular ion of the undecapeptide, substance P (MH^+ , m/z 1346). The curve is a combination of two competing processes: a fast process with a rate of 30 s^{-1} and a slow process with a rate of 0.7 s^{-1} . The fast process is significantly faster than the collision frequency (10^{-4} s^{-1}) associated with the analyzer chamber pressure (10^{-10} torr). It is also strongly mass dependent being faster for lower massed gramicidin S (Table 1), and too rapid to be observed for the tetrapeptide valyl-alanyl-alanyl-phenylalanine (m/z 407). The rapid process is most likely due to the excess energy associated with FAB ion formation. Experiments are currently being performed to vary the conditions during ion formation and to determine their effect on the rate of the fast process.

In contrast, the slow process is essentially mass independent within the mass range we have studied (i.e. 500 to 1400 amu). Additional experiments must be performed to determine the source of this process.

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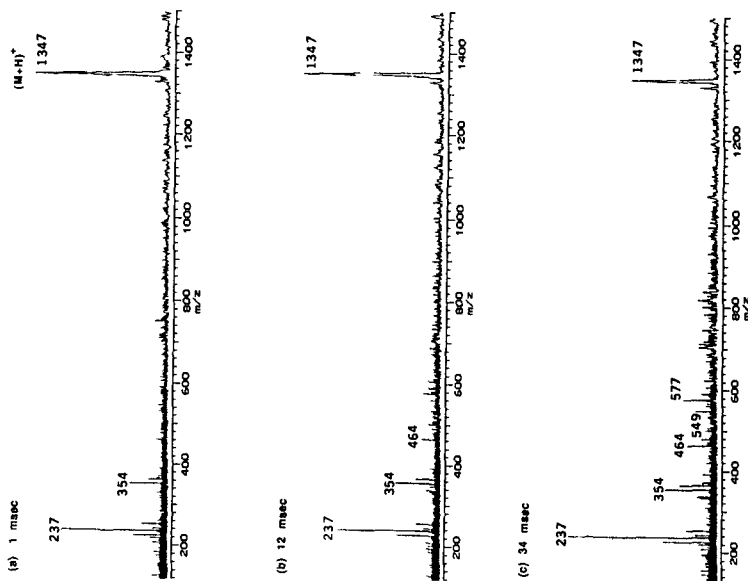
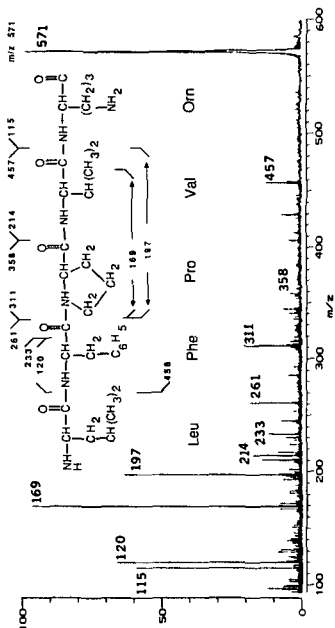
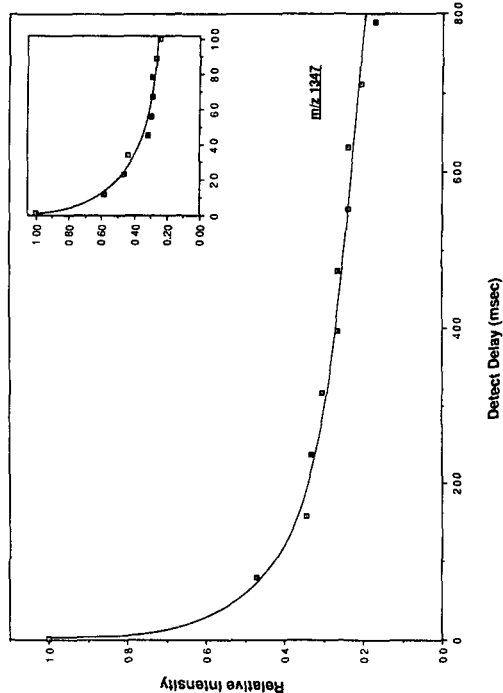


Figure 1 (upper left): The photodissociation spectrum and the structure of the m/z 571 fragment of Gramicidin S produced by FAB. Figure 2 (lower left): The decay of the relative abundance of the molecular ion of substance P as a function of detect delay time. Figure 3 (above): Representative spectra of substance P at three different detect delay time.



Metastable Decomposition of Substance P



Excited State Proton Transfer in Laser Mass Spectrometry
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The purpose of these investigations is to determine the analytical utility of excited-state proton transfer (ESPT) as an ion generation process in resonant laser mass spectrometry (LMS). Many aromatic compounds will undergo a functional group-specific change in pKa upon excitation to the S_1 state (1). ESPT has been cited in many resonant LMS investigations as a possible ion generation mechanism, however, no direct evidence for this process has been obtained prior to this study (for instance, see reference 2).

The laser desorption of both 1- and 4-fluorene-carboxylic acid with LiCl yields significant abundances of $(M-2H+3Li)^+$ ions (Figure 1) under LAMMA 1000 detection conditions at power densities around 10^8 W/cm², a wavelength of 266 nm and total energies of 9-12 μ J. The trillithiated molecular ion is formed by hydrogen-lithium exchange on the 9'-CH₂ bridge whose pKa changes from 20.5 in the S_0 state to -8.5 in the S_1 state (3) (equation 1). The abundances of the trillithiated ions probably provide a low estimate of proton detachment because proton reattachment should occur more readily than lithium attachment due to the greater mobility of the proton.

The laser desorption of some hydroxybenzoic acids with LiCl demonstrate that the lifetime of the excited state influences ion formation as well as pKa change. Salicylic acid forms $(M-2H+3Li)^+$ ions almost exclusively while p-hydroxybenzoic acid forms equal amounts of $(M-H+2Li)^+$ and $(M-2H+3Li)^+$ ions under approximately the same desorption conditions described above (Figures 2 and 3). The differences in the relative abundances of these ions formed upon irradiation may be attributed to the longer lifetime of the zwitterionic phototautomer of salicylic acid formed in the S_1 state when the pKas of the hydroxy and carbonyl groups invert (equation 2). However, if these ion formation processes were governed by ground state behavior just the opposite trend in ion abundances should be observed because the hydroxy pKa of the alpha isomer should be 4 units larger than the 4' isomer (Table 1). In order to verify this hypothesis, 2- and 4-hydroxybutanoic acid were codesorbed with LiCl under almost similar conditions of irradiation. These two compounds were chosen because they should mimic the ground state behavior of the hydroxybenzoic acids at a desorption wavelength of 266 nm. It is expected, based on the pKa data given in Table 1, that 4-hydroxybutanoic acid will yield more trillithiated molecular ions than 2-hydroxybutanoic acid and indeed this is what is observed (Figures 4 and 5). Many of the spectra show the 4' isomer forms the $(M-2H+3Li)^+$ ions exclusively while the 2' isomer forms only $(M-H+2Li)^+$ under the same desorption conditions. The differences in the desorption characteristics of the hydroxybutanoic and hydroxybenzoic acids verify that the molecular ion formation mechanism of the latter are governed by an excited state. Scheme 1 describes how excited state lithiation takes place.

In the next set of experiments we sought to ascertain direct evidence of protonated molecular ion formation by ESPT. 9-d₂-4-fluorene-carboxylic acid was synthesized in the same manner as 9-d₂-fluorene (5). The deuteration was determined to be 97% complete by EI mass spectrometry. The deuterated compound was codesorbed with several amino acids for the purpose of comparing the abundances of $(M+D)^+$ and $(M+H)^+$ ions (equation 3). It is known that the lattice energies of the amino acids are much larger than their neutral sublimation and gas-phase protonation energies (6). This means that the direct desorption of a preformed $(M+H)^+$ ion would not be thermodynamically favored over the plasma formation of a $(M+D)^+$ or $(M+H)^+$ ion. However, no $(M+D)^+$ ions were observed in these experiments. Furthermore, the codesorption of 9-d₂-4-fluorene-carboxylic acid and LiCl yielded no substantial abundances of $(M-(H+D)+3Li)^+$ ions. The reason for this is that deuterium loss is much slower than hydrogen loss. Murdoch and coworkers (7) determined that the kinetic isotope effect is almost 10 for lithium exchange with a deuterium on the CH₂ bridge of fluorene. Additionally, it was concluded in early fluorescence studies of fluorene that anion emission was not observed because hydrogen loss was slow with respect to fluorescence (2).

The investigations currently being undertaken are like those described above except with the acid and amine functional groups being deuterated and the 9' bridge is left hydrogenated.

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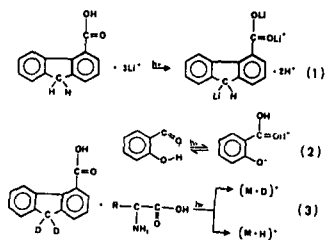
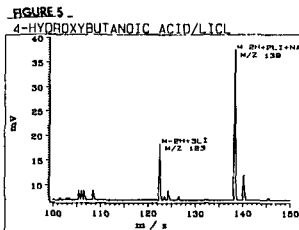
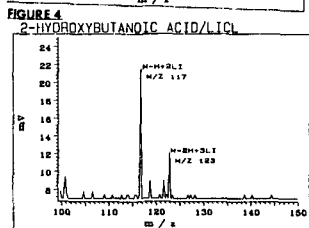
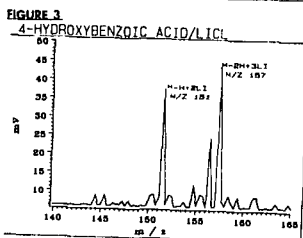
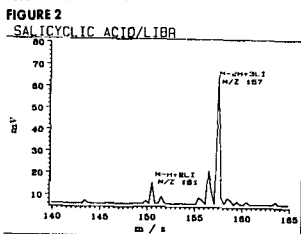
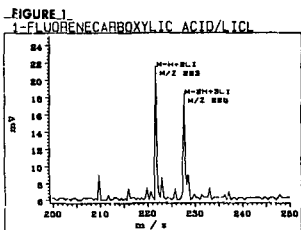
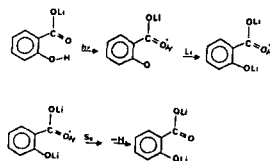


TABLE I

ACID	OH group	pKa	COOH group
Salicylic Acid	14	--	3.0
Benzoic Acid	--	--	4.2
Phenol	10	--	--
p-hydroxybenzoic acid	7	--	4.6
2-hydroxybutanoic acid	7	--	3.8
4-hydroxybutanoic acid	7	--	4.9

SCHEME I

Mechanism of Excited State Lithiation



DRY FAB MASS SPECTROMETRIC TECHNIQUES INCREASE PRACTICALITY
OF AND ACCESS TO TRACE METAL ANALYSIS IN MAN
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The absorption of trace elements, when they are consumed in a normal diet, depends upon a number of basic physiological and chemical interactions. The transport of a specific trace metal may be under direct homeostatic control (Cotzias 62) and the loss of a trace metal from the systemic circulation may also be regulated (Cousins 84). Determining these interactions is made more complex by specific interactions of the trace metal with components in food and with other metals and anions (Davis 80, Aggett 83). These interactions can take place in either the GI track or in the plasma after absorption (Solomons 82, Turnlund 84, Sandstrom 87).

Stable (non-radioactive) isotopes do not present the same health concerns as radioactive compounds but are considerably more difficult to measure (Hachey 87, Janghorbani 80). The analysis of isotope enrichment of trace metals in human nutritional research has been traditionally carried out using either neutron activation analysis (NAA) or a variety of different mass spectral techniques including ICP and thermal ionization (TI) mass spectrometry (Turnlund 84). In 1983, Smith showed that dry fast atom bombardment (FAB) could be used for the analysis of stable calcium isotopes in urine.

After the original report by Smith (83), three other laboratories, including ours, have reported on isotope analysis techniques using dry FAB/MS and have used them to carry out clinical studies (Peirce 87, Pratt 87, Lehmann 88). This approach has the advantage that it can be carried out on a shared, organic instrument; that it has adequate precision (1%); and that it has proven capability for Ca, Fe, Cu and Zn. This approach required a sample that is relatively free of species such as Na and K that compete for ionization in the FAB ionization beam.

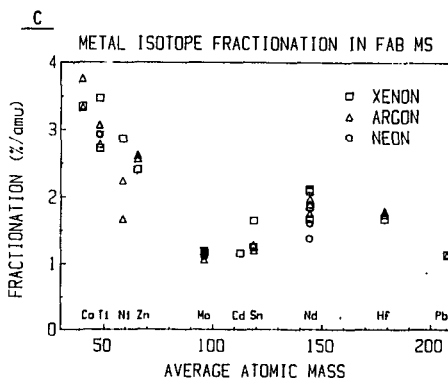
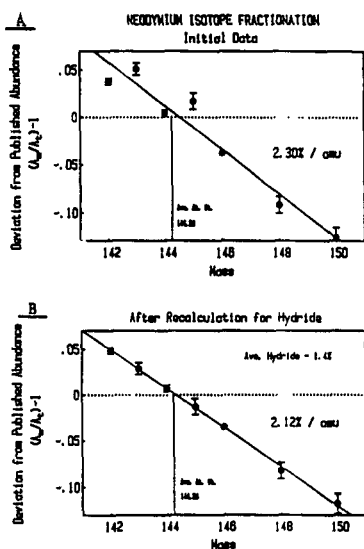
When our laboratory began to experiment with FAB for the analysis of the trace element Zn, it was not clear that this technique would have adequate sensitivity because of the low levels of this metal in the human diet (i.e. mg in contrast to gm levels of calcium). For this reason, we began our research using fecal samples where the concentrations would not be limiting.

In the initial experiments it was clear that we had adequate sensitivity to measure Zn in the expected concentration range. This sensitivity was evaluated by using pure zinc standards in acid solution. However, other metals found in the biological matrix (in particular Na, K, and Ca), suppressed the Zn signal to such a degree that the analysis could not be completed without a chemical separation step. A simple ion exchange technique that had been described by Kraus (53) and used for separation of trace elements by Turnlund (82) was employed. In this technique, divalent metals are retained by the ion exchange resin as $(MCl_3)^-$ and after other metals are removed in the initial wash, the Cl concentration is reduced in steps so as to allow each metal to elute as its neutral metal chloride (MCl_2) .

A separate problem, that also led to suppression of the Zn signal, was caused by the standard FAB target. The stainless steel of the target contributed significant peaks from Cr, Fe and their oxides. This problem was initially overcome by wrapping pure gold foil around the target. Recently, we have fabricated a target out of pure silver rod and this is the system we are presently using. We have reported the observed improvement in zinc signal strength as each of the above steps were included in the analysis (Peirce 87).

Although the sensitivity was adequate for our fecal studies, we noted that the observed isotope ratios did not match the theoretical. Initially, we believed the deviation from the theoretical was due to instrument parameters and we launched an investigation into the cause.

After a rather detailed study of as many of the ion optic parameters that could be changed on our VG 7070 EHF, we concluded that our observed isotope ratios were caused by fractionation during the FAB ionization process (Miller 86). This hypothesis led to a separate study of this phenomenon. A summary of this work has been reported (Miller 87). A similar relationship has been reported in the SIMS literature (Shimizu 82, Jull 82). Fractionation plots (see Figures A, B & C) have been completed for a number of elements. The negative slope of the line indicates that the lighter isotopes are, apparently, more abundant than the heavier isotopes. The value of the slope (e.g. 2.12‰/amu for Nd) is a measure of the magnitude of the fractionation. The regression line passes through "0" deviation at the average molecular weight of the specific element. Similar plots have been reported in the SIMS literature (Schwarz 87).



A separate observation taken from these fractionation plots shows that FAB metal ions are affected by "hydride" peaks. These are most often seen when there is a small isotope peak preceded by a larger isotope by 1 amu. For example, Nd-142 and Nd-144 +H contributes to the Nd-143 and Nd-145 peaks, respectively. The hydride contribution can be corrected out of the fractionation regression (see Figures A & B). However, this process of hydride formation leads to a greater error in the measured isotope ratios and should be considered when one is selecting isotopes for enrichment studies. Further work needs to be done in understanding how to minimize or eliminate this effect.

Results from recent patient studies using the techniques described above have exceeded our initial expectations. For example, we have been able to detect an effect of standard dye markers on Zn absorption (Johnson 89). The absorption of Zn during normal and stress situations (i.e. pregnancy) has been easily measure (English 89). The cumulative fecal excretion curves also reveal a positive slope. We believe that this is due to metal that has been absorbed and is being

lost from the circulation back into the GI track. A study where a subject was given dual isotopes (i.v. & oral) supports this hypothesis. These data show the fecal content after an oral dose of Zn-68 isotope and a simultaneous dose of Zn-70 isotope i.v. The fact that the slopes of these two curves are very close in magnitude would suggest that they are both a measure of the same biological activity (resecretion).

Recently, two additional laboratories have reported the use of dry FAB ionization for the measurement of Cu, Zn and Fe isotopic enrichment in human absorption studies (Pratt 87, Lehmann 88). Although we recognize that methods such as thermal ionization mass spectrometry are more precise than the methods that we are using, the increased sample throughput, the availability of organic mass spectrometers fitted for FAB ionization and the normal biological variation of trace metal absorption in different subjects offset the precision issue. Clearly, there are many applications where the FAB method is competitive. The availability of a large number of organic mass spectrometers fitted for FAB ionization provide a means of sharing the results of our research to other facilities.

In spite of the exciting and very encouraging success that we have experienced using this method, there is much that is yet to be accomplished. Progress must be made in the area of required sample size. As mentioned earlier, the fecal samples provide adequate sample quantities (0.5-2.0 ug) for our present studies. However, studies involving infant plasma or urine will only provide 1/10 of the sample amount for analysis. For example, we presently need 15 to 30 ml of blood for our adult investigations. For infants, we must reduce this requirement to no more than 1 ml and, ideally, to 0.2 ml. In order to reach this goal we will explore, at least, the following: (1) data acquisition using selected ion monitoring; (2) improvement in ionization efficiency; (3) improvements in ion collection; (4) improved computer models to aid in both the interpretation of our data and in the development of a better experimental strategy.

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NEW DEVELOPMENTS IN NEGATIVE THERMAL IONIZATION MASS SPECTROMETRY AND ITS APPLICATIONS

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Introduction

Due to its advantages negative thermal ionization mass spectrometry (NTI-MS) has increasingly been used for isotope ratio determinations during the last few years. The principles and preparation techniques for NTI have been described elsewhere [1]. Up to now, procedures for isotope ratio determinations have been developed for the following elements or species of these elements: B, C, N, O, S, Se, Te, Cl, Br, I, Mo, W, Tc, and Re. The main advantages of NTI-MS are the selective ionization method, relatively high ion currents, only a few interferences and, normally, simple sample preparation techniques.

Improvement in selenium isotope ratio measurements

Because of the importance of selenium as a toxic as well as an essential element the improvement of the isotope ratio measurement of this element and its applications is an important topic. Using a double-filament (Re) ion source and about 30 µg barium oxide on the ionization filament Se^- ion currents of approx. 10^{-12} A could be observed. This is valid for ionization filament temperatures of 940-990°C using 0.5 µg selenium as selenious acid. The ion current could be increased by a factor of 40 when a mixture of the selenium sample and 10 µl of a silica gel suspension were deposited on the evaporation filament. We believe that the silica gel prevents the evaporation of selenium at a temperature too low for a sufficient formation of Se^- ions.

Whereas the precision of selenium isotope ratio determinations of samples with natural isotopic composition lies in the range of 0.3-0.6% for the old technique [2], the new technique results in precisions in the range of 0.1-0.3%. With the silica gel technique it should be possible to reduce the uncertainty of the atomic weight of selenium, which is presently due to the insufficient precision and accuracy in the determination of selenium isotope abundances. Other fields of application are the determination of natural isotope variations, selenium trace analyses with IDMS and bioavailability studies. In three sediments (BCR standard reference materials No. 277, 280 and 320) selenium concentrations of $(2.03 \pm 0.06) \mu\text{g/g}$, $(0.73 \pm 0.03) \mu\text{g/g}$ and $(0.198 \pm 0.003) \mu\text{g/g}$ could be analysed with NTI-IDMS. These results agree well with the certified values.

Formation of PO_x^- and AsO_x^- ions

A new development is the production of negative thermal oxide ions of the monoisotopic elements phosphorus and arsenic. These ions were formed from phosphates and phosphites or from arsenates and arsenites in a single-filament (Re) ion source after depositing the sample compound, 30 μg $\text{Ba}(\text{OH})_2$ and 5 μl silica gel suspension on the filament. The optimum filament temperature is 1250°C where high ion currents ($> 10^{-10}\text{A}$) of PO_2^- and PO_3^- or of AsO_2^- and low intensities of AsO^- and AsO_3^- were produced. The intensity ratio $\text{PO}_2^-/\text{PO}_3^-$ depends on the temperature and increases with higher temperatures.

In principle, the formation of PO_x^- and AsO_x^- ions could be used in the following fields of application: production of phosphorus and arsenic containing ion beams, semiquantitative determination of these elements by their ion intensity, indirect quantitative analyses with IDMS using spike compounds labelled with ^{18}O and direct oxygen isotope ratio measurements in phosphates and arsenates. For the last two topics there is a problem due to possible oxygen isotopic exchange between the sample oxygen and the oxygen traces in the ion source under the high temperature conditions. This isotopic exchange reaction can influence the measured isotope ratio. Additional investigations must therefore be carried out to gain better control of this effect. Table 1 shows the results of an oxygen isotopic ratio measurement and the isotopic abundances, which were calculated from these data. ^{16}O in the phosphate sample of natural isotopic composition was determined with a relative standard deviation of 0.002%, ^{18}O with 0.5%.

Sulfur isotope ratio measurements

We developed a NTI-MS method for the direct measurement of sulfur isotope ratios in inorganic solids, e.g. in sulfates and sulfides, which also works well with sulfuric acid. Up to now, two different methods have been established for precise isotope ratio determinations of sulfur. The first one is the measurement of SO_2 or SF_6 in a gas mass spectrometer with a precision of about 0.05%. The second one is positive thermal ionization mass spectrometry using AsS^+ ions for detection with a precision of 0.1% [3]. The disadvantage of these two methods is the fact that all sulfur compounds must be converted into SO_2 , SF_6 or As_2S_3 .

Our new method does not require a conversion of the sulfur compound to be analysed. A double-filament (Re) ion source is applied. The sample is mixed with 5 μl of a silica gel suspension and then deposited on the evaporation filament. A solution of 30 μg $\text{Ba}(\text{OH})_2$ is deposited on the ionization filament and evaporated to dryness. Using a few micrograms of sulfur as sulfate or sulfide S^- ion currents up to 10^{-11}A could be observed at the detector side of the mass spectrometer MAT 261 under the optimum temperature for the ionization filament of 1100°C (the evaporation filament is normally not heated).

SO^- and SO_2^- ions could also be detected but with relatively low abundances. Measuring the isotope ratio $^{34}\text{S}/^{32}\text{S}$ with $(\text{NH}_4)_2\text{SO}_4$, Na_2S and Ag_2S samples relative standard deviations of 0.1-0.5% could be obtained.

The major topics we plan to investigate with the new NTI technique for sulfur are natural isotope variations, sulfur traces with IDMS and biochemical reactions with ^{34}S labelled compounds.

Tungsten isotope ratio measurements

Besides the measurement of non-metals with NTI-MS this method can also be used to analyse some of the transition metals using MO_x^- ions for detection. The formation of positive Na_2WO_4^+ ions had formerly only been used in a few cases for isotope ratio determinations of tungsten. Due to the low ion current obtained this is not a sufficient technique. Our NTI technique uses a double-filament (Re) ion source with about 5 μg lanthanum oxide on the ionization filament. Any tungstate compound can be used on the evaporation filament. With a 100 ng sample WO_3^- ion currents of $>10^{-11}$ A could be achieved for hours using the compact thermal quadrupole mass spectrometer THQ.

Tungsten is one of 21 elements in the periodic table where its atomic weight is uncertain by 0.015% and more due to the uncertainty of the isotopic composition of these elements. Our measurements of the isotopic abundances of tungsten with NTI show that this technique leads to improved data. The relative standard deviation we obtained was always much better than the uncertainty of the IUPAC data (Table 2) and it is also much better than most of the results given in the literature. NTI-MS can therefore be used in the future to improve the atomic weight of tungsten by a calibrated measurement. Up to now, we have applied this method for the determination of very low tungsten concentrations in different matrices with IDMS.

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Table 1. Oxygen isotopic composition measured in a sodium phosphate sample

Ratio or Abundance	Value
$\text{P}^{17}\text{O}^{16}\text{O}^-/\text{P}^{16}\text{O}_2^-$	0.00081 ± 0.00002
$\text{P}^{18}\text{O}^{16}\text{O}^-/\text{P}^{16}\text{O}_2^-$	0.00411 ± 0.00001
Abundance ^{16}O	$(99.754 \pm 0.002)\%$
^{17}O	$(0.040 \pm 0.001)\%$
^{18}O	$(0.206 \pm 0.001)\%$

Table 2. Isotopic abundances (%) of W measured with NTI-MS

Isotope	NTI	IUPAC
^{180}W	0.12 ± 0.00	0.13 ± 0.03
^{182}W	26.39 ± 0.03	26.3 ± 0.2
^{183}W	14.47 ± 0.06	14.3 ± 0.1
^{184}W	30.63 ± 0.02	30.67 ± 0.15
^{186}W	28.39 ± 0.02	28.6 ± 0.2

HIGH SENSITIVITY ISOTOPE DILUTION (IDMS)
USING THERMAL IONIZATION, RESONANCE IONIZATION,
AND SECONDARY IONIZATION MASS SPECTROMETRIES--TIMS, RIMS, AND SIMS.

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Isotope dilution mass spectrometry (IDMS) is a technique of proven high accuracy that is used routinely at NIST (formerly NBS) for the certification of elemental concentrations in Standard Reference Materials (SRMs). We have recently been investigating the use of mass spectrometric methods other than the traditional thermal ionization method for IDMS. Specifically, we are interested in improving our sensitivity for the broadest range of elements while maintaining the accuracy inherent to the technique.

IDMS is based on the addition of a known amount of enriched isotope, called the "spike" to a sample. After equilibration of the spike isotope with the natural element in the sample, chemical separation of the element from the matrix is typically done. This separation need not be quantitative, a decided advantage for the technique. The isotopic ratio defines the elemental concentration and this ratio will not change with element loss. Mass spectrometry is used to measure the altered isotopic ratio(s).

IDMS is an inherently accurate technique because the sources of systematic error are readily identified and assessed. The major sources of systematic errors are: chemical "blank" or contamination which occurs in chemical processing, handling, and analysis of samples; dissolution or equilibration problems; isobaric interferences in the mass spectrometer, both elemental and molecular; and, physical or instrumental processes that change measurement efficiencies between isotopes of an element. For most analytical work at the part-per-million level and above, these potential sources of systematic error can be made negligible (e.g. for blank or background problems) and/or can be controlled. As the concentration levels drop below parts-per-million to parts-per-billion and parts-per-trillion, these sources of systematic error become much more important and much less easy to control. However, mass spectrometric instrumentation with differing strengths (sensitivity, selectivity, ratio measurement precision, etc.) can be used advantageously for ultratrace IDMS analysis. Two experiments of several that we have done are presented here. Both illustrate IDMS analysis of oyster tissue, SRM 1566a. These examples demonstrate advantages of SIMS and RIMS relative to TIMS, and the trade-offs in precision that may be made. At the ultratrace level, precision will often be limited by material variability. Thus, reduced precision can be tolerated if accuracy can be more readily obtained.

Secondary ion mass spectrometry (SIMS) is a sensitive, as well as isotopically selective, method for the direct analysis of solid materials. It is therefore surprising that only a few investigations of IDMS-SIMS have been described to date. We have made a comparison between SIMS and TIMS for the concentration measurement of uranium and thorium by IDMS. The chemical procedures for the separation and measurement by TIMS of U and Th have already been established (1) and provide a baseline against which SIMS results can be compared. We have analyzed oyster tissue and prepared samples that were split and analyzed by the two techniques.

The chemical procedure consists of spiking with ^{233}U and ^{230}Th , wet ashing, and anion exchange separation of the U and Th from the matrix and each other. The samples were dried onto a pyrolytic graphite substrate for SIMS analysis. No difficulty was encountered in generating adequate signal for the nanogram amounts of U and Th that were loaded from the samples and the picogram amounts from the blanks. The spectral background was less than a few counts per second and the samples were not exhausted during measurement. Three or four independent ratio measurements were made from different regions of each dried deposit. The variance in the ratio measurement for a set of ratios on a single spot did not significantly exceed that from counting statistics. However, the spot-to-spot variability was larger than would be from counting statistics alone for most of the samples. The results are shown in Table I.

Table 1. Isotope Dilution Results, SRM 1566a.

	Uranium (ng/g)		Thorium (ng/g)	
	SIMS	TIMS	SIMS	TIMS
OT-1	128 24	129.60	33.30	33.33
OT-2	129 91	-	35.59	-
OT-3	130 96	129.65	42.21	42.39
OT-5	-	130.03	-	32.74
OT-6	-	128.82	-	33.80
Blank-1	10.0 pg	-	43 pg	-
Blank-2	-	9.7 pg	-	176 pg

The agreement between SIMS and TIMS is about 1% or better. It can be concluded that neither technique has difficulty making quantitative measurements down to the chemical blank level. For Th the TIMS measurements near the blank level are hindered by a loading blank problem--Th that is in the Re filaments used in the measurement. The ratio of Th loading blank to sample Th increases with temperature and thus, lower temperatures (lower signals) were deliberately used. This problem seems to be readily avoidable with SIMS since the substrate material is basically inert in the measurement. The Th results illustrate the greater variability of concentration that can be expected among samples at the ultratrace level. The OT-3 concentration was significantly higher than the other samples. Although initially tempting to ascribe this result to contamination at some point before the mass spectrometry, neutron activation analysis of this same material confirms a relatively large between bottle variance for this material.

Resonance ionization provides elemental selectivity which is extremely useful when isobaric interferences are limiting in the measurement. It is not so surprising that number of IDMS-RIMS investigations are small, considering the limited number of RIMS instruments. We have applied our RIMS instrument to extensive Os/Re isotopic measurements (2). We have recently determined iodine in oyster tissue using RIMS. It is instructive to compare the technique with IDMS-TIMS procedures that have been applied in the past in our laboratory (3).

The oyster tissue samples were spiked with ^{129}I , wet ashed under controlled conditions, and separated by coprecipitation with silver chloride. For RIMS the analyte was dried as silver ammonium iodide upon a single tantalum filament from which iodine was thermally desorbed in the RIMS instrument. A single-color, 2-photon resonant plus 1-photon ionization scheme was used to form positive iodine ions. Long-lived iodine signals were achieved from 100 ng of iodine.

The ratio measurement precision for RIMS was 1.0% as determined from replicate measurements of the spike, the spike calibration samples, and the oyster tissue samples and was 1.0%. Measurement precision among samples was 1.9% for the spike calibration and 1.4% for the oyster tissue. TIMS results for SRM Powdered Milk at comparable concentrations to the oyster tissue (PM, 3.376 ppm; OT, 4.444 ppm) demonstrated 0.15% precision among samples. The blanks were higher in the TIMS experiment, 45 ng, relative to the RIMS experiment, 11 ng. However, this cannot be ascribed to the differing mass spectrometric techniques.

These RIMS measurements did not exploit the selectivity of the photo-ionization process. However, the sensitivities of RIMS and TIMS were comparable and, again, the limit was the chemical blank. The precision of RIMS was a factor of 10 poorer than TIMS. However, the IDMS-RIMS precision was still a factor of 10 better of the second technique to be used in the certification, neutron activation analysis. We felt that the RIMS was advantageous relative to TIMS in two ways, both related to the thermal, triple-filament procedure. First, we avoided the need to prepare and condition the LaB_6 ionization filament--RIMS was simpler in sample loading. Second, we avoided heating the source to 1000°C --memory effects, a problem for iodine, could be more easily controlled.

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RIMS APPLIED TO ENVIRONMENTAL AND NUCLEAR RESEARCH

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Laser resonance ionization mass spectroscopy (RIMS) is used in a variety of spectroscopic schemes which allow the study of optical resonances with high sensitivity, selectivity and resolution. It is well suited for the detection, identification and investigation of small amounts of material. Therefore RIMS is in many cases the method of choice for trace analysis as, e.g., for environmental studies, and in nuclear research as for the determination of nuclear ground-state properties [1].

1. Introduction

RIMS is based on stepwise, resonant excitation of atoms or molecules with laser light followed by photo-ionization. This process is called resonance ionization spectroscopy (RIS) [2,3]. The selectivity of this technique can be increased by mass spectrometry of the ions created in the optical resonance.

The sensitivity of this method is affected by a couple of factors:

- The efficiency of the source for production of free, neutral atoms
- The initial occupation probability of the atomic ground state
- The spatial overlap of the atomic beam with the RIS laser beams
- The temporal overlap of the atomic beam with the RIS laser beams
- The excitation probabilities of the RIS path
- The transmission of the mass spectrometer
- The efficiency of ion detector
- The nuclear half live in case of the investigation of short-lived nuclei.

The overall detection efficiency can be defined as the ratio of the ions detected in resonance by the ion detector to the number of atoms in the sample under investigation. It is given by the product of all the factors listed above. A low detection limit requires an optimization of each factor.

The overall selectivity is given by the product of the selectivity of each resonant excitation step and the resolving power of the mass spectrometer. The latter is typically of the order of 10^3 . The former can be estimated by comparing the density of the atomic levels (about one per eV) with the line width generally given by the Doppler broadening (about 1 GHz). Therefore the selectivity can be estimated to be of the order of 10^5 per resonant excitation. This gives an overall selectivity of about 10^{13} for a two-step excitation followed by mass spectrometry.

In the following three experiments will be described having RIMS as an essential ingredient: The first one concerns the study of nuclear ground-state properties of unstable Au and Pt isotopes; the other two are trace-analysis experiments for the detection of Pu and Tc and ^{89}Sr , ^{90}Sr in the environment.

2. Ground-state properties of gold and platinum

The nuclei near $Z=80$ and $N=104$ have attracted considerable attention following the discovery of a big change in the ground-state mean-square charge radius between ^{187}Hg and ^{186}Hg , which was interpreted as an onset of strong deformation at $A \leq 185$ [4]. A measurement of the isotope and/or isomer shift represents the most straightforward and model-independent signature for shape transitions or shape coexistence. A RIMS experiment was designed to determine the isotope shifts of short-lived Au and Pt isotopes, the neighbours of Hg in the periodic table of elements. Since no target ion-source system existed at the on-line mass separator ISOLDE at CERN for a direct production of these isotopes, they had to be obtained as daughters of Hg isotopes, which can be produced with yields up to 10^{10} atoms per second and mass number by a $\text{Pb}(p,3\text{pxn})\text{Hg}$ spallation reaction. Because of the short half-lives and the small production rates of the isotopes far from stability, the investigations had to be performed in an on-line mode. The Hg ion beam was implanted into a graphite wheel. After the Hg isotopes had decayed into Au or Pt these were desorbed by a pulsed Nd:Yag laser to create a pulsed atomic beam. The atoms were excited and photo-ionized via a 3-colour, 3-step resonant process by the light of three tunable dye lasers pumped by a second Nd:Yag laser. In case of Pt, the frequency-doubled output of the same Nd:Yag laser was used for the last step. In order to increase the signal-to-noise ratio, the photo ions were detected and mass-selected by a time-of-flight (TOF) spectrometer using an ion reflector. A background counting rate of typically one event per 1000 laser pulses was observed.

The overall detection efficiency has been determined to be $3 \cdot 10^{-5}$ (defined as the ratio of ions detected by the mass spectrometer to the number of atoms collected on the wheel). This represents an increase in efficiency by a factor of about 1000 compared to the use of an oven producing a continuous atomic beam.

With this technique the hyper fine structure and isotope shift of the short-lived, neutron-deficient isotopes ^{184}Au and ^{183}Au as well as of Pt down to ^{183}Pt have been measured [5,6].

3. Trace analysis of plutonium and technetium

Another way to reach a high temporal overlap of the RIS laser beams with the atomic beam is to use a laser with high repetition rate ν_{rep} . Since a thermal ion traverses the region of interaction with the laser light within about 10^{-4} s the duty cycle comes close to unity for $\nu_{\text{rep}} = 10 \text{ kHz}$. This strategy was followed up at Mainz for trace analysis of Pu: One or two Cu-vapour laser ($\nu_{\text{rep}} = 6.5 \text{ kHz}$) pump two or three dye laser. As in the Au/Pt experiments, the interaction takes place in the acceleration region of a TOF spectrometer. Pu samples containing between 10^{10} and 10^{12} atoms of ^{239}Pu electrolytically deposited on a Re filament have been investigated. From these experiments an overall detection efficiency of $4 \cdot 10^{-6}$ has been determined.

Plutonium is one of the most serious radioactive poisons in the environment. Therefore very sensitive and selective methods are required to study the ecological behaviour and migration in the environment. The detection efficiency corresponds to a detection limit of about 10^7 Pu atoms in the sample, which is 40 times more sen-

sitive than α -spectroscopy [7,8]. This method has been applied to technetium at a similar level of sensitivity ($2 \cdot 10^{-8}$). The detection of extremely small amounts of technetium is also important in connection with the measurement of the high-energy solar neutrino flux via ^{98}Tc formed in molybdenum ores. In test experiments, samples down to 10^8 atoms of technetium could be measured. Furthermore it has been demonstrated that molybdenum is suppressed by a factor of $5.2 \cdot 10^{-8}$ at the technetium resonance [9].

4. Trace analysis of ^{89}Sr , ^{90}Sr

The trace analysis of ^{89}Sr and ^{90}Sr is very important in the evaluation of nuclear reactor accidents. Since the conventional ^{90}Sr -determination takes at least one week, a fast but also sensitive method is required. This method must be extremely isotope-selective because of the large surplus of stable Sr isotopes. The set up currently constructed at Mainz aims at a detection limit of 10^8 radioactive atoms in 1 mg natural strontium. Therefore an isotopic selectivity of almost 10^{11} is required. This will be achieved by the combined application of different techniques: Depletion of stable Sr in the sample by use of a medium-current isotope separator, production of a 50 keV-ion beam by surface ionization, mass separation, neutralization by charge exchange in collinear geometry and RIS. The most important step in this experiment will be the use of collinear spectroscopy [10]. The mass-separated ion beam will be neutralized in a Cs gas cell and the atoms will be ionized again by RIS. Using the collinear beam technique the resonances are Doppler shifted in dependence of the mass of the isotopes leading to an isotope separation of the order of some GHz. Furthermore the Doppler broadening is largely reduced. Using cw laser an extreme resolution of $\Delta\nu/\nu = 10^{-8}$ has been achieved [11]. A selectivity of the order of 10^8 to 10^9 is expected. Adding the previous mass separations and a further selectivity by depletion of the unwanted stable Sr by optical pumping the overall selectivity will be sufficient for the measurement of $^{89,90}\text{Sr}$.

The examples given show that RIMS is a valuable and in many cases indispensable technique in environmental and nuclear research.

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Synthetic Silicon Isotope Mixtures for
Absolute Isotope Abundance Ratio Measurements

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An isotope abundance ratio measurement is called absolute when it is based on a calibration by means of quantitatively synthesized mixtures of chemically purified highly enriched isotopes. The isotope (i) ratios R_i thus obtained yield an absolute atomic weight A_r when combined with the nuclidic masses M_i of the element under study using the formula :

$$A_r = \frac{\sum_{i=1}^n R_i M_i}{\sum_{i=1}^n R_i}$$

The atomic weight of a nearly perfect Silicon crystal, $A_r(\text{Si})$, resulting from this study, is used in a redetermination of Avogadro's Constant N_A according to the formula :

$$N_A = \frac{A_r(\text{Si})}{\rho \cdot a_0^3/n}$$

This redetermination is a joint PTB-CBNM effort where PTB stands for Physikalisch Technische Bundesanstalt, FRG and CBNM for the Central Bureau for Nuclear Measurements, Belgium.

The representative isotopic composition of natural Silicon is given as follows : ^{28}Si , 92.23%; ^{29}Si , 4.67%; ^{30}Si , 3.10% [1]. The isotope ratios $^{29}\text{Si}/^{28}\text{Si}$ and $^{30}\text{Si}/^{28}\text{Si}$ are then respectively 0.050 6 and 0.033 6.

The enriched Silicon isotopes, needed to prepare the synthetic mixtures, are delivered by Oak Ridge National Laboratory in the Silicon dioxide, SiO_2 , form. The "Silicon 28 isotope" is over 99.5% enriched and both "the 29 and 30 isotopes" have an enrichment of over 96%. These SiO_2 materials are converted into Cesiumhexafluorosilicate, Cs_2SiF_6 , the precursor compound. It is from these compounds that portions are withdrawn for gravimetric mixing, for assaying and for impurity determination. The Cs_2SiF_6 mixtures are converted into Bariumhexafluorosilicate, BaSiF_6 , that releases Silicontetrafluoride, SiF_4 , on heating. SiF_4 allows the most precise Silicon isotopic measurements. The flow diagram of the chemistry is shown in Fig. 1. The Cs_2SiF_6 compound will be discussed in more detail. Note that a part of the enriched Cs_2SiF_6 's is converted to BaSiF_6 directly to allow the measurements of their Silicon isotopic composition. The natural samples, of high purity, are also converted to BaSiF_6 .

For a needed total uncertainty, from chemistry and abundances of the enriched isotopes, of around 5×10^{-4} on the ratios of the mixtures the uncertainty due to the chemistry i.e. assay, impurity determination and mass determination, should not exceed 1×10^{-4} for each of these three components. As a consequence, the precursor compound, Cs_2SiF_6 , must have, and indeed has, some unique properties :

- it attains a stable mass when dried at 300°C

- it gains only 0.008% in mass when the relative humidity is varied from 0 to 80%
- its solubility can be reduced by lowering the temperature of its aqueous HF solution and by addition of ethanol. CsF is more soluble and does not cause problems
- it is convertible to BaSiF₆ by adding BaCl₂ to its hot aqueous HF solution
- it can be accurately weighed to a few µg, in a range of a few g to 0.2 g, in a controlled atmosphere
- it can be examined for impurities by spark source mass spectrometry
- it is crystalline with an average crystal size of 2 µm
- and the inclusions in the crystals are limited and removable on heating at 300°C.

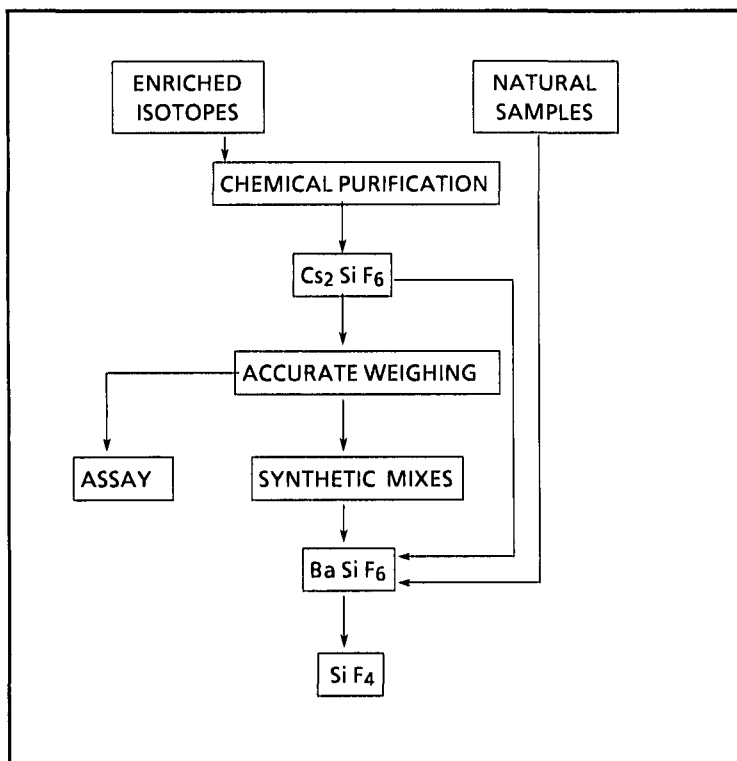


Fig. 1

The Cs₂SiF₆ compound is prepared out of the SiO₂ enriched isotope by taking this in solution with high purity HF. The solution of 0.25 M H₂SiF₆ in 0.5 M HF thus obtained is filtered and purified on a strongly acidic cation exchange column (BioRad 50W x 8, 100-200 mesh). The Cs₂SiF₆ is precipitated by adding Cesiumchloride, CsCl. Finally, the Cs₂SiF₆ is reprecipitated, filtered and dried at 300°C.

Two synthetic mixtures are prepared and they bracket the natural (WASO 17.2 crystal) ratios as shown in Table 1.

	$^{29}\text{Si}/^{28}\text{Si}$	$^{30}\text{Si}/^{28}\text{Si}$
MIX 1	- 0.284%	- 2.862%
MIX 2	+ 1.659%	+ 2.604%

Table 1

The results of assay, impurity determination and mass determination of the enriched Cs_2SiF_6 's together with 2s uncertainties are compiled in Table 2.

Enriched Material	Assay		Impurity determination		Mass determination	
	Result (%)	Unc. (%)	Result (ppm)	Unc. (ppm)	Approx. mass (g)	Unc. (μg)
$\text{Cs}_2^{28}\text{SiF}_6$	99.996 2	0.002 4	13	50	1.400	5
$\text{Cs}_2^{29}\text{SiF}_6$	6 5	3 6	37	50	0.070	2
$\text{Cs}_2^{30}\text{SiF}_6$	4 8	1 2	30	50	0.045	2

Table 2

The resulting 2s uncertainty contributions, from the chemistry and the abundance determination of the enriched isotopes, on both ratios of e.g. synthetic mixture 2 are expressed in 10^{-5} in Table 3. Those resulting from the chemistry are considerably smaller than the 10^{-4} requested.

Ratio	Total	Mass spec.	Assay	Impurities	Mass determinations
$^{29}\text{Si}/^{28}\text{Si} :$ $5.155\ 3 \times 10^{-2}$	62.7	62.5	2.0	2.1	2.7
$^{30}\text{Si}/^{28}\text{Si} :$ $3.433\ 4 \times 10^{-2}$	37.6	37.3	2.0	2.1	4.0

Table 3

Using these synthetic mixtures an absolute atomic weight of a Silicon single crystal (WASO 17.2) is determined as 28.085 382 with a 2s uncertainty of 1.67 ppm.

This atomic weight and uncertainty are used in a redetermination of Avogadro's Constant N_A .

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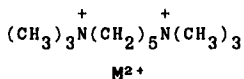
FACILITATING DESORPTION OF MULTIPLY CHARGED IONS

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Mass spectrometry of multiply charged ions has grown in importance both from intrinsic interest in obtaining confirmatory evidence for theoretical predictions of cation and anion stability, and for more immediately practical advantages offered by the potential for extending instrumental mass range through reduction of m/z . The high energies accessible for multiply charged ions with ordinary accelerating potentials also present interesting possibilities, particularly in the context of high-mass MS/MS.

Although there are instances of multiple ionization of both gas-phase and solid-phase samples (including field and plasma desorption), most approaches successful in M.S. sampling of multiply charged organic cations and anions have exploited the facile ionization of polar materials in solution (e.g., thermospray, electrospray, electrohydrodynamic, and, occasionally, fast atom bombardment). This reliance on solution chemistry provides an opportunity for fundamental studies of ion-solvent systems and sampling mechanisms [1,2], but can complicate optimization and spectral interpretation, especially for true "unknown" samples. Even in simple model systems, a choice must often be made between optimum overall sensitivity and optimum sensitivity for multiply charged ions. Of course, the more experimental parameters available (e.g., primary beam or spray interface parameters), the more complex the job of optimization. Reasoned effort at optimization can make orders-of-magnitude differences in sensitivity.

Among the solution chemical factors affecting sensitivity to multiply charged ions are ion pairing, solvation/desolvation, and redox chemistry. The first two can be seen to interact in the electrohydrodynamic (EH) MS of the diquatery ammonium ion M^{2+} in glycerol solutions containing various sodium salts as supporting electrolyte [3].



There is a direct correlation between the overall sensitivity to M^{2+} and the intensity ratio $i(MX^+)/i(M^{2+})$, where the numerator is the intensity of the ion pair involving M^{2+} and the anion X^- . Here, the reduced solvation of the ion pair results in increased sampling efficiency; overall sensitivity is increased when the abundance of multiply charged ions is reduced by employing a counterion that pairs extensively in glycerol (e.g., NO_3^-). Of course, pairing of singly charged ions (e.g., $(\text{CH}_3)_4\text{N}^+$) reduces sensitivity by depleting "pre-formed" ions in solution. This is evident even in FAB spectra (Table 1), despite the complications introduced by atom bombardment (*vide infra*).

The effect of solvation is also evident when comparing spectra obtained from a mixture of M^{2+} and $(\text{CH}_3)_4\text{N}^+$, with the ESA set to pass either ions with full acceleration energy, or ions with lower energy [4]. In the latter case, dications dominate. Due to relatively strong solvation, they are sampled with their solvation sphere partially intact; subsequent in-flight desolvation reduces ion kinetic energies. This effect accounts at least in part for the greater success of electrospray and thermospray experiments employing quadrupole, rather than sector, instruments.

When multiply charged ions are generated by ion association (rather than salt dissociation) in solution (as in most electrospray experiments), charge may be enhanced by selecting a thermodynamically preferred cation for attachment. For example, both poly(ethylene glycol) [5] and 18-crown-6 [6]

show a strong preference for potassium relative to other alkali metals.

Efforts to sample multiply charged ions can be frustrated by electrochemical reduction. This can result both from intrinsic solvent chemistry (e.g., the quantitative reduction of $\text{Ru}(\text{bpy})_3^{3+}$ to the corresponding $\text{Ru}(\text{II})$ complex by solvent glycerol [7]), and from beam-induced reduction (e.g., the further reduction of the $\text{Ru}(\text{II})$ complex to $\text{Ru}(\text{I})$ in FAB [8,9]). In the latter case, the extent of reduction can be mitigated [10] by employing a matrix with improved radical scavenging capabilities (e.g., m-nitrobenzyl alcohol, Figure 1), by reducing beam flux (Figure 2), and/or by increasing beam energy.

Comparison of EH, FAB, field desorption, and thermospray MS of the divalent cation of tubocurarine [2] suggests that high fields in the ionization region (either applied, as in EH; or self-developed during field evaporation, as in electrospray and thermospray) facilitate sampling of dications. This may account in part for the recent remarkable advances in high-charge electrospray MS, and for the ability of EH MS to sample doubly negative ions without fragmentation or oxidation. Due to the absence of perturbing effects from bombardment or droplet evaporation, EH spectra generally reflect relative solution equilibria in comparing similar systems. Note, for example, the reflection of relative pK_a s in comparing the spectra of Indigo Carmine and Bromcresol Green (Table 2)

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Table 1: Effect of Ion Pairing
on FAB Sensitivity

Counterion	Relative Sensitivity
NO_3^-	22
Cl^-	29
NO_2^-	42
OAc^-	100

Table 2: EH Negative Ion Mass Spectra^a

Indigo Carmine MW: 466 (Na_2 Salt) (pK_a : Strong Acid)		Bromcresol Green MW 698 (pK_a : 4.7)	
$[\text{M}+\text{Go}-2]^{2-}$	100%	$[\text{M}+\text{Go}-4]^{2-}$	20%
		$[\text{M}+\text{H}+\text{Go}-2]^-$	15%
		$[\text{M}+\text{Na}+\text{Go}-2]^-$	65%

^a G_n denotes retention of n
glycerol solvent molecules.

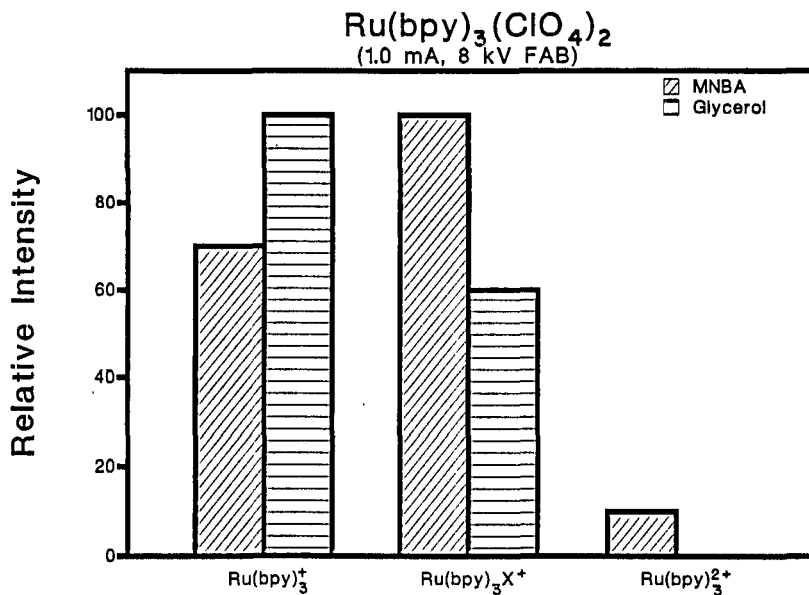


Fig. 1 Comparison of principal analyte molecular ion intensities from FAB spectra obtained with different matrix solvents.

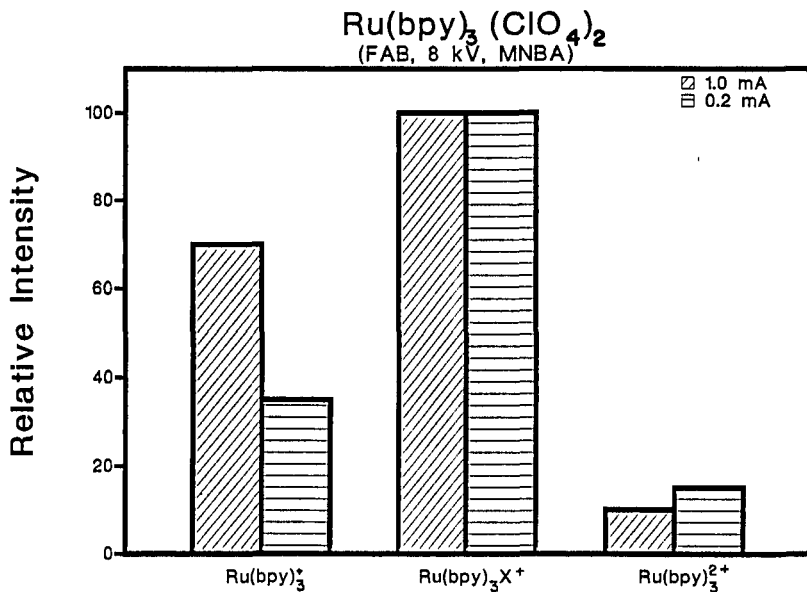


Fig. 2 Comparison of principal analyte molecular ion intensities from FAB spectra obtained with *m*-nitrobenzyl alcohol at two different FAB gun emission currents.

SOME MASS SPECTROMETRIC IMPLICATIONS
OF MULTIPLY CHARGED IONS

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In the past few years non-traditional techniques including Field Desorption (FD), Plasma Desorption (PD), Fast Ion Bombardment (FIB or SIMS), Fast Atom Bombardment (FAB), Electrohydrodynamic (EH), Aerospray (AS or APIE), Thermospray (TS) and Laser Desorption (LD) ionization have been able to produce unfragmented molecular ions from parent species of ever increasing size and complexity. Along with this increase in ion size has been an increase in ion charge. Even so, because the ions with more than one charge are usually in the minority, mass spectrometrists still tend to think almost entirely in terms of singly charged ions. In the last year large ions with extensive multiple charging have begun to demand more attention because Electrospray (ES) ionization has shown a remarkable ability to produce them in great abundance. Here we call attention to some emerging aspects multiple charging that seem at once interesting and important.

1. Formation. Although there is no unanimity as to the ion formation process that seems to be shared by ES, AS and TS the most widely accepted mechanism assumes that an already existing ion is desorbed from a droplet by an electric field whose strength is determined by the density of charge on the droplet's surface. The solvation forces binding that ion to the droplet surface are roughly proportional to its size. The lifting force required for desorption is proportional to the number of its charges. Ergo, the bigger the ion the more charges it must attach in order to become desorbed and observable. In sum, multiple charges are not only consequent to ES ionization of big molecules they are responsible for it. A corollary of this desorption model is that the greater the initial charge/mass ratio in a newly formed droplet, the greater are the charge multiplicity and the size of the ions that can be formed ions, the efficiency of forming them and, therefore, sensitivity of species detection. ES achieves higher charge multiplicity and analytical sensitivity than either AS or TS because its initial droplets have a higher charge/mass ratio. It follows that any attempt to "assist" ES atomization of sample liquid, e.g. by thermal or aerodynamic forces, is in this perspective counter-productive because it inevitably decreases the charge/mass ratio of the initial droplets. To date, experience seems to confirm these conjectures.

2. Mass Analysis. Because most mass analyzers measure m/z , the mass/charge ratio, of the ions they process, multiple charging extends their effective mass range by a factor equal to the number of charges per ion. Thus, ions with molecular weights up to 130,000 have been analyzed with quadrupole mass filters whose nominal upper limit for ion mass is around 1600 daltons. Indeed, all the evidence thus far strongly suggests that for the reasons already mentioned, m/z values for the largest ions that can be produced by desorption from droplets will never be more than 4 kDa or so. It seems to follow that high resolution should have a higher priority than high mass capability in evaluating an

analyzer. Therefore, a marriage between an ES source and an FTICR analyzer would seem to be made in a mass spectrometrists' idea of heaven. Because of their modest m/z values, ES ions of the largest mass would have the high cyclotron frequencies that make for high resolution and reduced residence time requirements. Moreover, ions with a large number of charges moving at high velocity would induce a large signal in the detection circuit. In addition to the reduced residence time they would have a larger momentum than would singly charged ions having either the same mass or the same m/z . Therefore, their trajectories should be less perturbed by collisions with background molecules than would singly charged ions of the same mass or the same m/z . Consequently, multiple charging may relax the requirement for ultrahigh vacuum that has been the bane of ICR mass analysis. The problems of transferring ions from ES source to an ICR cell remain to be solved but do not seem overwhelming.

Charge multiplicity in ES ions is always accompanied by peak multiplicity in their mass spectra. It turns out that each of the peaks is an independent measure of parent ion mass. Therefore, one can in effect "signal average" over those peaks and substantially increase the precision and confidence with which one can assign a mass value to the parent ion. The methods and advantages of such averaging will be set forth in some detail elsewhere. (1) Meanwhile, recent work in our group suggests that this peak multiplicity may also provide an intrinsic calibration of the analyzer's mass scale.

3. Detection. Very large singly charged ions have been known to present detection problems because they may require awkwardly high accelerating voltages to produce secondary electrons from a multiplier dynode. Because it's all a matter of m/z , the multiply charged ions from ES sources have been readily detected without significant post acceleration no matter how high their mass. Even so, problems arise for quantitative analysis because we do not yet know the dependence of secondary electron yield on the charge multiplicity, structure and incident energy of large ions. Further complications loom when one contemplates the very exciting possibilities of MS-MS with multiply charged ions. Upon collisional dissociation a singly charged parent ion is inevitably uniparous but a multiply charged parent can give birth to several daughter ions. Thus, information on composition and structure can be accumulated at a much greater rate from parent ions with multiple charges. Early results including those presented at this meeting are promising but suggest that challenging problems must be solved before MS-MS with multiply charged ions can become a routine procedure. One of them is to find methods for determining z for a fragment ion so that its mass m can be obtained from the m/z information provided by the second analyzer. Another is to develop software capable of processing and interpreting the veritable torrent of data that will result from MS-MS analysis of large multiply charged ions.

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**ELECTROSPRAY IONIZATION AND COLLISIONS INDUCED DISSOCIATION OF
LARGE MULTIPLY CHARGED IONS**

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Tandem MS of *singly charged* molecular ions greater than ~3,000 dalton is generally precluded due to inefficient ionization and, most importantly, insufficient collisional excitation to promote dissociation on the observational time scale (10^{-6} to 10^{-3} s). Electrospray ionization (ESI) has been shown to efficiently produce multiply charged molecular ions of biopolymers¹ of at least 130 kDa.² Recently, we demonstrated that CID spectra can be obtained for multiply protonated molecular ions of mellitin (Mr 2,846), yielding extensive sequence information.³

We have observed that the internal energy (E_i) of macromolecular ions can be adjusted over a broad range by varying an electric field on the vacuum side of the sampling orifice. Tandem mass spectra were obtained with a triple quadrupole instrument using an atmospheric pressure ESI source.^{2,3} The ($M + 15H$)¹⁵⁺ molecular ions of cytochrome c (m/z 825) were selected. Increasing the bias of the orifice related to the first focusing region results in frequent low energy collisions, allowing molecular ions of $m/z < 1,000$ to be heated to (quasi-thermal) E_i sufficient to result in complete dissociation. Figure 1 shows attenuation ($\log I/I_0$) vs. collision gas thickness (T_g) for internally "cold" and "hot" molecular ions at three different translational energies (E_t). Cold cytochrome c molecular ions require $T_g > 1 \times 10^{14}$ cm⁻² before substantial dissociation (> 1%) is observed; in contrast, at this T_g more than 95% of the "hot" ions have dissociated. CID cross sections for hot ions (from the linear regions of Figure 1) are $\sim 10^{-13}$ cm², while they are negligible for cold ions. Clearly the cold molecular ions require numerous collisions before CID can occur, whereas hot ions (collisionally excited in the interface) require few additional collisions.

Figure 2 compares CID spectra for different E_i and a laboratory frame E_t of 1500 eV, or 4.84 eV center of mass frame. Remarkably, for cold ions a significant peak is observed at $\sim m/z$ 773 (consistent with the 16+ molecular ion), probably due to charge stripping. Negligible fragmentation is observed, consistent with Figure 1. Since only a fraction of the center of mass energy is converted to molecular ion E_i , it appears that no single collision provides sufficient excitation for dissociation until high E_i values are reached. The "warm" and "hot" ions show 80% and 99% dissociation, respectively, with extensive fragmentation observed across the spectra. Noteworthy fragments from hot molecular ions are the iron-porphyrin complex ($m/z \sim 617$) and numerous peaks at low m/z .

The present results have profound implications for the extension of practical tandem MS to large molecules. Multiply charged ions allow relatively high E_t to be obtained, even in quadrupole mass spectrometers. Additionally, the ESI-vacuum interface provides a high pressure region where collisional heating (to arbitrarily large E_i) is obtained without creating a large E_t spread that would degrade spectrometer performance. In light of these developments, we propose that the limitations of tandem MS are more accurately defined by $m/z < 3000$ (rather than $m \leq 3000$ dalton).

Acknowledgment

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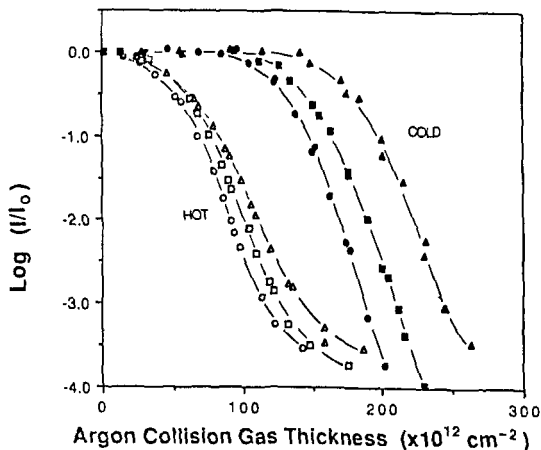


Figure 1. CID efficiency in terms of attenuation of the 15+ protonated cytochrome c molecular ions (m/z 825) without collisional heating in the interface ("cold"), and with heating ("hot"), at CID translational energies of 12090 eV (Δ), 1500 eV ($\square$), and 1800 eV ($\circ$).

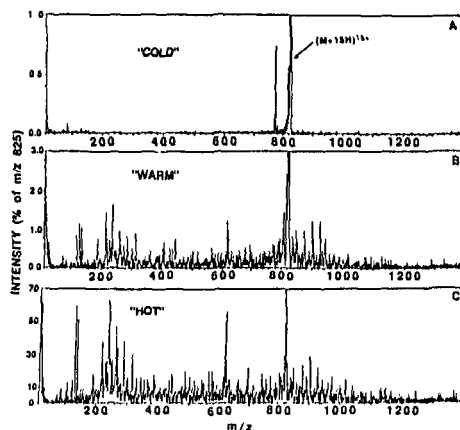


Figure 2. CID mass spectra for (A) "cold", (B) "warm", and (C) "hot" 15+ protonated molecular ions of cytochrome c for $T_g = 10^{14} \text{ cm}^{-2}$ and a collision energy of 1500 eV.

252 MULTIPLY-CHARGED PROTEIN IONS IN
CF-PLASMA DESORPTION MASS SPECTROMETRY

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INTRODUCTION

The specific issue that is being addressed in this study is to determine what factors influence the formation of higher charge gas phase protein molecular ions. In particular, a comparison is made of the conditions for ion formation by the electrospray method vs. the ^{252}Cf -PDMS technique. We shall first look at protein ionization and ion pair interactions in solution and will postulate what happens to the ion pairs when the solution becomes an evaporating aerosol. We then shall then continue the analysis to what happens to the ion pairs when a thin solid film of the protein is formed and subjected to vacuum conditions. Finally, using as a guide, experimental observations from ^{252}Cf -PDMS measurements, we shall determine the fate of the protein ion pairs in the solid state when the matrix is subjected to an intense energy pulse, e.g. the energy deposited by a nuclear fission fragment.

CHARGE STATES OF PROTEINS IN SOLUTION

The degree of protonation of a protein in solution is dependent on pH and the number of charged residues in the protein. For the formation of positively charged species, the three basic amino acids (arginine, lysine, and histidine) are the major sites of protonation. At physiological pH, the more acidic amino acids (aspartic and glutamic acid) are negatively charged so that most proteins have only a small positive or negative charge. Within the vicinity of the charge sites, there is a corresponding counter ion and both the charged site and counter ion are surrounded by a solvation sphere which shields the ion pairs to such an extent that the counter ions are fairly mobile.

CHARGE STATES OF PROTEINS IN THE GAS PHASE (ELECTROSPRAY)

In the process of the formation of a protonated gas phase ion, at least one of the ion pairs associated with the three basic residues must separate as ions rather than as a neutral species. The two competing gas phase reactions: $\text{R-NH}_3^+ \text{X}^- \rightarrow \text{R-NH}_3^+ + \text{X}^-$ and $\text{R-NH}_3^+ \text{X}^- \rightarrow \text{R-NH}_2 + \text{HX}$ determine whether a particular site protonated in solution will also be a protonated site in the gas phase molecular ion contributing to its overall charge. One of the remarkable aspects of the electrospray results is that the final charge state strongly correlates with the number of basic residues in the protein. This means that in the evaporation of the microdroplets, the ion pair dissociation favors the first reaction, the separation into two separated ions even in a sequence of dissociations where the residual protein is gaining a higher charge. This is not the case for ^{252}Cf -PDMS.

CHARGE STATES FOR PROTEINS IN THE GAS PHASE (^{252}Cf -PDMS)

The charge state distributions for protein molecular ions formed by ^{252}Cf -PDMS are in general

much smaller. In the 5000 - 10,000 u region, the singly-protonated ion dominates but there is a small doubly-protonated component. For proteins in the 13,000 -20,000 u range, the singly-charged component is small and the degree of protonation extends to as high as 4 protons. The charge distribution is matrix dependent. Results obtained for insulin adsorbed on polyethylene terephthalate show only the singly-protonated species while the spectrum for insulin adsorbed on nitrocellulose shows a significant doubly-protonated ion.

ROLE OF ION-PAIR DISSOCIATION

We shall now examine some of the reasons as to why there is such a difference in the charge state distributions for the two methods. There are obviously differences in the ion-pair dissociation reactions with the electrospray ionization favoring charge separation to a higher degree of protonation. One of the reasons for this is that in the evaporation of the microdroplet, the ions retain their solvation sphere for a longer period of time which means that the ions are farther apart in the beginning stages of the ion-pair dissociation. It is easier for a solvated ion-pair to dissociate into separated ions. In ^{252}Cf -PDMS, however, the protein is in the solid state. Much of the solvation sphere is lost due to evaporation and the ion-pairs are more tightly bound to each other. It is more difficult to form separated ions and the alternative reaction, proton transfer to the counter ion resulting in the formation of a separated neutral pair, plays a more important role. Thus, the final degree of protonation is much less.

There is also an additional factor relating to the degree of internal excitation of the protein and the instability of higher charge states for excited molecules. For the electrospray ionization, the degree of internal excitation is extremely low whereas for ^{252}Cf -PDMS, the energy dissipation from the fission track can easily transfer energy to the protein molecule. A measure of the stability of multiple charges within a polymer is a property called the Debye shielding length. When the internal excitation of the polymer is low, the Debye shielding length is small and the polymer can accommodate a higher number of charges. The number decreases as the internal energy of the polymer increases. This is another reason why the charge state distribution for protein molecular ions in ^{252}Cf -PDMS is smaller.

ROLE OF MATRIX IN CONTROLLING DEGREE OF INTERNAL EXCITATION IN ^{252}Cf -PDMS

The major way that the degree of internal excitation can be controlled in ^{252}Cf -PDMS is through the modification of the substrate. The crucial feature is the coupling of the substrate to the protein. For a highly hydrophobic surface such as poly(ethylene terephthalate) the protein is strongly bound through the hydrophobic domains that reside on the surface of the protein. When this matrix is excited, a considerable amount of energy is coupled into the protein through the substrate/adsorbate link. This results in a broad spectrum of metastable ions, a weak molecular ion and no doubly charged ion. If a nitrocellulose matrix is used, the metastable component is considerably reduced, the singly-protonated molecular ion intensity increases by a factor of 6 and a doubly protonated species appears. If the substrate is a loose assembly of small molecules, a similar pattern is observed and the internal excitation of the protein is still smaller.

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MULTIPLY CHARGED NEGATIVE IONS: A CRITICAL REVIEW

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Most elements in the periodic chart can accommodate an extra electron to form a stable negative ion. Only a few elements (some exceptions are rare gas atoms, Be and Mg) cannot permanently bind an excess electron. The electron affinities for the great majority of molecules lies in the region between -2 eV (unbound state) to +4 eV. On the other hand, all atoms and molecules can accept an additional electron for fleeting periods of time (milli-sec to femto-sec) in the form of compound states or resonances. For example, an electron can be temporarily bound to an atom via a shape or Feshbach resonance. These states are seen as resonance features in electron-atom scattering cross sections or as autodetaching (metastable) ions in beam experiments. Some of these metastable ions can live for many micro-seconds [e.g. $\text{He}^-(1s2s2p)^4\text{P}$] and have served as useful ionic species in accelerator applications. Much is known and understood about the structure of singly charged negative ions of atoms and small molecules. On the other hand, very little is known about multiply charged negative ions.

The Milliken oil-drop experiment amply demonstrates that multiply charged objects can exist as stable or metastable entities. Thus, it is natural to ask, "How *small* an entity is capable of binding two extra electrons?... can an atom, in particular, form a long-lived doubly charged negative ion?" Doubly-charged atomic negative ions were first reported in 1960.⁽¹⁾ Since that time there have been many claims of observations of multiply-charged atomic and molecular negative ions using a variety of mass spectrometers (omegatron, ICR, magnetic sector, quadrupole, etc.).⁽²⁾ There is considerable evidence for the existence of long-lived ($\gtrsim 1\mu$ sec) or stable multiply charged negative ions of large molecules. Most reports involve large molecular negative ions with electronegative groups as part of the molecule. The conventional wisdom is that electrons can reside in localized "spots" (electronegative groups) at sufficiently large distances within the molecule so as to minimize the Heitler-London repulsion energy. In all cases, the lifetimes of these gas-phase anions must be greater than a few micro-seconds in order to be detected with most mass spectrometers discussed in this talk.

Multiply charged anions are well known to exist in liquids and are stabilized by the surrounding medium. Therefore, it is not surprising that there are reports of doubly charged molecular anions from clusters in nozzles,⁽³⁾ ion spray,⁽⁴⁾ or electrohydrodynamic ionization,⁽⁵⁾ or field ionization from liquid surfaces.⁽⁶⁾ Very recently, doubly charged anions were detected by collisionally induced "ion-pair" formation from singly charged negative ions.⁽⁷⁾ Dissociation of negative ions (e.g. RN_3^-) into ion pairs (e.g. $\text{R}^{2-} + \text{Na}^+$) is also invoked in some electrodynamic studies. The first report of a doubly negative charged "large molecule" (dimer of benzo [cd] pyrene-6-one) is credited to Dougherty.⁽⁸⁾

Thermospray and ion spray mass spectroscopy at atmospheric pressure has proven most effective at producing multiply charged negative ions. The data suggests that multiply charged anions are produced primarily by evaporation in the ion spray and by both evaporation and gas-phase chemical ionization in the thermospray techniques. Covey *et al.*⁽⁴⁾ have used ion-spray mass spectroscopy to produce a synthetic DNA molecule with *eleven* excess electrons.

The evidence for short lived *atomic* negative ions is not so conclusive.⁽⁹⁾ Upper limits on the ratio of X^{2-} to X^- using various ion sources give values ranging from 10^{-7} ,⁽¹⁰⁾ 10^{-12} ,⁽¹¹⁾ to 10^{-16} .⁽¹²⁾ Many of the claims are not reproducible or are known to be flawed. Their possible existence carries the most practical significance since multiply charged atomic ions could be used to greatly extend the energy of tandem accelerators around the world.

Certain classes of hexafluoride molecules are known to possess electron affinities of up to ten electron volts.⁽¹³⁾ There is recent theoretical evidence that stable or perhaps metastable MF_6^{q-} ($q = 2, 3$) ions may exist. One interesting possibility is PtF_6^{2-} . Semi-empirical calculations of the enthalpy of formation of PtF_6^{2-} from a crystal lattice $[K_2PtF_6] \rightleftharpoons 2K^+ + PtF_6^{2-}$ yields an electron affinity for PtF_6^{2-} of 3.9 ± 0.6 eV.⁽¹⁴⁾ Recent SCF-MO and CI calculations⁽¹⁵⁾ on AuF_6^{q-} ($q = 0, 1, 2$, and 3) suggest that AuF_6^{2-} lies lowest [$EA(AuF_6) \simeq 9.6$ eV] in energy; however, the AuF_6^{2-} potential energy surface lies below that of AuF_6^- at the internuclear separation ($Au - F$) of AuF_6^{2-} . This result and other experimental observations suggest that hexafluoride molecules may accept more than one extra electron to form a long-lived or perhaps stable multiply charged negative ion. Aside from the importance to fundamental physics, the existence of stable or long-lived ions such as PtF_6^{2-} , UF_6^{2-} or AuF_6^{2-} would have great practical importance.

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INTRODUCTION

Environmental samples pose very challenging problems because the analytes are often at trace levels in complex mixtures and cover a wide range of compound types. Historically, the non-polar, more volatile compounds have been targeted because these are amenable to established capillary GC/MS determinations. Capillary gas chromatography is very useful for its high separation efficiencies which facilitate separation of complex mixtures. Electron ionization is useful because it provides structural information which is helpful for characterizing the identity of unknown compounds. However, when the compound is either too polar or involatile to be amenable to GC/MS, serious analytical problems arise.

HPLC is a natural complement to capillary GC because the former can chromatographically separate many of the compounds not amenable to GC. However, without the combined sensitivity and selectivity of mass spectrometry, HPLC does not offer much help for identifying unknown compounds in environmental samples. The obvious answer to this problem is on-line LC/MS. However, most traditional approaches to LC/MS have produced chemical ionization mass spectra which are very simple and do not provide information useful for structural characterization. Tandem mass

spectrometry(MS/MS) can provide this additional structural information.

Typically MS/MS can provide mixture analysis capability and structural information via collision-induced dissociation(CID) of parent or fragment ions. Both of these features of MS/MS complement LC/MS. The mixture analysis capability is useful because HPLC does not provide the high separation efficiency available from capillary GC so HPLC peaks often have co-eluting components. Thus MS/MS mixture analysis in these instances is helpful. In addition, mild ionization conditions characteristic of most LC/MS systems benefits from the CID process which generates fragmentation patterns characteristic of the compound's structure and thus allows structural characterization. Thus it could be said that there is more reason to do LC/MS/MS than GC/MS/MS!

We have applied the technique of MS/MS on an atmospheric pressure ionization mass spectrometer(APIMS) for the study of a variety of polar, involatile compounds of environmental interest. These include, in particular, sulfonated azo dyes and phenoxyacetic acid herbicides. We have found that API LC/MS/MS is a viable, routine approach for detecting and characterizing these compounds in environmental wastewaters.

EXPERIMENTAL

A TAGA 6000E tandem triple quadrupole mass spectrometer (Sciex, Inc., Thornhill, Ontario, Canada) was interfaced to an ion spray LC/MS interface maintained at ambient temperature and -3kV which forms highly charged micro droplets in the vicinity of the API sampling orifice. The HPLC separations were often carried out with a Brownlee Micro Pump equipped with a 1mm x 15 cm microbore HPLC column packed with reversed-phase C18 packing with an eluent flow maintained at 40 microliters per minute through a gradient profile capable of providing a separation of the target compounds. Alternatively, conventional 4.6mm x 10cm columns of similar packing

material were used at one milliliter flow rates, but with a post column "T" which split the effluent about 20:1 to pass about 40-50 microliters per minute to the ion spray interface.

In addition to on-line LC/MS/MS the same ion spray interface may be used to couple capillary zone electrophoresis (CZE) to the mass spectrometer. Typically, 100 μ m x 50-75cm fused-silica capillary columns without a bonded stationary phase inside were used with the anode held at about 30kV and the cathode held at 3kV. The capillary was charged with appropriate aqueous ammonium acetate buffer with either methanol or acetonitrile to accomplish the separation. Both of these separation inlet systems could be used in the MS/MS mode.

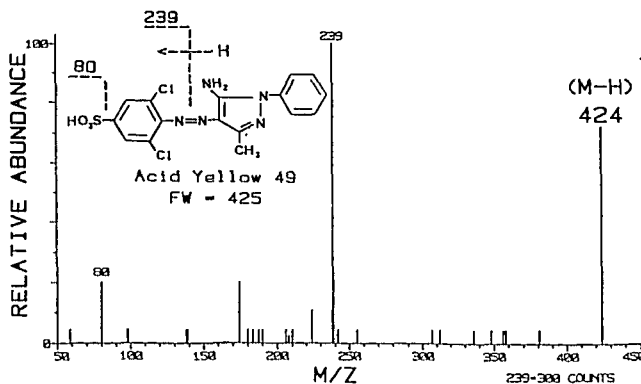
RESULTS AND DISCUSSION

A variety of sulfonated azo dyes with as many as six sulfate groups were readily characterized using both LC/MS/MS and CZE/MS/MS. Solid phase extraction of fortified water and municipal wastewater following treatment were analyzed by LC/MS/MS in the negative ion mode. The APIMS system is very sensitive for these compound with 10pg detection limits in the SIM mode. In the single MS mode abundant deprotonated molecular ions are obtained with no evidence for fragment ions. Under LC/MS/MS conditions an abundant fragment ion at m/z 80 is observed in addition to weaker fragment ions at higher mass characteristic of the azo linkage and the parent dye. Quantitative analyses were carried out using an internal standard under LC selected reaction monitoring (SRM) conditions and showed that these compounds could be readily quantified by this approach.

CZE/MS/MS offers the same type of mass spectral information as that described above, but the separation efficiency provided by this inlet system is much greater than that obtained from HPLC. However, the low sample volume (5-15nL) injected in CZE makes it impractical for trace analyses where the sample amount is limited. However, it is possible that CZE could be the premier separation method for complex mixtures where low part per million or greater levels were available.

CONCLUSIONS

LC/MS/MS and CZE/MS/MS are both very useful for providing on-line separation and identification of polar, involatile components in complex environmental samples. The detection limits can be as good or better than typically found for GC/MS, and compounds can be characterized which would be impossible to handle by conventional approaches. There would appear to be a real need and a bright future for tandem mass spectrometry when it is combined with liquid inlet systems and mild ionization conditions applied to environmental problems.



APPLICATIONS OF TRIPLE QUADRUPOLE GC/MS/MS TO THE
DETERMINATION OF CHLORINATED DIBENZO-p-DIOXINS
AND DIBENZOFURANS IN COMPLEX ENVIRONMENTAL SAMPLES

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The analysis of chlorinated dibenzo-p-dioxins (CDD) and dibenzofurans (CDF) in complex matrices, which oftentimes contain chemical interferences at concentrations several orders of magnitude greater than those of the CDD and CDF, requires extensive sample cleanup prior to instrumental analysis. Limitations in sample cleanup place additional demands on the instrumentation.

The difficulties in the analysis of environmental samples make selectivity and sensitivity important considerations for the method of choice. Capillary column high resolution gas chromatography / high resolution mass spectrometry (HRGC/HRMS) has been the standard method for analyzing CDD and CDF in complex matrices such as sediments, effluents and biological tissues.

Until recently, tandem mass spectrometry (MS/MS) has been able to meet the requirements for selectivity but has been approximately one order of magnitude less sensitive than HRGC/HRMS methods. Optimization of tuning parameters using 1 ug of the analyte of interest in a direct insertion probe (DIP) [1] rather than PFTBA resulted in sensitivities comparable to the HRGC/HRMS technique reported previously [2]. Table 1: compares the limits of detection, reported in 1986, by three manufacturers of triple quadrupole mass spectrometers, using PFTBA or PFK for tuning, to the limits of detection that were obtained when dioxins and furans were used for tuning and to limits of detection obtained by HRMS. Most of the limits of detection obtained by the MS/MS dioxin tune method are between one and two orders of magnitude lower than when PFTBA or PFK is used for tuning and almost as low as those obtained by HRMS.

Collision induced dissociation breakdown graphs were used initially to optimize parameters such as collision energy and collision gas pressure. The breakdown graphs for PFTBA, 2,3,7,8-Cl₄DD and 2,3,7,8-Cl₄DF plotted vs. collision gas pressure are shown in Figure 1. The optimum collision gas pressure for the dioxin and furan was 3.3 mtorr while that for PFTBA was 0.7 mtorr. Figure 2: shows the breakdown graphs of PFTBA and four Cl₄DD congeners. The optimum collision energy for PFTBA was 25 eV while those for the dioxins were between 20 and 28 eV. The variation in optimum collision energy for the four Cl₄DD congeners implies that the relative response factors within a congener group are dependent on collision energy. Figure 3: shows the mass chromatogram of a mixture of three Cl₄DD congeners (1,2,3,4-, 2,3,7,8-, and 1,2,7,8-). Injection A was run with parameters set to optimize 2,3,7,8-Cl₄DD while injection B was set to optimize 1,2,3,4-Cl₄DD. As expected, the signal strength of 2,3,7,8-Cl₄DD was enhanced in injection A and the signal strength of 1,2,3,4-Cl₄DD was enhanced in injection B. In order to obtain reproducible relative response factors, a 2,3,7,8-containing (the most toxic) congener was chosen for each group. These are shown in Table 2.

Each probe sample was prepared by evaporating 1 ug standards of a furan and the corresponding dioxin with the same number of chlorines in the same sample cup. Each of the five cups (Cl₄DF/DD to Cl₈DF/DD) was inserted in turn into the mass spectrometer using the DIP and parameters such as collision gas pressure, collision energy, electron energy, lens voltages, quad offsets, etc. were optimized for both the furan and dioxin.

Another factor that increased the signal strength of the dioxins and furans was elevation of the source temperature to 250°C. This enhanced the signal strength of the congeners of higher chlorine substitution. Also, a high trap current (300 uA) and a Finnigan 4500 ion volume instead of the normal TSQ-70 ion volume was used to increase

the flux of the ionizing electrons into the ion source.

The advantages obtained by using the dioxin tune method include: good sensitivity - less than 1 picogram of dioxins and furans can be detected for all congeners; infrequent tuning - about 3 to 4 times a year @ 200 injections / month with source cleaning every six months; and selectivity - less cleanup is required because of the reduced chemical noise.

Example 1 shows the mass chromatogram of a 500 fg standard injected on a 30m SE-52 capillary column with a split/splitless injector. Note the signal to noise is about 10:1. Example 2 shows 320 fg of 2,3,7,8-Cl₄DD injected on the same column using the "DOW" only cleanup. Example 3 shows a comparison of a fish extract run on both a ZAB-2F and TSQ-70 mass spectrometer. Note that the chromatogram of the sample determined by MS/MS is much cleaner than that determined by HRMS at 12,000RP. Example 4 shows the mass chromatograms of an effluent sample. Note that the relative response factors obtained in the GC/MS/MS mode, when 2,3,7,8- dioxins and furans are used for tuning, are very similar to those obtained in the GC/MS mode.

- Schellenberg D.H. et al, Rapid Comm. in Mass Spectrom., 1,111,(1987).
- Taguchi V.Y. et al, Anal. Chem., 60, 1429, (1988).

TABLE 1: MOE MS/MS COMPARISON
LIMIT OF DETECTION

CONGENER	MANUFACTURER A	B	C	PRESENT METHOD	HRMS
Cl ₄ DD	2	2	8	0.3	0.2
Cl ₅ DD	5	4	9	0.5	0.2
Cl ₆ DD	10	10	23	0.3	0.2
Cl ₇ DD	15	15	19	0.3	0.2
Cl ₈ DD	20	25	38	0.6	0.4
Cl ₄ DF	4	3	7	0.3	0.2
Cl ₅ DF	5	4	11	0.3	0.2
Cl ₆ DF	10	8	21	0.2	0.2
Cl ₇ DF	15	10	20	0.5	0.2
Cl ₈ DF	20	20	19	0.5	0.4

ALL DATA IN PICOGRAMS

FIGURE 1: COLLISION ENERGY VS %TIC
PFTBA

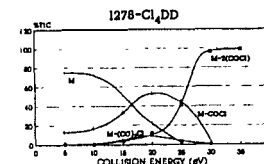
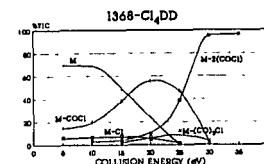
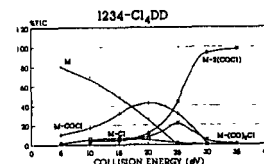
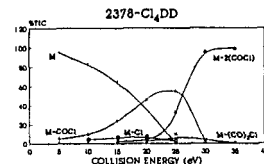
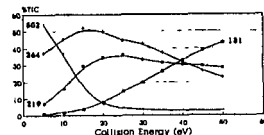


FIGURE 2: COLLISION ENERGY VS %TIC
PFTBA

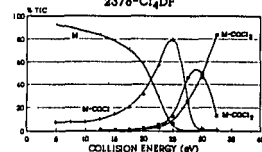
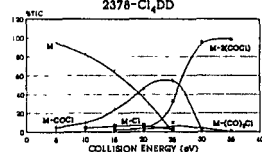
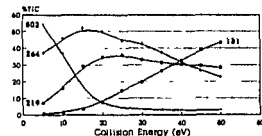
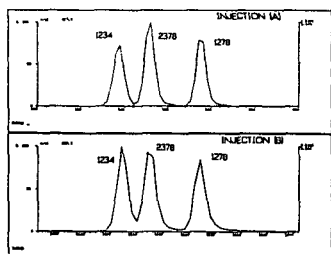


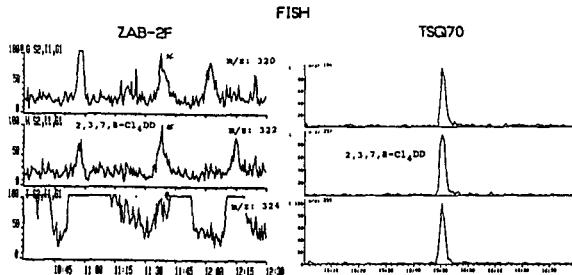
TABLE 2:
CONGENERS USED FOR
TUNING IN MS/MS

- 2,3,7,8-Cl₄DD
- 1,2,3,7,8-Cl₅DD
- 1,2,3,4,7,8-Cl₆DD
- 1,2,3,4,6,7,8-Cl₇DD
- Cl₈DD
- 2,3,7,8-Cl₄DF
- 1,2,3,7,8-Cl₅DF
- 1,2,3,4,7,8-Cl₆DF
- 1,2,3,4,6,7,8-Cl₇DF
- Cl₈DF

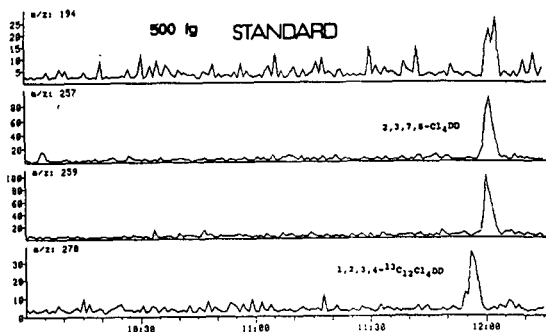
FIGURE 3:



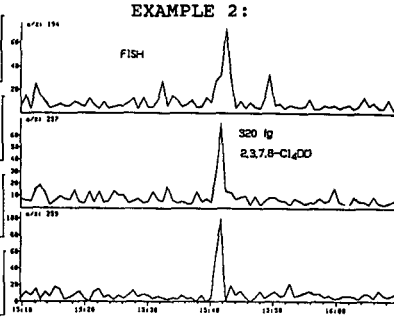
EXAMPLE 3:



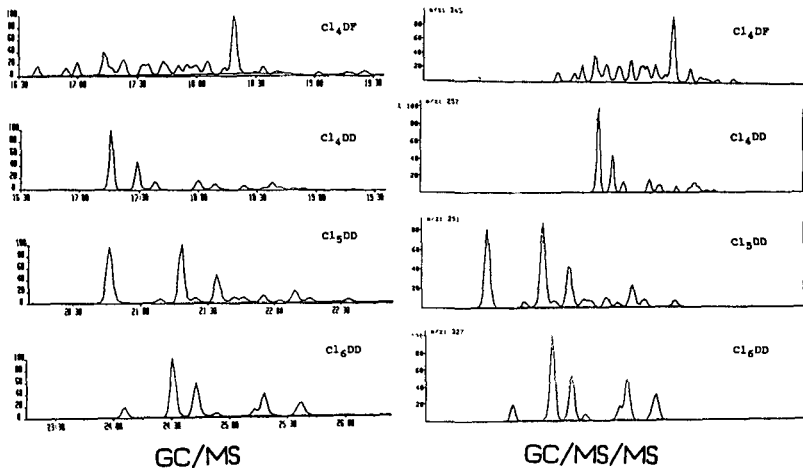
EXAMPLE 1:



EXAMPLE 2:



EXAMPLE 4: COMPARISON OF RELATIVE RESPONSE FACTORS



CHOOSING BETWEEN HRMS AND MS/MS: ENVIRONMENTAL APPLICATIONS

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What would you do if asked to analyze small quantities of contaminants in a complex environmental sample? You, as the Analyst, would then appreciate any information pertaining to the type, origin and history of the sample that would facilitate your selection of the analytical methodology. The expected analyte concentration domain also constitutes a useful parameter in the selection process. However, most often, this type of information is not available and the decision on how to process the sample will have to be made almost in an arbitrary manner. What you are looking for in the analytical procedure is its ability to detect with certainty and quantify accurately the trace components in a reasonably short span of time. The approach should be capable of segregating the analyte(s) of interest from the co-extractables, which could be either specific or non-specific interferences, with the highest precision and recoveries as possible.

If a specific type of interference is expected to be present, then you can make an "educated" choice by selecting appropriate fractionation techniques such as the type of chromatographic separations (e.g., gravity flow, HPLC and GC) and the selectivity of the detection system (e.g., HRMS and MS/MS). Of course, you can design the procedure so that these two major aspects (fractionation and analysis) of the analytical scheme can be used in concert to optimize your chances of reaching the goals defined earlier.

Here, we would like to consider more specifically the determination step when a mass spectrometer is used as the detection system. The two facets of the mass spectrometer that are considered below are high-resolution mass spectrometry (HRMS) and tandem mass spectrometry-mass spectrometry (MS/MS). Our objective is not an attempt to demonstrate the general superiority of one approach over the other but to examine specific environmental applications showing the advantages of, what is increasingly becoming the most two common options offered to Analysts for the determination of trace components. Tetrachlorinated dibenzo-*p*-dioxins (TCDD) will serve as the contaminants in this illustration.

The sample introduction can be optimized to allow further separation between the analytes and potential interferences. The combination of gas chromatography with mass spectrometry is particularly powerful during the measurement of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. The mass spectrometer can either be operated in the selected ion monitoring mode (SIM) where ions of a given elemental composition are measured or in the selected reaction monitoring mode (SRM) where the parent (molecular ion) ions are transmitted -- with the help of the double focusing mass analyzer (MS1) -- in the collision cell area. There, collisionally induced daughter ions (i.e., M-COCl) are formed and subsequently mass-analyzed by the quadrupole analyzer (MS2) if a hybrid mass spectrometer (EBQQ configuration) is used.

Unless the Analyst/Operator has knowledge of the type of interferences present in the sample extract, the he/she cannot decide whether one approach is more appropriate than the other. Let's consider the case whereby the sample is known to contain polychlorinated biphenyls (PCB) -- in particular the hexa- and heptachlorinated congeners -- which are

known to interfere with the measurement of TCDDs. If the analysis consists in monitoring the molecular ions of TCDDs (SIM) with the mass spectrometer resolution set at 10,000, one would then expect traces of heptachlorinated PCBs not to interfere during the assay. Indeed, the loss of two ^{35}Cl from the molecular ion of an hexachlorobiphenyl isomer produces a fragment ion that requires a resolving power of 12,500 to distinguish TCDD from the PCB. If however, the Operator is required to monitor, in addition to the molecular ion of TCDD, the fragment ions formed inside the ion source (e.g., $\text{M}-\text{COCl}$), then under SIM conditions this would require impractical resolving powers exceeding 300,000 to segregate TCDDs and the PCBs. In this latter case, SRM would constitute the selection of choice.

Figures 1 and 2 illustrate another example, which was obtained from a paper pulp effluent sample, where severe interferences caused by methyl and ethyl tetrachlorinated dibenzofurans are seen at m/z 320 and 332. Increasing the mass spectrometer resolving power from 10,000 to 18,000 (Figure 1) helps in the elimination of the interferences resulting from the elution of the methylated species. However, as shown in Figure 2, the interferences created by the ethylated compounds are not removed by SIM (note that a resolving power of 85,000 would be necessary) whereas their contributions are substantially minimized in the MS/MS experiment.

In most other situations, the nature of the interferences is unknown. For example, Figure 3 was obtained from a municipal incinerator ash extract under SIM conditions. The figure shows a series of signals, obviously not attributable to tetrachlorinated species, which are eluting in the vicinity of TCDDs. When the chromatograms (Figure 3) generated by analyzing the sample under SIM (at 10,000 and 18,000 resolving powers) and SRM (MS/MS) conditions are compared, the effect of the interferences is significantly reduced by MS/MS analysis whereas no improvements could be noted after increasing the mass spectrometer resolution.

So far the two examples above show that the two techniques are complementary and contribute to improving the quality of the conclusions reached by the Analyst when used in conjunction. This is all fine as long as time is not taken into consideration. Indeed, on numerous occasions, there has been a tendency to overstate the advantages of MS/MS over HRMS in terms of specificity inciting the Analyst to reduce the need for "extensive" sample preparation prior to analysis. We have found that this approach should be reconsidered since, regardless of the instrument configuration (HRMS or MS/MS), the ion source sensitivity can vary with time as large quantities (hundreds of ng per second) of non-specific interferences are eluting of the GC column (Figure 4). These results show that no matter how selective the detection system can be, there will remain a need for adequate fractionation (cleanup) procedures before the determination step when gas chromatographic and mass spectrometric techniques are used [Tondeur et al, Biomedical and Environmental Mass Spectrom. 14, 449-456 (1987); Anal. Chem. 56, 1344-1347, 1984].

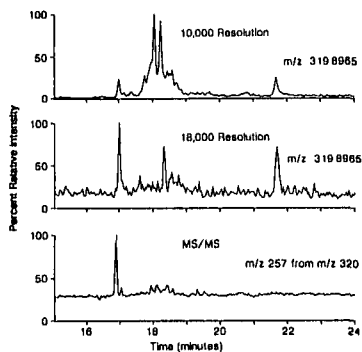


Figure 1: Comparison of HRGC/MS and HRGC/MS/MS
on Pulp Effluent Extract

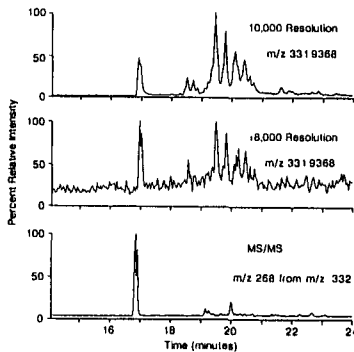


Figure 2: Comparison of HRGC/HRMS and HRGC/MS/MS
on Pulp Effluent Extract

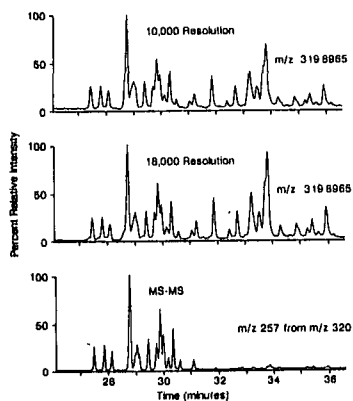


Figure 3:
Comparison of HRGC/HRMS and HRGC/MS/MS
on Municipal Incinerator Ash Extract

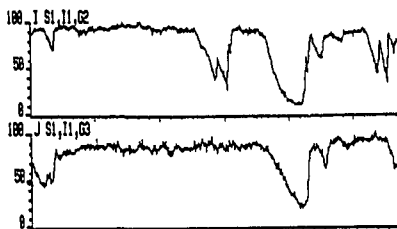


Figure 4: Quality Control Selected Ion Current Profiles Showing a
Series of Non-Specific Interferences Eluting within the
PCDD/PCDFs Retention Time Windows.

DEVELOPMENT OF A MOBILE ION TRAP MASS SPECTROMETER SYSTEM FOR
ENVIRONMENTAL MONITORING

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The objective of the work reported here is to develop a compact, mobile mass spectrometer with EI, CI and MSⁿ capabilities. Moreover, the mobile MS system should be capable of analyzing gases, vapors and aerosols in air at low ppb levels while allowing positive identification of individual components in complex mixtures.

In view of the size and weight restrictions inherent in the mobility requirement, a special miniaturized version of the Finnigan MAT Ion Trap Mass Spectrometer (ITMS®) was constructed in close collaboration with the manufacturer (Figure 1).

This MINITMASS (Miniaturized Ion Trap Mass Spectrometer) system is equipped with automatic gain control [1], automatic reaction control [2], selective mass storage [3] and axial modulation [3] options, in addition to the required EI/CI [4,5] and MSⁿ [5] operating modes.

The 2x2x2 ft MINITMASS module is mounted within a 6 ft high mobile rack together with all gas supplies, pumps, inlet controls and a PC 80386 workstation (Figure 1). The entire system requires approx. 1000 W of ac power. A 6x7x8 ft. self-contained mobile laboratory module, which fits on a standard 3/4 ton pick-up truck (Figure 2), enables operation of the MINITMASS in remote locations and rugged terrain. Also, the MINITMASS system can be remotely controlled from a distance up to several miles using a second PC workstation, and Carbon Copy® software.

A direct air sampling inlet enables repetitive collection of 10⁻⁵ microliter air samples at 15-60 sec intervals. Use of "transfer line chromatography" provides additional resolution and specificity for complex mixtures (Figures 3 and 4). Minimum detectable levels of gases and vapors in air are in the low to medium ppb range without the use of concentration devices (Figure 5). Alternatively, a special automated inlet enables electrostatic precipitation of air particulate matter on a ferromagnetic filament with subsequent analysis by Curie-point pyrolysis (GC)/MSⁿ. An example of the analysis of a simulated biological aerosol (albumen solution) is shown in Figure 6.

The system has been successfully operated in the desert (at temperatures up to 100 F), as well as under a variety of indoor conditions, e.g., for monitoring laboratory-scale combustion reactors.

ACKNOWLEDGEMENTS

The help and advice of A. Peter Snyder, Michael S. Story, Michael Weber-Grabau, JoAnn S. Lighty, Eric G. Eddings, Eric R. Lindgren and David W. Pershing is gratefully acknowledged. Financial support for this work was received from the Advanced Combustion Engineering Research Center (funded by NSF, State of Utah, 23 industrial participants, and US DOE), the DOD Chemical Research Development Engineering Center (CRDEC) and Finnigan-MAT Corp.

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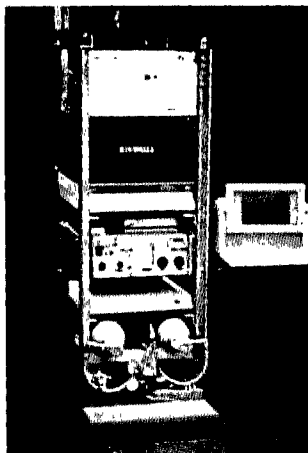


Figure 1. MINITMASS (Miniaturized Ion Trap Mass Spectrometer) system.



Figure 2. Mobile laboratory module on 3/4 ton pick-up truck.

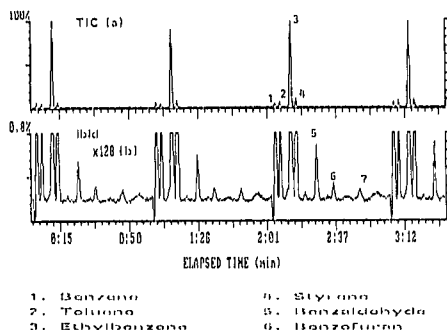


Figure 3. Transfer line GC/MS TIC profile of combustor effluent during ethylbenzene test run. For identity of peak labeled (?) see Figure 4.

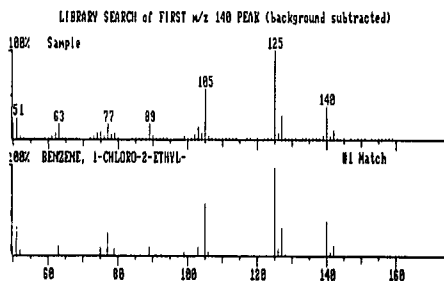


Figure 4. Library search result for unknown peak in Figure 3. Estimated concentration approx. 50-100 ppb.

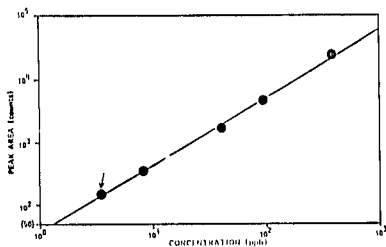
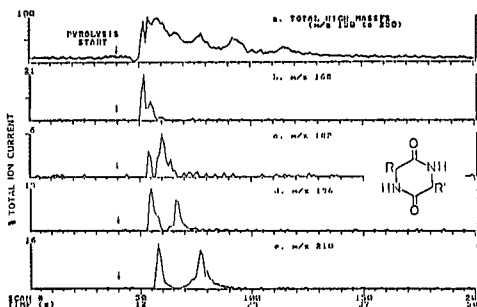


Figure 5. Linear response of MINITMASS system (with vapor sampling inlet) to trace levels of toluene in air over several orders of magnitude. Arrow points to 3.5 ppb (200 fg) sample.



ANALYSIS OF XENOBIOTIC CONJUGATES BY MS/MS

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Tandem mass spectrometry has proven to be very useful for a number of reasons. These reasons include: 1) mixture analysis; 2) increased fragmentation; 3) new information contained in MS/MS spectra not found in MS spectra; and 4) complex molecules often fragment into their constituent building blocks. The advantages of MS/MS over MS are especially useful in conjunction with soft ionization techniques which yield molecular ion species with little fragmentation and, thus, little structural information. With the advent of FAB, the determination of polar pollutants, such as surfactants, has become significantly easier than was the case with ionization techniques such as field desorption.

Surfactants are ideal examples of the utility of combining MS/MS with soft ionization techniques for determining the nature of polar environmental pollutants. They are ubiquitous in modern society and are usually very complex mixtures. The existence of both anion, cationic, amphoteric and nonionic surfactants increases the complexity of their determination.

Arquard 2C-75 is a typical cationic surfactant whose nominal structure is dimethyldidodecylammonium chloride. The FAB/MS spectrum of this surfactant demonstrates the complexity of the surfactant (Fig. 1). Not only is the nominal structure present (m/z 382) but a complex series of homologous compounds are also observed. The MS/MS spectrum of the major species (m/z 382, Fig. 2) contains a series of alkane losses from the molecular cation which can be used to identify the alkyl group in the molecule. The losses of alkanes that are observed are not observed in the MS spectrum and are an example of a new fragmentation. The MS/MS spectra of the homologs, eg. m/z 466, Fig. 3, show a more complex pattern. Losses of C_{12} , C_{14} , C_{16} , and C_{18} chains are observed which indicates that this ion is a mixture of a C_{12} and C_{18} containing quaternary ammonium ion and a C_{14} and C_{16} containing quaternary ammonium ion.

This example demonstrates the ability of the combined techniques of FAB and MS/MS to determine the structures of components of very complex mixtures.

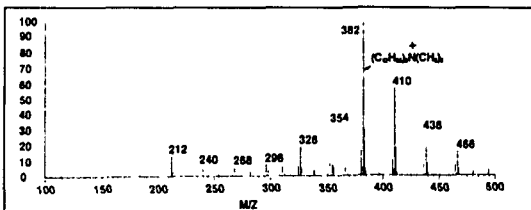


Fig. 1 Positive ion FAB spectrum of Arquard 2C-75.

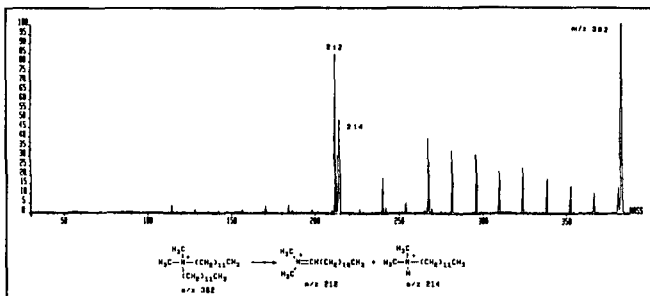


Fig. 2 MS/MS spectrum of m/z 382 from Arquard 2C-75.

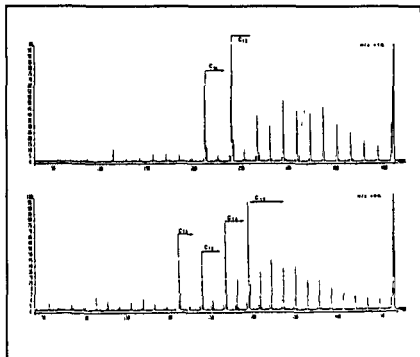


Fig. 3. MS/MS spectra of (top) m/z 410 and (bottom) m/z 466 from Arquad 2C-75.

In the environmental sciences there is a need not only to monitor pollutants in the environment, but also a need to determine the fate of the pollutants once it enters living organisms. It is this interaction between xenobiotics, i.e. pollutants, and living organisms which defines our concerns about most of these compounds and is the province of the environmental health sciences. A major key in understanding the effects of xenobiotics on organisms is the determination of the products of the covalent interactions between the xenobiotic and biomolecules.

These xenobiotic-biomolecule adducts are typically isolated as mixtures of polar, often thermally-labile, molecules. The xenobiotics can interact within living organisms in

several ways, including 1) binding to cell constituents and, thereby, modifying the function of the biomolecule, and 2) conjugating with polar molecules as part of the bodies mechanism for excreting xenobiotics. Often the conjugates are also toxic to the cell.

An example of the potential product of the interaction of a xenobiotic with a cell constituent is N⁶[1-(2-hydroxyacenaphthenyl)]adenosine. The FAB/MS/MS spectrum of this modified nucleoside is shown in Fig. 4. The fragmentations observed are characteristic of modified nucleosides, which include formation of the modified base, RBH⁺, by loss of the sugar, formation of the parent nucleoside, BSH⁺, by loss of the modifying group and formation of the ion corresponding to the modifying group, R⁺. A number of ions resulting from water loss from the primary diagnostic fragment ions are also observed. This example illustrates the propensity of complex biomolecules to fragment into their constituent parts which provides information allowing the analyst to determine their structures.

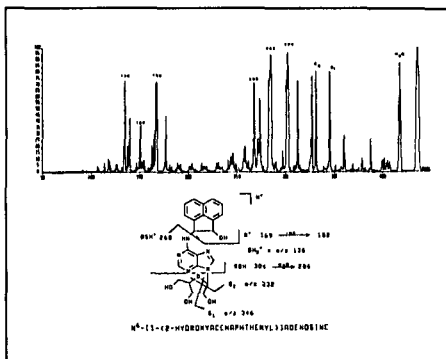


Fig. 4. MS/MS spectrum of hydroxy-acenaphthene and adenosine.

Metabolic activation of molecules to polar metabolites followed by conjugation is a typical mechanism for excretion of xenobiotics from an organism. Both the metabolically activated compound and the final conjugated product can also be toxic. A common mechanism for excretion is conjugation with the tripeptide glutathione followed either by direct excretion or by hydrolysis to the cysteine conjugates or N-acetylcysteine conjugates which are then excreted.

An example is the cysteine conjugate of benzo[a]pyrene epoxide. The FAB/MS/MS spectrum of this compound (Fig. 5) is dominated by an ion attributable to the xenobiotic moiety readily identifying the xenobiotic. The loss of 121 dalton from the molecular ion to form this ion readily identifies the conjugating group as cysteine.

In conclusion, FAB/MS/MS is a powerful tool for both the analysis of pollutants in the environment and for determining the nature of the interactions of pollutants with biomolecules within the body.

ACKNOWLEDGEMENT

The surfactant samples were provided by Dr. P. A. Lyon of the 3M Co. The modified nucleoside was provided by A. Bartoszak and Prof. A. Gold of the University of North Carolina-Chapel Hill. The cysteine adduct was provided by Dr. L. T. Burka of NIEHS - Division of Toxicology Research and Testing.

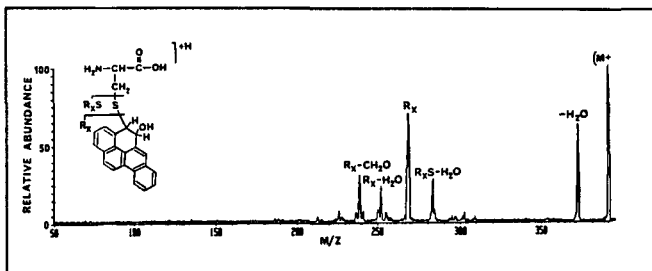


Fig. 5. MS/MS spectrum of the (M+H)⁺ ion of the cysteine-benzo[a]pyrene epoxide adduct.

DETECTION OF LARGE IONS IN FTMS

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The multichannel detection capability of Fourier transform mass spectrometry (FTMS) over a wide mass range makes possible enhanced sensitivity for measurements involving high resolution, pulsed ionization, and tandem MS. However, the shorter RF transients obtained with high mass peptide ions reduces both the detection efficiency and resolution for these species. Sensitivity for high mass ions can be substantially improved by remeasuring ions hundreds of times. Measurement of multiply charged ions greatly extends the mass range of FTMS and makes accurate mass measurements of peptides above mass 10,000 possible.

A unique characteristic of FTMS detection which has not previously been taken advantage of is the non-destructive nature of imaging the cyclotron ion current. Elimination of the quench pulse commonly used in an experimental sequence results in substantial increases in sensitivity for high mass ions with multiple signal averaging events. This effect is shown in Figure 1, where molecular ion species of gramicidin S (MW 1141) formed by plasma desorption (1) show a signal-to-noise (S/N) enhancement of 35x with quench off. This enhancement increases with the number of scans such that a S/N improvement of >100x can be obtained with 1000 scans.

This is made possible by x,y collisional deactivation of previously excited ions back to the center of their orbits where they are available for subsequent redetection. The time required for this process decreases with increasing pressure and lower excitation power (corresponding to a smaller ion orbit), consistent with a collisional mechanism. For gramicidin S at $\sim 2 \times 10^{-9}$ torr, the remeasurement efficiency is nearly 100% after 180 s. This high efficiency makes possible the impressive gains in signal observed. For n signal averaged spectra, a S/N gain of $n/2$ is possible. This corresponds to a time savings of $1.59/n^{0.67}$. Thus, 7 spectra measured with quench off produces the same S/N as 100 spectra with quench on.

For only one ionization event, remeasurement can still be used to enhance the signal. Figure 2a shows a mass spectrum of gramicidin S formed by a single laser shot and detected once. Detecting these same ions five more times (without further ion production) results in a S/N enhancement of 3.1x (Figure 2b).

In addition to the sensitivity enhancement possible with remeasurement, the relaxation time provides an indication of the collisional cross section of various ionic species. For example, to obtain a remeasurement efficiency of 97% for Cs_7I_6^+ (m/z 1692) and $(M + \text{Na})^+$ of gramicidin D (m/z 1905) requires 290 s and 32 s respectively at a pressure of 3×10^{-8} torr. While this difference is larger than indicated by their relative physical dimensions, this could explain why high mass CsI clusters have much longer transients (resulting in higher resolution spectra) than peptide ions of comparable mass.

Preliminary results obtained in collaboration with Hunt and Shabanowitz using electrospray ionization (ESI) (2) in a modified QQ-FTMS (3) show abundant multiply charged molecular ions of a variety of peptides up to $\sim 17,000$ MW (Figure 3). Resolution for myoglobin ($M + \text{H}_1^{17}$) is ~ 700 at 10^{-8} torr indicating these high energy multiply charged species do not collisionally dephase and relax faster than a singly charged ion at a comparable m/z ratio. Reduction of pressure to 10^{-8} to 10^{-9} torr should increase sensitivity and greatly extend the lengths of the transients making high resolution, accurate mass measurements of peptide ions >10,000 a.m.u. possible.

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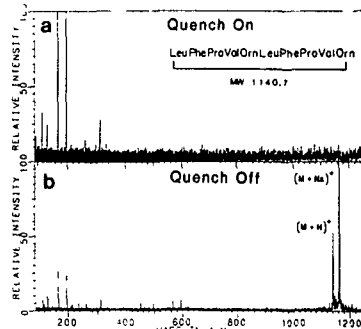


Figure 1: ^{252}Cf -PD spectrum of gramicidin S using 45 s ion collection time and 40 scans (30 min. total) with a) quench on, and b) quench off.

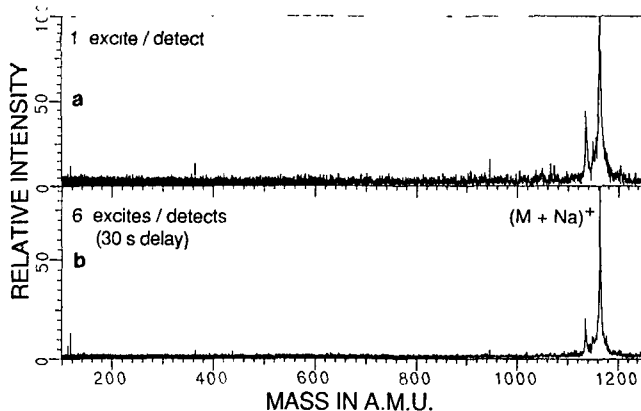


Figure 2: Laser desorption spectra of gramicidin S using a single ionization event and a) detecting once b) detecting 6 times with 30 s delay between excite/detect events.

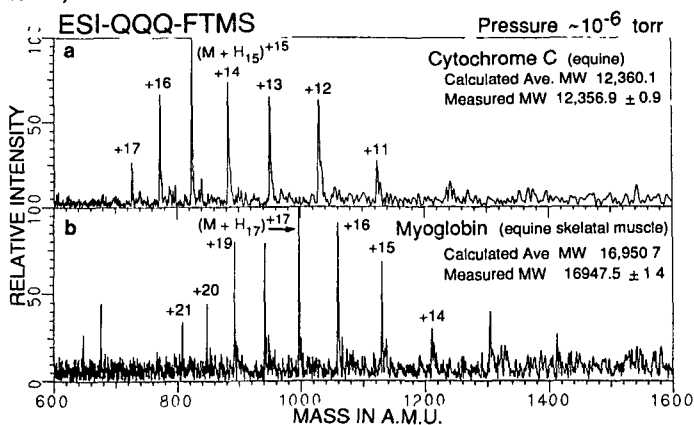


Figure 3: ESI-QQQ-FTMS spectrum of a) cytochrome C and b) myoglobin.

**TWO DIMENSIONAL FT-ICR AND ITS
REALIZATION ON A PC BASED INSTRUMENT**

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The technique of two dimensional FT-ICR mass spectrometry was first introduced by Pfandler *et al.* [1]. The techniques of 2D FT-ICR are very new. While its underlying principles have not been fully understood, it has great potential in that, in theory, it allows one to examine all the ion-molecule reactions in a chemical system in one experiment. Future development of the method requires a break-through on theoretical as well as experimental aspects.

This work offers a simple model to interpret the physical significance of the modulation of the ions signals in the additional dimension. According to the model, the additional dimension (which is introduced as the duration between the end of the first excitation pulse and the beginning of the second) determines the velocity of the primary ions just before the reaction period. If the excitation level is sufficiently high, the combination of the 2D excitation pulses will cause ejection of the primary ions at the beginning of the reaction period. Therefore, the primary and the product ions will be modulated with the primary ion's cyclotron frequency in the same phase. Even if the excitation level is low enough to avoid ion ejection, the velocity of the primary ions is still modulated. Starting from assumptions about the ion-packet and assuming negligible phase diffusion during the 2D excitation, a mathematical expression for the velocity modulation can be derived from the Lorentz equation [2]. The expression clearly shows that the velocity modulation is not sinusoidal.

The modulation of ion signals is complicated. It requires information on (1) the ion-loss mechanisms; (2) the ion-velocity dissipation; (3) the ion-molecule reaction kinetics; and, (4) the detection mechanisms for thermally-excited ions. From the examination of a simple chemical system which conserves charge and follows a rigid sphere kinetic model, it can be concluded that the modulation of the primary ion velocity can cause the modulation of ion signals. The ion-signal modulation is essentially not sinusoidal and, therefore, harmonics exist in the 2D spectra.

2D FT-ICR has been implemented on our home-built instrument [3]. According to the model outlined above, the Fourier transformation should be carried out step-wise in order to recover all the information. The instrument is operated at frequencies above the Nyquist frequencies in both dimensions. The spectra (Figure 1 and 2) obtained show the broad-band nature of the 2D technique.

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Figure 1

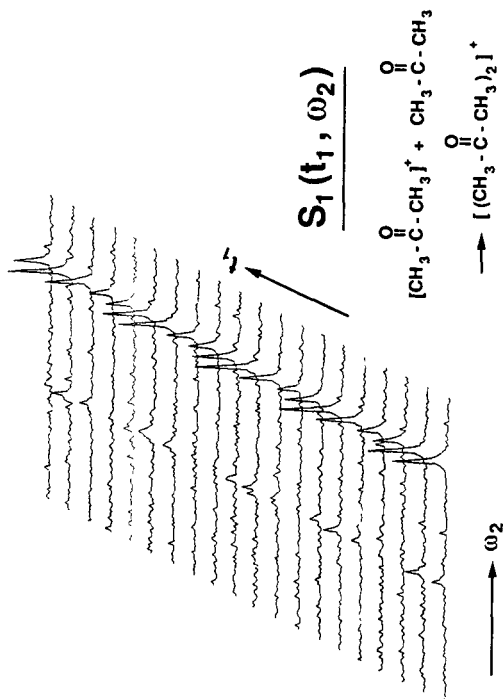
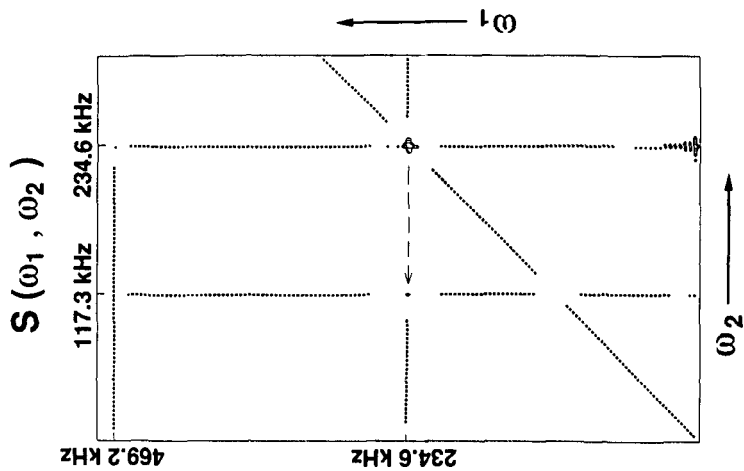


Figure 2



MINIMIZATION OF SPACE CHARGE EFFECTS IN AN FTICR TRAPPED ION CELL

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Suspended trapping pulse sequences provide a convenient mechanism to alleviate space charge effects generated during FTMS detection of poorly controlled, transient, or fluctuating neutral populations. These pulse sequences are distinguished by an event during which trapping voltages are set to ground potential to facilitate longitudinal ion flight along magnet field lines. Although suspended trapping pulse sequences have found some application in FTMS, most notably for ion introduction from external sources, their analytical utility has yet to be exploited. As will be described, manipulation of electrodes along the path of ion formation in the FTMS vacuum chamber accesses an array of experiments which improve mass calibration, increase sensitivity, and extend dynamic range, all with modest changes in hardware and software.

Initial suspended trapping experiments were devoted to an understanding of longitudinal ion motion in the vacuum chamber of the Nicolet FTMS 2000. As indicated in Figure 1, five electrodes placed at right angles to the magnetic field both constrain ions in potentials wells and direct the electron beam. For this electrode configuration in a conventional dual cell experiment, electrode 2 is used as a gate to pass an electron beam which is collected at electrode 5. Electrodes 2, 3, and 4 serve as analyzer trap, conductance limit, and source trap for the dual cell. Trap voltages are maintained throughout the pulse sequence except during the beam event when electrode 3 is grounded to equilibrate ion populations between the analyzer and source trap. An FTMS pulse sequence was written to select any combination of potentials between ± 10 V at electrodes 2 through 5 during the beam and subsequent delay events in the experiment sequence. Among the advantages of suspended trapping observed with this sequence are ion accumulation in a high pressure storage trap to maximize duty cycle for sensitivity gains in chromatographic experiments, a 1000-fold dynamic range improvement for fluctuating neutral populations, and wideband low ppm error mass calibration from a single calibration table independent of ionization conditions.

To gain an appreciation for the flexibility which suspended trapping experiments bring to FTMS experiments, an understanding of longitudinal ion motion between the various electrodes during the pulse sequence is required. In Figure 2, the flight of ions generated by electron ionization from a single section cell established between electrodes 3 and 4 is shown to occur over a several hundred microsecond period. (In an earlier experiment, Dunbar used this approach to determine the kinetic energy of photodissociated fragment ions in the cell (1).) Figure 2 also includes a repeat of the EI ion flight experiment except that a large well between electrodes 4 and 5 is allowed to accumulate ions prior to suspended trapping. This is easily accomplished because electrode 5 is usually biased at +5V during the beam event and can serve a dual role as collector for the electron beam and also as a trap plate for this second reservoir of ions. Note that while ions exit the analyzer cell over several hundred microseconds during the suspended trapping event, ions from the larger well enter the cell and sustain the ion signal for several milliseconds.

Figure 3 provides evidence of the utility of suspended trapping for simplified mass calibration. In the case where trapping potentials are sustained, excess ions

which contribute to a local electric field decrease the measured cyclotron frequency. In contrast, following a several milliseconds suspended trapping time, the identical initial ion population is replaced by a detected population which makes a negligible contribution to the electric field term for the effective cyclotron frequency. In effect, suspended trapping spectra exhibit an invariance to both initial ionization conditions and neutral populations.

Suspended trapping should extend the qualitative dynamic range in which varying neutral populations produce spectra of good quality. Presently, mixture components for GC/FTMS analysis must be within a relative concentration range of 10^2 to ensure detection of low abundance components without space charge distortion in the spectra of large abundance compounds. Figure 4 contrasts conventional and suspended trapping GC/FTMS spectra of naphthalene and methyl naphthalene mixture in the ratio of 1:10,000. To observe a spectrum for 100pg of naphthalene injected on column, a 50 uA, 30 ms electron beam was employed. As expected these ionization conditions yielded grossly distorted spectra of the overloaded methyl naphthalene with sustained trapping. In contrast, the suspended trapping spectrum exhibits no distortion and no frequency shift due to the large initial ion cloud.

These preliminary experiments indicate the potential of suspended trapping to improve the reliability of FTMS as a detector for fluctuating neutral populations. Work continues in the evaluation of its performance for FTMS detection of chromatographic effluent and for detection of transient ion populations generated by laser and thermal desorption.

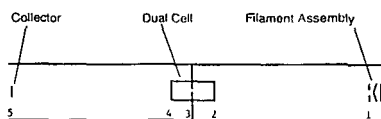


Figure 1. Electrode configuration in the Nicolet FTMS-2000 dual cell vacuum chamber.

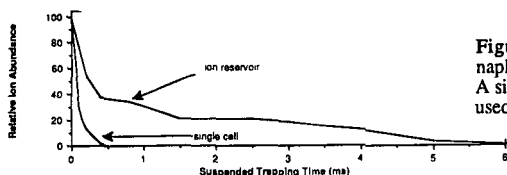


Figure 2. Flight of naphthalene molecular ion from a single section cell (electrodes 3 and 4, Figure 1) and for ions in the cell and large reservoir (electrodes 3 and 4, and 4 and 5.)

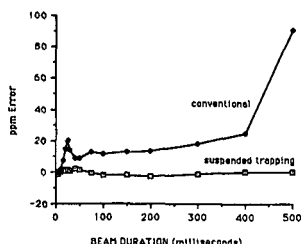


Figure 3. Mass measurement error (ppm) for naphthalene molecular ion as ion population increases. A single low ion density PFTBA calibration table is used for all measurements.

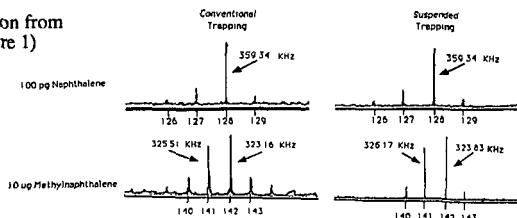


Figure 4. Comparison suspended and conventional trapping GC/FTMS from the injection of 100pg naphthalene and 10ug methyl naphthalene.

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EXACT AND NEARLY EXACT INTERPOLATION OF APODIZED FT-ICR SPECTRA

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All experimental Fourier spectra (FT-NMR, FT-ICR, FT-IR etc.) are defined at only a finite number of points and some sort of data manipulation is required to measure the true peak location/intensity if a spectral peak falls between two points of the discrete spectrum, *as it always does*. Some time ago¹ we developed a simple algorithm for *exactly* interpolating the true peak location from the *unapodized* discrete magnitude Fourier lineshape. This algorithm also extends to the exact interpolation of certain absorption-mode lineshapes². We have now extended this methodology to *apodized* FT-ICR lineshapes. This procedure completely obviates the systematic digital error inherent in apodized discrete Fourier spectra³.

We have also developed a family of interpolating functions, the KCe functions, which provide nearly exact interpolation

$$KC_e = (a\omega^2 + b\omega + c)^e$$

The parabola ($e = 1$) the Lorentzian ($e = -1$) and the magnitude-Lorentzian ($e = -1/2$) functions are members of this family and previously have been used for the approximate interpolation of apodized lineshapes⁴. The optimal KCe function varies with the apodizing window. Compared to the parabola which was shown⁴ to be the best interpolating function for apodized lineshapes, the optimal KCe function is one to two orders of magnitude superior. That is, the optimal KCe function has a *maximum* residual error which is one to two orders of magnitude lower than that of the parabola.

We have also determined the random *frequency* errors which come from the random *amplitude* errors in the spectrum. The efficacy of the KCe functions is so high that for spectra with a signal-to-noise ratio of 100, the residual systematic error is less than the random error. For all intents and purposes then the KCe functions are "exact". We have also shown that the random error increases if the spectra are zero-filled. For the parabola, zero-filling is desirable until it reduces the systematic error to below the random error. For the KCe functions, the residual systematic error is less than the random error and no zero filling gives the lowest total error.

Our recommended interpolating functions are KC5.5 for the Hanning window, KC6.6 for the Hamming window, KC9.5 for the Blackman-Harris window and KC11.5 for the Kaiser-Bessel window. The Kaiser-Bessel window is an estimate based upon extrapolation of the other recommendations. We also recommend *not* zero-filling spectra, prior to KCe interpolation.

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An External Ion Source Providing Ion Accumulation and Trapping
of Ions with Broad Energy Range for
Ion Cyclotron Resonance Spectrometry

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In ICR spectrometry there are three different possibilities described in the literature to transfer ions generated in an external source outside the room temperature bore of the superconducting magnet. They can be characterized by the average kinetic energy of the transferred ions. Kofel et al. (1) described ion transfer at low kinetic energy of only a few eV for general applications. Alford et al. (2) employed a kinetic energy of a few hundred eV to transfer externally generated metal cluster ions. The deceleration was performed with a pulsed decelerator, which limits the mass range. Kofel and McMahon (3) utilized the same energy range to sample ions from a high pressure source. They used a cylindrical decelerator. The ICR spectrometer manufactured by Spectrospin employs high ion energy of more than 1 kV (4). According to the Lagrange-Helmholtz equation the opening angle, the radius, and the kinetic energy of an ion beam are connected. For a given ion beam radius small opening angles are obtained at high ion energy. On the other hand, the trapping efficiency of externally generated ions is inversely proportional to the kinetic energy distribution.

Best suited as external ion sources are ion sources with a high ion yield like the Extrel ion source for quadrupole mass spectrometers. This source generates ions of a fairly broad energy range and operates best at ion energies around 30 eV.

To trap efficiently ions of a broad energy range and with kinetic energies of up to 100 eV the "screening" of the trapping electrodes of the ICR cell introduced by Marshall (5) was applied to a cylindrical ICR cell.

The experiments were performed with a modified Spectrospin Fourier transform ICR spectrometer (4) with a 7.02 T superconducting magnet. The room temperature bore of the magnet is 89 mm. The instrument is controlled by an Aspect 3000 minicomputer. A differentially pumped vacuum system is used. In the ICR chamber the pressure is kept constant at 2×10^{-9} mbar. In the external source sample pressures between 1×10^{-7} and 8×10^{-6} mbar are utilized. Ions are externally generated with a crossed-beam Extrel ion source, modified for axial beam operation. The source gives best ion yields at ion energies between 10 and 50 eV. The ions are transferred to the ICR cell with this energy with the aid of a guiding lens and deflection system designed for operation at low energies. The ions are trapped in the ICR cell by pulsing the trapping plates between the trapping potential and an optimized potential of a few volts. Trapping potentials of up to 100 V are employed.

With the trapping potentials at 100 V, a mass shift of less than 0.001 amu is generated for nitrogen molecular ion. The mass shift depends linearly on the trapping potential. In the pressure range from 1 to 9×10^{-6} mbar the ICR signal intensity depends linearly on the sample pressure in the external ion source. At higher pressures saturation is observed. The transfer efficiency shows a broad maximum, which depends on the ion kinetic energy as shown in Fig. 1 and the ion density in the beam. The maximum transfer efficiency is at 40 eV ion kinetic energy. The maximum trapping efficiency is found for trapping potentials of 30 - 40 V, correspondingly.

The pulse sequency described above can also be utilized to accumulate ions. The ICR signal intensity increases linearly with accumulation time, as shown in Fig. 2, until the trapping capacity of the ICR cell is exhausted.

In small band mode high resolution of 3×10^6 for the Argon ion at a pressure of 2×10^{-9} mbar has been observed. The performance in broad band mode is not degraded.

SIMION (6) calculations show that the trapping and the ion accumulation are due to oscillations of the ions perpendicular to the magnetic field induced by the grids.

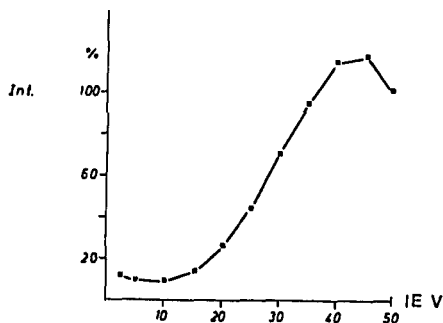


Fig.1.

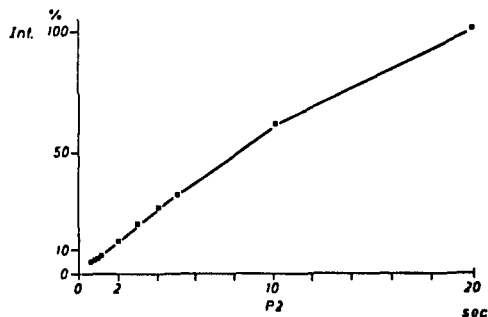


Fig.2.

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Funding of work by the Deutsche Forschungsgemeinschaft is acknowledged.

THE EFFECTS OF PRESSURE ON ION EXCITATION IN FOURIER TRANSFORM
ION CYCLOTRON RESONANCE (FT-ICR) SPECTROMETRY

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In the past two years we have developed a series of experiments on the TAMU two-section cell FT-ICR to study ion excitation and detection. These experiments involve exciting (rf electric field of discrete frequency, sum of individual frequencies, or by sweeping the rf field over a range of frequencies) the ion population (formed by electron impact ionization) to a radius larger than the aperture in the conductance limit. Ions which are excited to larger radii do not partition to the analyzer cell when the potential applied to the conductance limit is grounded.¹ These types of experiments can be extended to permit high-resolution ion partitioning by phase-specific ion excitation (HIPPIE), which enhances the utility of ion ejection techniques for mass-selective ion chemistry studies.²

The mass-selection methods are most useful for ion molecule reaction chemistry studies and collision-induced dissociation. Because ion chemistry studies require relatively high pressures ($>10^{-7}$ torr), ion excitation can be altered by collisional relaxation (of the translational energy) and the chemistry of a particular system can be altered by collisional excitation (increasing the internal energy of the ion). Another aspect of ion ejection that has been not been evaluated in detail deals with the efficiency of the process. For example, if one ejects ions from the ion cell and then introduces a delay in the experimental sequence to allow ion molecule reactions to occur, the abundance of the mass-selected ion decreases and low abundance ions appear in the ICR spectrum. Oftentimes the ions that "grow" into the spectrum correspond to ions which were ejected. Presumably, the ions "grow" into the spectrum because the ion ejection process is not completely quantitative. That is, a small fraction of the ions are not ejected by the rf excitation, but rather the abundance of the ion is reduced to a value which is below the dynamic range of ion detection.³ *Such considerations become very important for processes such as CID where the efficiency of converting parent ions to fragment ions is low (a few per cent. at best!).*

There are several factors which can strongly influence ion excitation. Two of which are: (i) the extent of ion excitation is greatly influenced by the location of the ion in the ion cell, especially for the cubic ion cell where rf field inhomogeneities are large; (ii) at pressures of 10^{-6} to 10^{-7} torr ion-neutral collisions can lead to collisional relaxation. Collisional relaxation is an important consideration for low power rf excitation where excitation times are long and when ions are excited to high energies (10 eV or greater) and the time between collisions becomes very short ($<10^{-3}$ s).

It is difficult to quantitate the problems described above by performing single cell experiments, but such effects are readily observed in ion partitioning experiments performed on a two-section cell instrument. Figure 1 contains a series of mass spectra obtained on our two-section cell instrument. The region displayed includes m/z 28 (N_2^+) and m/z 29 (background signal). The first mass spectrum was obtained by simply partitioning the ions to the analyzer and detecting. The subsequent spectra were obtained by exciting the m/z 28 by applying an rf signal at the cyclotron frequency for a variable period. Note that the relative abundance of m/z 28 decreases as the rf excitation period is increased (the signal decreases because some fraction of the m/z 28 ions are excited to radii which exceeds the dimensions of the conductance limit aperture), but the m/z 28 signal is not completely eliminated as it should be if all the ions were being uniformly excited. If the pressure of N_2 is reduced (from 10^{-6} to 10^{-7} or 10^{-8}) the signal at m/z 28 is eliminated after the ion has been excited to approximately 4 eV. On the basis of the dimensions of the conductance limit aperture, ion partitioning should cease when the ion has been excited to approximately 4.4 eV.

The results of these experiments clearly illustrate the limitation of rf excitation for quantitative measurements and for enhancement of dynamic range to measure low level signals. Careful attention must be given to any experiment involving ion ejection---ion ejection is not an alternative to quenching the cell by a DC voltage pulse.

The experiment described above points out an important limitation of single section cell experiments for performing CID and mass-selected ion molecule reaction chemistry. Specifically, ion ejection is not an adequate technique for quenching the ion cell of unwanted ions. This problem can be eliminated by performing CID and mass-selected ion molecule chemistry in a two-section ion cell. For instance, by using a two-section ion cell all the ions in one cell can be eliminated by performing a DC voltage quench and ions from the other cell can be selectively partitioned according to mass, energy, and/or phase to the "clean" cell. Once the desired ions are trapped in the "clean" cell the chemistry experiments can be performed.

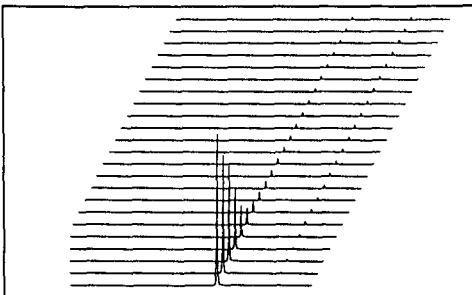


Figure 1. Partitioning profile for N_2^+ (m/z 28) at 5×10^{-6} torr. N_2^+ ions were irradiated on resonance (110 Vm^{-1}) for 60-500 μs prior to partitioning to an ion free analyzer region.

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SIMULATED RESONANCE EXCITATION OF TRAPPED IONS

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Preliminary findings are presented of a simulation study of resonance excitation of gaseous ions stored within a quadrupole ion trap [1]. The simulation program calculates the trajectories of positively- or negatively-charged ions stored in an ion trap operated in the common mode wherein the end-cap electrodes are grounded. The simulation can be performed for ions of any mass-to-charge ratio either in a collision-free system or in the presence of a chosen collision gas at a selected pressure [2-4]. The output of the simulation is obtained as (i) temporal variations, (averaged usually over one RF cycle), of kinetic energy, axial position, and radial position, (ii) two-dimensional representation of an ion trajectory in the form of axial (z) versus radial (r) position, and (iii) frequency analysis of any chosen time domain of ion trajectory.

The arrangement chosen for computer simulation of resonance excitation allows an excitation signal of any amplitude and frequency to be applied to both end-cap electrodes such that these electrode potentials are in phase with one another. The excitation signal can be initiated at any chosen time and phase. In Figure 1 is shown an example of the temporal variation of ion kinetic energy.

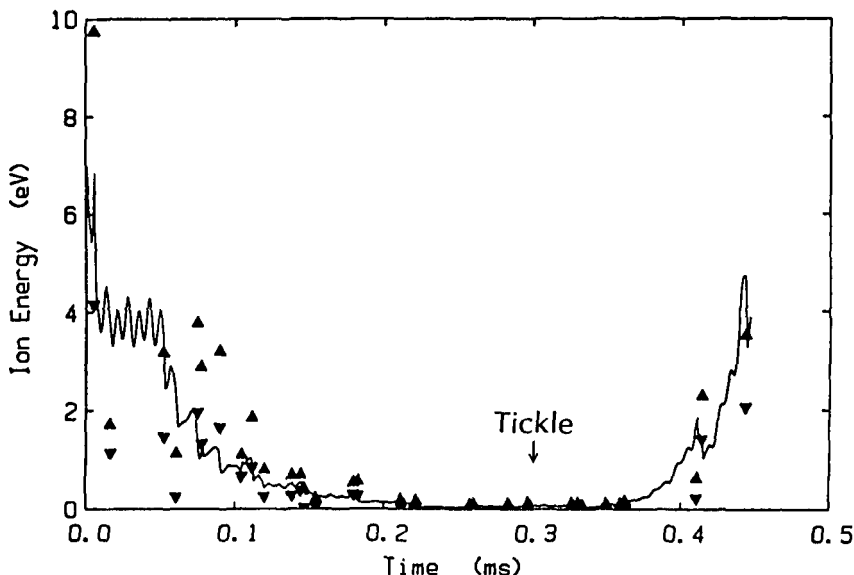


Figure 1. Calculated temporal variation of ion kinetic energy (averaged over one RF cycle) in the presence of neon as collision gas. A delayed auxiliary potential is imposed after 0.3ms of storage time. Working point ($a_z = 0$, $q_z = 0.1955$); m/z 134; $r_0 = 1$ cm; $V = 268$ V; drive frequency 1 MHz; neon pressure = 7 mtorr; initial position, $x = y = z = 5 \times 10^{-6}$ m; initial velocities, $\dot{x} = 1 \times 10^3$ m s $^{-1}$, $\dot{y} = \dot{z} = 0$; auxiliary potential of 3.5 V oscillating at $\Omega_z/2$ (69.6536 kHz); 32 collisions are shown wherein the solid upright and inverted triangles represent, respectively, the instantaneous ion kinetic energies before and after each collision.

Initially, the ion loses kinetic energy from an initial energy of 7 eV in successive momentum-dissipating collisions with neon. While the kinetic energy is averaged over one RF cycle, the instantaneous values of ion kinetic energy upon and immediately following a collision are shown in Figure 1. Collisional focusing of the ion at the ion trap center is concomitant with ion thermalization. At 0.3 ms of the simulation, the excitation signal or tickle is initiated; the tickle voltage, of amplitude (p-p) 3.5 V, is oscillating at the fundamental axial secular frequency. Ion kinetic energy is enhanced gradually in competition with momentum-dissipating collision until at 0.45 ms the ion is ejected. In this example, the ion is ejected radially [5].

The simulation programme permits calculation of ion trajectories over a wide range of conditions and has revealed a variety of features of ion trap behavior. In a collisional system, ion kinetic energy upon ejection is sensibly constant for a given working point within the stability diagram, while the tickle fluence (amplitude x irradiation period) required for ion ejection increases with collision gas pressure. In view of the observation for the experimental conditions of Figure 1, that radial ejection is induced upon axial excitation, attention has been directed to the frequency dependence of ion excitation and the analysis of frequency components of ion motion in collision-free systems.

Results obtained with four ions for which the initial coordinates and velocities are representative of an ensemble indicate that, for the ideal case of a single ion in a trap with infinite electrodes, interpretation of ion motion in terms of a three-dimensional Lissajous figure with independent axial and radial components is incorrect. Ion irradiation at the fundamental axial ($\omega_{z,0}$) and radial ($\omega_{r,0}$) secular frequencies does lead to ion ejection but does not lead exclusively to axial and radial ejection, respectively.

Irradiation at $\omega_{z,0}$ can induce a frequency component of ion motion which is initially of a frequency lower than $\omega_{r,0}$ and increases to the value of $\omega_{r,0}$ upon prolonged irradiation. This frequency component is designated as an *induced* frequency. The higher order secular frequencies are only weakly absorbed. Strong absorption of resonance irradiation was observed with a series of frequencies, $\omega_{u,n}^* = (n + \beta)\Omega$, where $u = r$ and z , $n = 0$ and 1 , β is a function of the working point, and Ω is the RF drive frequency. Of these frequencies, which have been designated as *new* frequencies, $\omega_{z,0}^* = \beta\Omega$ is the most strongly absorbed and absorption of this *new* frequency occurs more readily than that of $\omega_{z,0}$.

Further work is underway in an effort to gain understanding of these observations and to investigate the behavior of trapped ions at working points throughout the stability diagram and in collisional systems.

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**NEGATIVE ION CHEMICAL IONIZATION IN AN ION TRAP MASS SPECTROMETER
USING REAGENT ANIONS INJECTED FROM AN EXTERNAL ION SOURCE**

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We have been pursuing the analysis of polyatomic anions in an ion trap mass spectrometer (ITMSTM). During the course of this work we have reported on the formation of reagent anions within the ITMS [1], collision-induced dissociation of polyatomic anions in the ion trap [1,2], anion/molecule reactions in the ITMS [2], and the coupling of an external ion source for the injection of anions into the ion trap [3]. We have noted that the number of reagent anions formed within the ion trap by conventional means is roughly 10^3 lower than the positive ions formed from the same reagent gas species [2]. This is due to the fact that electrons are not stored under conditions used to store ions. The slow electron number density is always, therefore, relatively low in the ITMS. For this reason, we have been developing tactics for negative ion analysis which do not rely on the formation of reagent anions or slow electrons within the ITMS.

The interfacing of an external ion source to an ITMS provides two possible approaches to negative ion analysis; i) the formation of **analyte** anions in the ion source followed by injection into the ITMS and ii) formation of **reagent** anions in the ion source, injection of the reagent anions into the ITMS, and formation of **analyte** anions by ion/molecule reactions in the ITMS. We have demonstrated the former approach [2,3] with an atmospheric sampling glow discharge ionization (ASGDI) source [4]. Such an approach is preferred in the real-time analysis for trace compounds in air, particularly for species which capture electrons. The latter approach is demonstrated here. This approach is appropriate when samples can be introduced into the ion trap in a more or less undiluted form, e.g. via a chromatograph or a solids probe. An advantage of this approach is the capability of selecting the reagent anion and therefore having better control over the mechanism and energetics of ionization than is possible in a high pressure chemical ionization source. We have demonstrated the utility of selected reagent cation chemical ionization in an ITMS. We demonstrate selected reagent anion chemical ionization here.

Figure 1 shows the mass spectra of ions injected into the ITMS from a glow discharge source using three different reagent gas mixtures. Note that a variety of anions can be readily created in the glow discharge ion source by changing the gas composition. The ITMS can be operated to mass selectively accept only one of these reagent ions and the ion trap can be "filled" with these ions orders of magnitude faster than by forming the reagent anions in the ITMS. To date, we have concentrated on the reactions of OH^- , O_2^- , and NO_2^- . We find that for 2,4,6-trinitrotoluene (TNT) (admitted via a solids probe) O_2^- reacts primarily by electron transfer, NO_2^- reacts primarily by proton abstraction, and OH^- shows both types of reactions. We have studied the kinetics of the reaction of NO_2^-/TNT in detail to demonstrate the desirability for large reagent ion number densities and long reaction times for low levels of analyte.

To demonstrate the ability to observe low sample concentrations, we admitted the plastic explosive RDX via an unheated solids probe. The saturated head space vapor pressure of this compound at room temperature is roughly 6 pptr. This constitutes an upper limit in number density in the ion trap of $\approx 1 \times 10^8 \text{ cm}^{-3}$. The actual number density within the ITMS is likely to be substantially less since the RDX is not allowed to equilibrate (i.e. the system is pumped at 350 L/s) and the end of the probe was situated several mm away from the ITMS (no teflon inserts were used). Figure 2 (top) shows the mass spectrum obtained when all of the reagent ions from the N_2O , H_2 , He mixture were injected into the ITMS and a reaction period of 1 s was used. A variety of ions appear from the relatively dirty vacuum system (we have

housed the ion trap in an unheated 6" cube). Two of the ions fall at masses known to be formed from RDX, viz. those that appear at m/z 129 and m/z 176. MS/MS confirms that these ions are indeed formed from RDX as the major daughter ions observed (m/z 85 from m/z 129 and m/z 102 from m/z 176) are known from our previous experience with RDX in the ITMS.

A disadvantage to the use of a non-pulsed external ion source, as used in this study, is that reactive neutrals that are formed in the ion source are constantly entering the vacuum system. These reactive neutrals can interfere with the desired reaction chemistry by reacting with the mass-selected reagent anion to form other anions. A pulsed ion source is therefore more appropriate. Nevertheless, the usefulness of the injection of reagent anions into the ITMS from an external ion source is clear from this work and should prove to be an important approach to the analysis of compounds that undergo fast ion/molecule reactions with anions.

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Research sponsored by the U. S. Department of Energy, Office of Basic Energy Sciences, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

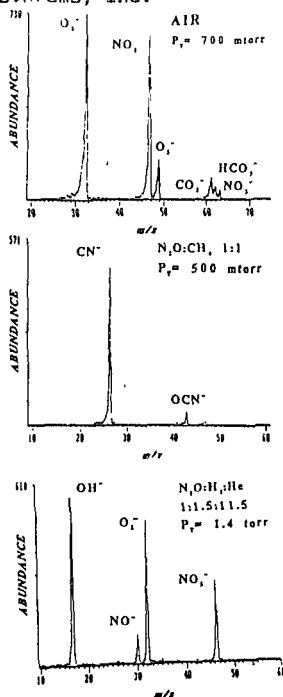


Figure 1

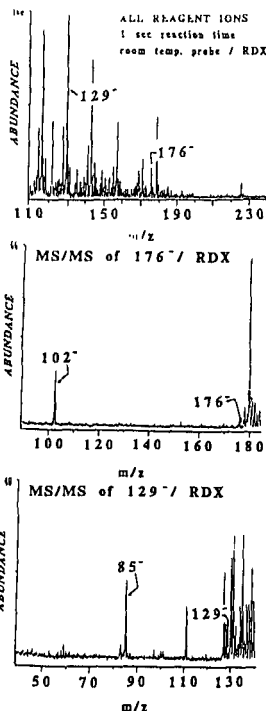


Figure 2

DAUGHTER ION FORMULA DETERMINATION BY MSⁿ EXPERIMENTS
ON NATURALLY OCCURRING ISOTOPES

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Naturally occurring isotopes like ¹³C and ³⁷Cl provide additional information that is useful in the interpretation of a mass spectrum. An MS/MS spectrum of a polyatomic parent ion m⁺ in conjunction with the MS/MS spectrum of the corresponding ¹³C or ¹⁵N containing ion (m+1)⁺ can be used to determine daughter ion elemental compositions.^{1,2} A limitation of this method is that the (m+1)⁺ peak must be composed mainly of the isotopic ion; if it has a significant content due to another ionic species the data is difficult or impossible to interpret.

This limitation can be overcome by the use of high resolution parent ion separation or by using one or more additional stages of mass spectrometry to generate the appropriate isobar for the final stage of MS/MS. The latter method can be carried out with an instrument capable of MSⁿ experiments with good MS/MS efficiency since the initial parent ion will be of small relative intensity when it contains the natural isotopic abundance. The Finnigan Ion Trap Mass Spectrometer (ITMSTM) seemed well suited for this type of experiment since it has been shown to be very efficient in performing MSⁿ experiments.

An example of this utility is described with the compound N,N-dimethylaniline. We have noted that (M-H)⁺, m/z 120, from this compound shows a couple of interesting daughter ions due to loss of 17 and 29 daltons. It cannot be determined by MS/MS alone with the ion trap whether or not these neutral fragments contain the nitrogen atom. The normal experiment of taking the MS/MS spectrum of (m+1)⁺ was not possible since this peak is composed predominantly of the molecular ion. However, the molecular ion fragments efficiently to (M-H)⁺ in the MS/MS spectrum obtained with the ITMS. Thus, an MS³ experiment can be performed to obtain the daughter ion formula from the natural abundance of the isotopes. The left spectrum in Figure 1 is an MS³ spectrum obtained starting with the ¹²C molecular ion and the right spectrum is the corresponding experiment starting with the natural ¹³C isotope of the molecular ion.

The m/z 120 ion in the ¹³C spectrum results from a competing process in the experiment sequence (vide infra) and is not important in the analysis of the data. Looking only at the peaks below m/z 120, the two spectra are almost identical except for the mass shifts. The Table below lists the calculated ¹²C/¹³C ratios for the two possible isobars at each of the main daughter ions along with the experimental results, which have been corrected for contributions from adjacent peaks. This data shows that the neutral lost in each of these reactions contains the nitrogen. While this is not unexpected for the m/z 77 ion which can be formed by a simple cleavage, a rather complex rearrangement must occur for the formation of m/z 103 (loss of NH₂) and m/z 91 (loss of CH₂NH). We are continuing to study this system to determine the reaction mechanism and product ion structure.

Besides the ¹²C/¹³C ratio of the daughter ion peaks of interest, there are other features of these spectra to be noted. First, it is desirable to obtain both daughter ion spectra under identical conditions so that appropriate corrections can be made when there are adjacent peaks. To get the initial parent ion intensities of m⁺ and (m+1)⁺ to be equal in their respective experiments, it is necessary to ionize for approximately eleven times longer in the (m+1)⁺ experiment. However, increasing the ionization time increases the relative contribution of other reactions taking place. In this case a competing reaction to electron ionization is self-protonation to give an isobaric species to the ¹³C containing molecular ion. The MS/MS spectrum of this species is different from that of the molecular ion because of the additional dissociation pathways from the protonated species (e.g. a base peak at m/z 107). Also, this competing reaction makes it difficult to obtain an initial intensity of the ¹³C containing parent ion that is equal to the ¹²C parent ion in the complementary MS/MS spectrum.

Another feature of the data is the large m/z 120 peak in the ^{13}C spectrum. This peak provides evidence of another reaction taking place during the first stage of MS/MS in the experimental sequence, charge exchange. This is a potential problem in any trapping instrument when the neutral analyte is continuously present in the reaction region. The result here is that the m/z 121 selected for the second stage of MS/MS contains isobaric ions, the ^{12}C molecular ion along with the ^{13}C (M-H) $^+$. The charge exchange reaction is not readily apparent in the first stage of the ^{12}C experimental sequence since it mainly produces (91.2%) the same ion, along with some (8.8%) ^{13}C molecular ion.

Looking only at the MS^3 spectrum of the ^{12}C ion, it might be deduced that the m/z 120 ion undergoes charge exchange and proton transfer with the neutral to give the ions at m/z 121 and 122, respectively. However, looking at the MS^3 spectrum of the ^{13}C ion, it can be seen that the respective ions formed are m/z 122 and 123. This suggests that the ^{13}C species retains the charge in the ion/molecule reaction and that one and two hydrogens are transferred to it from a neutral reactant.

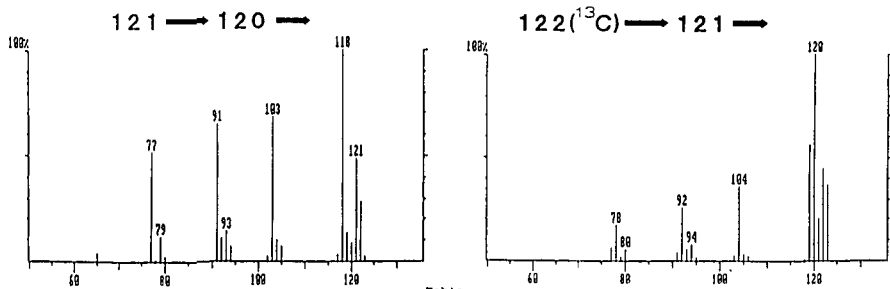
A limitation to this procedure is that it will be applicable, for the most part, only to peaks that have a significant abundance in the final MS/MS spectrum or peaks that do not have adjacent peaks. This is due to the statistical considerations associated with the fact that a limited number of ions can be stored in the ion trap (10^3 - 10^6). Thus small peaks in MS^3 or higher spectra will consist of a few hundred to few thousand ions. Therefore, statistical fluctuations in ion signal will be relatively large and determining accurate $^{12}\text{C}/^{13}\text{C}$ ratios will be difficult.

In conclusion, the use of multiple stages of mass spectrometry (greater than two) should prove useful in many cases in which isobaric or possibly isomeric interference precludes the use of the conventional MS/MS approach. Such circumstance is likely for the ITMS which typically affords unit mass resolution for both parent ion selection and daughter ion measurement.

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Figure 1



m/z	Formula	Calculated $^{12}\text{C}/^{13}\text{C}$	Experimental $^{12}\text{C}/^{13}\text{C}$
103	$\text{C}_7\text{H}_5\text{N}^+$	0.14	0.03
	C_8H_7^+	0.00	
91	$\text{C}_7\text{H}_5\text{N}^+$	0.33	0.17
	C_7H_7^+	0.14	
77	$\text{C}_7\text{H}_5\text{N}^+$	0.60	0.38
	C_8H_7^+	0.33	

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HOW TO USE A STANDARD QUADRUPOLE FILTER AS A TWO DIMENSIONAL ION-TRAP

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Ion confinement in the RF-only collision cell of a tandem quadrupole instrument, using the entrance and exit lenses as electrostatic mirrors is very efficient (1). With trapping Collisionally Activated Dissociation (CAD) efficiency is proportionnal only to the number of collisions (which is proportional to the product of target gas pressure by confinement time) between reactant ions and the collision gas, independently of the value of each parameters. Furthermore the absence of an added buffer gas allows to observe reactions at room temperature since the kinetic energy of ions near the quadrupole axe is low in comparison of thermal energy. An important application of this device is the access to thermodynamic equilibrium in ion molecule reaction (2). We have also demonstrated that selective ion rejection from the collision cell can be effected using an auxiliary radiofrequency field characteristic of the considered ion (3).

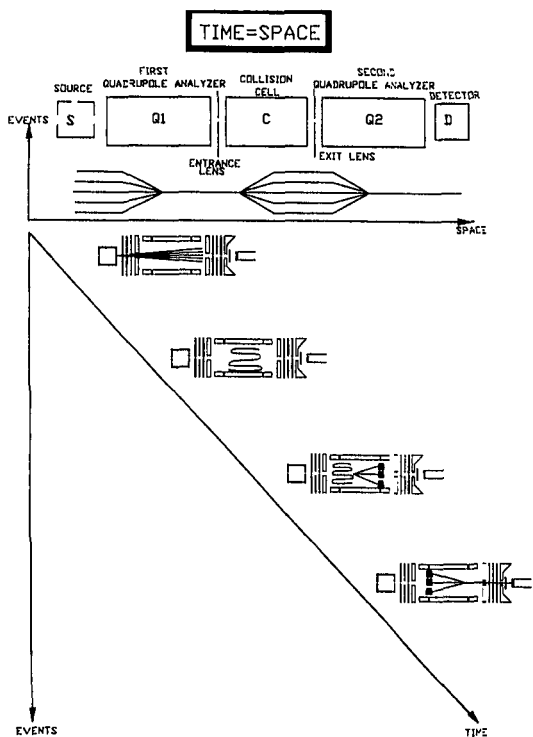
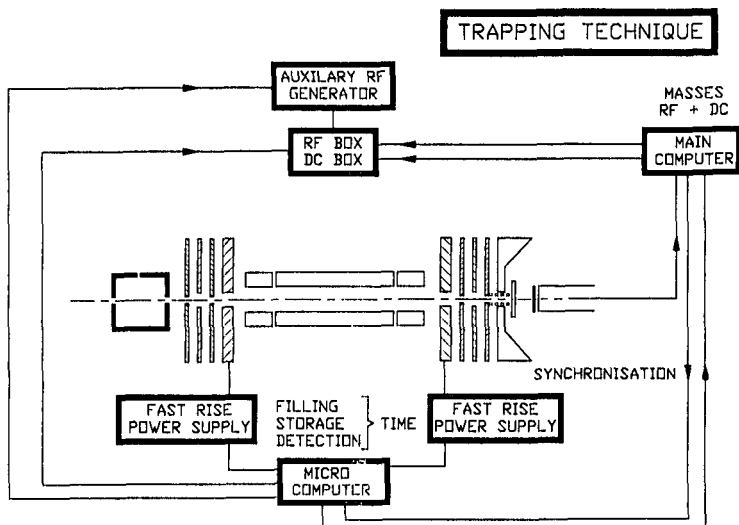
We wish to report here the use of a single quadrupole analyzer (scheme 1) fitted with prefilter at the front and at the rear of the filter and with an annular detector in order to perform ion selection and confinement process. In this instrument the mass selection for parent or daughter ion and the reaction or CAD steps are realized at consecutive time instead of being done at different place as in a tandem MS/MS instrument (scheme 2).

A typical experiment for ion-molecule reaction is described below: (i) filling of the trap (RF-only), (ii) parent ion selection (resolving quadrupole), (iii) reaction step (RF-only, and auxiliary field), (iv) daughter ion selection (resolving quadrupole), (v) ion detection.

Preliminary experiments show that resolution of the analyser increases with trapping time and that good kinetic data can also be obtained with this instrument although the reaction time is less precisely defined than when using the RF-only collision cell of a MS/MS instrument.

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INJECTION OF IONS INTO A QUADRUPOLE ION TRAP FROM A CHEMICAL IONIZATION SOURCE USING A DC QUADRUPOLE DEFLECTOR

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Commercially available quadrupole ion trap mass spectrometers (Finnigan ITD, ITS, and ITMS) are currently designed to permit ionization only within the ion trap by either electron ionization (EI) or low-pressure chemical ionization (CI). The ability to inject ions from an external ion source into an ion trap with simple line-of-sight (on-axis) ion optics has recently been reported (1-2).

In this work, off-axis injection of ions into an ion trap is demonstrated from a high-pressure CI ion source through a DC quadrupole deflector (3). Design criteria for this system were: non-differentially pumped system for instrumental simplicity, ion optics which are open to gas flow to minimize collisions of ions with neutral gas molecules; no line-of-sight between the ion source and the detector, to minimize neutral noise problems as well as to minimize resolution and sensitivity problems, and to preclude ion-molecule reactions inherent to operating an ion trap under high pressure conditions; exclusive pulsed injection of either positive ions or negative ions; ability to inject ions of low kinetic energy.

The quadrupole deflector ion injection system (Figure 1) was designed through the use of the SIMION ion optics modeling program. Since the quadrupole deflector is an energy analyzer, it would be expected to discriminate against ions created outside the ion source through collision-induced dissociation, CID, as they pass through the injection system. The quadrupole deflector does suffer from the lack of focusing in the direction parallel to the quadrupole rods. Through SIMION modeling, it was determined that the quadrupole deflector system offers excellent positional and angular acceptance in addition to its inherent energy filtering. Pulsed injection was effected by gating the voltages applied to the quadrupole deflector rods, thereby deflecting the ions 180° from the trap when injection is gated off.

Representative EI and methane electron-capture negative CI spectra of injected perfluorotributylamine ions with a nominal injected kinetic energy of 5 eV are shown in Figure 2. The ion at m/z 219 in the EI mass spectrum, not present in previous experiments, suggests minimal fragmentation for this low-energy ion injection experiment. The ion at m/z 197 is the result of an ion/molecule reaction of m/z 219 with water.

Experimentally determined transmission efficiencies were: 98% of ions which entered the quadrupole deflector could be deflected to the second tube lens, 60 to 70% of deflected ions are transferred through that tube lens to the entrance endcap, and 25% of deflected ions are actually transferred into the ion trap analyzer. The poor transmission through the endcap aperture is due to the defocusing of the ion beam which accompanies the sharp reduction in ion kinetic energy just prior to injection.

Detection efficiency can be defined as that percentage of total injected ions which are collected, stored, and ultimately detected. Figure 3 shows the detection efficiency of ion injection for argon 40^+ ions with various kinetic energies. Also shown is the multiplicative effect of detection efficiency with the transmission efficiency through the entrance endcap, illustrating the sensitivity advantage of low ion kinetic energies during injection.

Distortions in the quadrupolar field inside the quadrupole ion trap can result in non-linear resonances (4-5), experimentally seen as losses in sensitivity for certain combinations of RF and DC voltages. Relative minima for injected ions for various RF and DC voltages applied to the ring during ionization are found to correspond with theoretical third- and fourth-order distortions in the quadrupole field. The effects of non-linear resonances on injected ion spectra are shown in Figure 4: intensities of Br^- ions from bromobenzene and the resulting isotopic ratios are plotted as a function of RF voltage applied to the ring during injection (1 DAC $\sim 4 \text{ V}_{pp}$). These effects could be minimized by ramping the RF voltage applied to the ring during the ion injection pulse. Similar effects were seen using two different ion trap analyzers. Such non-linear resonances may result from field imperfections due to the holes drilled in the center of the ion trap endcaps for ion injection and detection. Optimal collection efficiency for injected higher mass ions ($m/z > 200$) occurs with applied RF trapping voltages corresponding to lower values of the Mathieu parameter q , where non-linear resonances are less prevalent; thus, injected higher mass ions do not suffer from this phenomenon.

This work demonstrates the utility of using a quadrupole deflector to separate a high pressure ion source from a mass spectrometer both in space and orientation, thus alleviating the need for differential pumping, as well as minimizing problems inherent to having a line-of-sight relationship between a high-pressure ion source and a mass spectrometer. Injection of ions from an external ion source opens up the possibilities for a wide range of new ionization and sample introduction techniques, including positive CI, electron-capture negative CI, fast atom bombardment (FAB), and any of numerous interfaces for liquid chromatography (LC/MS).

Acknowledgement.

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Fig. 1 Quadrupole Deflector Ion Injection System
(With Voltages for Positive Ions)

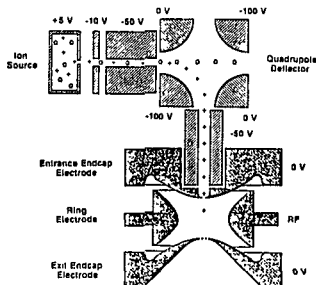


Fig. 2a EI Mass Spectrum of Injected Perfluorotributylamine Ions.

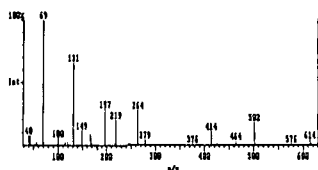


Fig. 2b EC-NCI Mass Spectrum of Injected Perfluorotributylamine Ions.

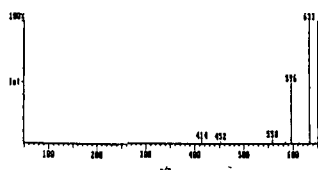


Fig. 3 Detection Efficiencies of Ion Injection System

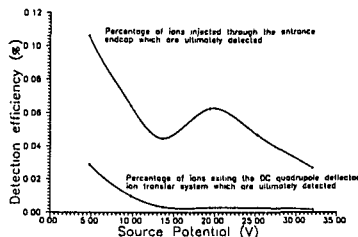


Fig. 4a Br^- Intensity as a Function of Injection RF Voltage

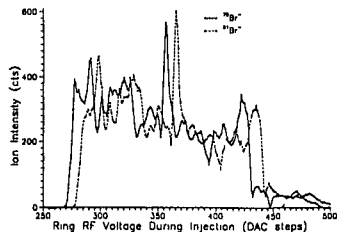
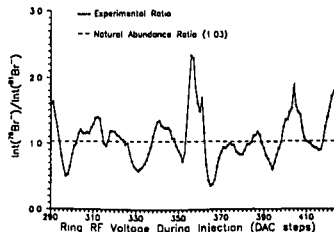


Fig. 4b Variation in Br^- Isotope Ratio as a Function of RF Voltage



A Historical Review of the On-line Combination
of Chromatography with Mass Spectrometry

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During the decade of the 50's, two important developments were taking place. One of these was the introduction of gas-liquid-partition-chromatography by James and Martin; the other was the development of mass spectrometry for a broader range of organic compounds (McLafferty, Meyerson, Sharkey, *et al*). Looking back 30 years later, it is easy to say that the on-line combination of the two instruments was obvious.

In actuality, the combination was not obvious. Gas chromatographs of that period usually ran at carrier gas levels of 25 to 50 cc/min. Mass spectrometers at that time were designed for analysis of petroleum fractions using a batch inlet. Vacuum systems were not optimized and scan mechanisms were deliberately slow to achieve maximum stability and precision. Thus, the suggestion that a mass spectrometer could be opened up to a gas chromatograph was modestly audacious to say the least, and required significant hardware modifications and operational compromises before the first crude experiments could be demonstrated.

In 1956, J.C. Holmes and F.A. Morrell presented a paper at the ASTM E14 meeting describing changes they had made to a consolidated 21-103B mass spectrometer. The inlet system was adopted to permit "an aliquot of the output of the column into the mass spectrometer"; the scan circuit was modified to give "a scan from approximately mass 12 to mass 100 in six seconds"; and the amplifier output was "fed to the Y input of the oscilloscope ... to present mass 28 to mass 44 in a single 13 second sweep".

Although today's sophisticated users might scorn this humble start, a small cadre of research workers foresaw the potential of the combination and in the next 2-4 years, several laboratories modified their magnetic mass spectrometers in a similar manner. In addition, the Bendix Time-of-Flight on the market at that time was immediately recognized as an instrument with certain built-in features that facilitated the combination, namely, a large, reasonable efficient pumping system and an electron multiplier (which was not standard in magnetic instruments of that period). Roland Gohlke at Dow was one of the first Bendix TOF users and his pioneering work demonstrated clearly that GCMS (as we now call it) was very practical for many real life problems.

Although instrument manufacturers were slow to respond to the need of larger pumping systems, several research groups were attacking the problem from two angles. Use of capillary columns for the GC separations provided carrier levels of 1-2 cc/min or less and thus improved efficiency of sample transfer. For packed column systems, a variety of enrichment devices, the so-called separators, were being developed in many laboratories. These were primarily of 3 types; jet separators (Ryhage-Stenhagen), effusion separators (Watson-Biemann) and membrane (Llewellyn-Littlejohn) separators. The first two of these performed the task of removing carrier helium from the GC effluent without significantly decreasing the quantity of sample in the flow. The membrane separator performed the task of enriching sample through a thin silicone rubber membrane due to the higher permeability of organic materials over the helium carrier gas.

Typical separator yields to the mass spectrometer were in the range 20-70% depending upon the GC flow conditions and mass spectrometer pumping systems. Considerable controversy occurred during the 60's over which separator was the best and occasionally, erroneous data was cited in an attempt to prove superiority. In the end, as is very evident today, the jet separator has been shown to have acceptable enrichment with minimal sample decomposition and is the only survivor in common usage.

Of course, use of capillary columns in the flow range of 1 cc/min. automatically results in a higher sample/carrier ratio. Even with the older pumping systems in use in the early 60's, 20 to 100% of the flow could be directed to the mass spectrometer so that use of separators was unnecessary.

By the end of the 1960 decade, most of the problems of GCMS interfacing were reasonably well understood. Electron multipliers were standard on all organic mass spectrometers, thus facilitating fast scanning. Some compromise was necessary for magnetic scanning, but this was not serious except for high resolution studies. Most commercial spectrometers had well designed differential pumping systems that would accept a gas flow in the range 10-30 cc/min. Future developments were to involve computerization, adaptation to special ionization methods, improvement of column technology, and of course, acceptance of the quadrupole. It was clear to everyone by 1970 that GCMS had arrived.

Wonderful! So now what about LCMS? Although liquid chromatography in several of its many forms had preceded gas chromatography by many years, it was not until the decade of the 60's that the technology was developed to permit what we now call high performance liquid chromatography (HPLC). Since most of the problems of GCMS had been resolved, it might be anticipated that LCMS should follow naturally. But nothing could be further from the truth.

The flow problems of GCMS involved handling 1-20 cc/min of gas into the spectrometer. In HPLC, a typical flow is 1 cc/min. of liquid but this amount will generate around 1000 cc/min. of gas (1244 for H₂O at STP). Furthermore, one important raison d'être for liquid chromatography is the separation of nonvolatile compounds. If a compound cannot be vaporized for separation in a GC column without decomposition, then it is highly probable that the same adverse fate will result if the compound is vaporized into a mass spectrometer. For LCMS, the problems of GCMS are exacerbated by one or two orders of magnitude. This is virtually a whole new ballgame.

The first published account of LCMS was by Tal'Rose, et al in 1968. In this work, a very small fraction of the LC effluent was leaked into a general purpose EI mass spectrometer with no special modifications. Benzene, biphenyl, and naphthalene were separated using heptane as the mobile phase. Aside from historical interest, the experiment caused little excitement due to the very small fraction of solute that was sampled and due to the volatile nature of the selected samples.

In 1974, 3 separate groups published results on 3 separate LCMS interface methods. Arpino, et al, modified an AEI MS-902 mass spectrometer with increased pumping capacity to accept 10-12 microliters/min. of LC effluent and showed an LCMS chromatogram of several steroids. Scott, et al, adopted a wire transport detector so that the wire passed through two vacuum locks into the ion source of a Finnigan 1015 quadrupole mass spectrometer and exited through similar vacuum locks on the other side. In spite of the limited amount of material that can be deposited on the wire, impressive results were shown for a fermentation extract sample. Horning, et al, used an API source to obtain spectra of MTH aminoacids. In their apparatus, the LC flow was

vaporized in an evaporation chamber prior to entering the API source where ionization occurred by either corona discharge or ^{63}Ni beta radiation.

Commercial adaptation of the wire transport and the direct introduction system followed in the latter half of the 70 decade. The wire transport was modified into a belt transport system to permit efficient transfer of solute into the ion source (Finnigan Corporation). The McLafferty DLI system was modified and marketed by Hewlett-Packard. These two commercial units were the basis of most LCMS studies up to the early 1980's.

Of course, other developments were concurrently in progress. A dimethylsiloxane membrane system was demonstrated by Jones and Yang (1975), but showed little promise due to low selective leverage between solute and solvent. Furthermore, tailing of relatively easy samples such as anthracene was excessive and presaged significant problems for less volatile materials. More important, several laboratories were actively searching for other methods, particularly methods involving nebulization of the solvent flow. Vestec investigated several processes for producing thermal nebulization; Futrell, using a similar apparatus, demonstrated mass spectra using sonic nebulization; Takeuchi used micro LC with pneumatic nebulization; and Iribarne and Thomson were showing direct ion evaporation using a pneumatic nebulizer with an atmospheric pressure induction electrode to enhance charging of spray droplets.

In 1980, a very important event occurred; thermospray ionization was discovered. Although this remarkable serendipity was anticipated by the theoretical studies on ion evaporation, it was first discovered in Vestal's laboratory when the filament of an experimental system failed and ion current was still observed. It was soon apparent that this was a soft ionization process that would produce ions from low volatility compounds. Equally important, the discovery stimulated the basic understanding of soft ionization processes and the realization that many of these occur through a similar mechanism, namely ion evaporation from aerosol droplets.

Within 2-4 years, thermospray was commercially available from several manufacturers and rapidly became the most popular LCMS interface. Further development of the belt transport system continued with significant interest in laser and ion bombardment. The data obtained by these desorption processes was quite acceptable but the sensitivity achieved was about 2 orders of magnitude less than acceptable. The DLI system lost much of its popularity to thermospray but continues to be used, particularly with micro LC which achieves a significant increase in sample utilization. But undoubtedly, an equally important development taking place in the early eighties, alongside thermospray, was the emergence of the various atmospheric ionization sources (Tsuchiya, liquid ionization; Fenn, Whitehouse, electrospray; Thomson, Iribarne, ion evaporation; Thomson, Danylewych-May, heated pneumatic nebulizer; Covey, Henion, ion spray). These atmospheric pressure techniques all have in common some form of nebulization and an electrode to assist in aerosol formation and sample ionization. They all give excellent soft ionization of a wide variety of samples with high sensitivity. Some of the operational modes, especially electrospray, have been shown to produce multiply protonated ions on high molecular weight materials. These multiply charged ions can be used to extend the molecular weight range of analytes above 15,000 Daltons even though the m/z range of the spectrometer is less than 2000 Daltons. The practical limit of this technique is not known at this time but is expected to possibly exceed 100,000 Daltons.

On the other end of the molecular weight scale, there has been renewed interest in obtaining electron impact data on compounds in the range 100-500 Daltons, particularly for environmental samples. Although the belt interface has performed this service for over

12 years with subnanogram quantities, the mechanical system is still perceived as a dinosaur lumbering through a delicate ion source and has never received widespread acceptance. Recent developments in the field of particle beam generation, as pioneered by Browner and co-workers at Georgia Institute of Technology, have been commercialized by Extrel (Willoughby) and Hewlett-Packard (Apffel) and offer an alternative interface method for obtaining EI spectra. These systems have demonstrated useful EI spectra for a wide variety of compounds, usually on samples in the range of 1-50 nanograms. Current trends show a strong orientation toward the particle beam system for environmental samples. As another alternative, there has been some effort toward generation of EI spectra using low flow columns (500 nanoliters/min. to 10 microliters/min.) in a DLI mode. These systems work satisfactorily but the paucity of commercial support for microliquid chromatography has discouraged any significant move toward this methodology.

GCMS started during the mid-50's, most problems were understood by the late 60's, and today it is almost a household word (at least it is in my house). LCMS started 15 years later, about 1970, but due to the very difficult obstacles that had to be overcome, has only started to be widely accepted in the past 3-4 years. But another very encouraging trend has been occurring concurrently during this decade of the 80's, namely, the interfacing of other chromatographic techniques to the mass spectrometer. The most dramatic of these new combinations has been the interfacing of capillary zone electrophoresis (CZE) and isotacophoresis by Smith and co-workers at the Battelle Pacific Northwest Laboratory. This group modified the capillary electrophoretic system so that the terminal end of the capillary was biased at 3-4KV rather than the conventional ground potential. Electro-osmotic flow provides a bulk liquid transfer of approximately 1 microliter/min. to the tip which is interfaced to the mass spectrometer in the configuration of an electrospray ion source (following Fenn, *et al*). Preliminary data show a real potential for analysis of complex biochemicals as well as other simpler molecules, especially salts.

Other separation systems that are being successfully interfaced to the mass spectrometer are super critical fluid chromatography, thin lay chromatography, ion chromatography, and yes, even countercurrent chromatography. The degree of interest in these various methodologies varies considerably, but an important message is being revealed. Even though interfacing a liquid separation system to a mass spectrometer will always be fraught with obstacles, there is now a clear demonstration that it is practical in many forms and that we have conquered our fear of these difficulties. The developments of the next decade will be spectacularly inspiring!

The Development of Selective Methods of Ionization

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Perhaps the first selective method of ionization in mass spectrometry was the technique of low voltage electron ionization, LVEI. The technique was first applied for the determination of low molecular weight inorganic gases.¹ However, the first substantial amount of work on the application of LVEI/MS to the analysis of complex mixtures was reported in 1956 for the analysis of unsaturated hydrocarbons in complex petroleum mixtures. The electron energy is adjusted to a compromise value sufficiently low to eliminate most (if not all) of the ionization from paraffins and sufficiently high to allow reasonable sensitivities for the unsaturated hydrocarbons.² Quantitative analyses on the compositions of synthetic mixtures agreed well with values from synthesis. The analyses of individual compounds and compound types agreed well with the much more time consuming analytical procedures then available.

One of the main problems in the analysis of complex mixtures is the absence of standards for the determination of calibration factors. It was shown in subsequent work that the sensitivities as molecular ions, M^+ , for aromatic hydrocarbons depended on the compound type, the number and length of the side chains, and the actual value of the electron energy.³

The combination of high resolution mass spectrometry with low electron energy ionization increased the selectivity of the analyses and provided much more detailed information about the composition of complex mixtures. The initial procedures required the manual recording of the peak heights for each component of every multiplet for hundreds of masses from the oscillographic paper from a high resolution mass spectrum.⁴ Improvements in the stabilities of the scan functions and the development of appropriate computer algorithms eliminated much of the spectacularly tedious work and while still allowing the identification of at least septuplets at the same nominal mass.⁵

Recently low voltage EIMS has been successfully combined with gas chromatography.⁶ Good agreement has been reported between the conventional batch introduction and gas chromatographic introduction using low voltage EIMS. The gas chromatographic separation enhances the interpretative capabilities of low voltage EIMS in general. A combination of low and high voltage GC/EIMS allows the differentiation between alkenes and cycloalkanes of the same molecular weight.

In chemical ionization mass spectrometry, CIMS, the sample ions are formed by chemical reactions of ions from the reagent gas with the sample molecules. Selective detection in CIMS involves changing the ionic chemistry (by changing the reagent ions) to be able to identify compounds with certain functional groups in the presence of other compound types. Perhaps the earliest reported example of selectivity in CIMS was the use of water as a reagent, H_3O^+ or $H(H_2O)^+$, as the reagent ion, to ionize only decanol in an equimolar mixture of decane and decanol.⁷

Since the sample ions are produced by gas phase ionic reactions, the rate constants for the ion/molecule reactions between the reagent ions and the sample molecules are the determining factors in the selectivity (if any) of a chemical ionization reagent gas system. The original CI reagent gas (and one that is still widely used) is methane, a non-selective reagent gas because of the large rate constants for the reactions of the two reagent ions, CH_4^+ and $C_2H_5^+$, with most molecules or classes of compounds. Using methane as an example, one may readily show the dependence of total

sample ionization (or sensitivity) on the rate constants for the ion/molecule reactions.

Under normal CI conditions, when the pressure of the sample is less than a few tenths of a percent of the pressure of the methane reagent gas and the extent of conversion of the reagent ions to product ions is no more than approximately 25%, one may write the differential rate equation for the formation of sample ions,

$$d\Sigma I_i^+ / dt = \alpha (k_{i+}[CH_5^+] + k_{is}[C_2H_5^+]) [Sample] \quad (1)$$

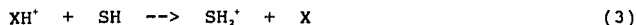
which may be solved with the usual approximations for a small extent of conversion to give

$$\Sigma I_i^+ = \alpha (k_{i+}[CH_5^+] + k_{is}[C_2H_5^+]) [Sample] \Delta t \quad (2)$$

The symbols in these equations have relatively standard definitions: I_i^+ = detected ion current for each product ion; α = proportionality constant between detected ion current and ion concentration in the source of the mass spectrometer, which is presumed (and generally appears) to be the same for all ions; k_i = rate constant for reaction of ion, i , with the sample; $[X]$ = molar concentration of X ; and t = ionic reaction time. From equation (2), one can see that the total sample ionization, ΣI_i^+ (for positive ions), is proportional to the sample concentration, the ionic reaction time, the concentration of reagent ions, and the rate constants for the reagent ion/sample molecule reactions. An analogous equation can be developed for the production of sample ions from reactions of negative CI reagent ions.

It is one of the generally accepted tenets of ion/molecule chemistry that endothermic reactions are slow and seldom observed, whereas exothermic reactions are at least probable. For simple reactions, like proton transfer and charge exchange, it is generally agreed that the exothermic reactions will occur rapidly to give product ions and the endothermic reactions will not occur, and hence no product ions will be detected. Consequently, one uses a thermochemical basis to identify selective CI reagents.

Some of the most commonly used CI reagent ions react by proton transfer,



or by charge exchange,



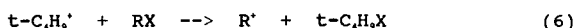
The heat of reaction for reaction (3) is

$$\Delta H = PA(X) - PA(SH) \quad (5)$$

that is, the proton affinity (or basicity) of the sample minus the proton affinity (or basicity) of the reagent (which may not always be the reagent gas itself). If ΔH is positive, the proton transfer reaction from the reagent ion, XH^+ , to the sample, SH , will not occur and no sample ions will be detected. Reagent ions, XH^+ , which are formed from strongly basic compounds (high proton affinities), X , will not produce sample ions from compounds, SH , that are less basic than X . If the sample, SH , is a stronger base than X , then the proton transfer reaction is exothermic and it is likely that the proton transfer reaction from XH^+ to SH will be fast and abundant sample ions, SH_2^+ or fragment ions, will be produced.

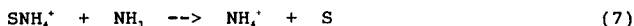
One of the first selective CI reagent gases was isobutane, because the dominant CI reagent ion, $t\text{-C}_4\text{H}_9^+$, is a weaker acid than the CH_4^+ reagent ion in methane; that is, isobutene is a stronger base than methane. The methane CI mass spectra of alcohols contain virtually no ROH^+ ions, and it was expected that the isobutane CI spectra of aliphatic alcohols would give more abundant ROH^+ ions than the methane CI spectra.⁹ Although the extent of fragmentation in the isobutane CI mass spectra of aliphatic alcohols is less than in the methane CI mass spectra, virtually no protonated molecules were observed for the higher alcohols. Although moderately abundant $(\text{M} - \text{H})^+$ ions are observed (which are useful for molecular weight determination), the dominant ions are the alkyl ions, R^+ . The sensitivities appear to be somewhat low for the alkanols in general and for the 1-alkanols in particular. It was similarly noted that the isobutane CI spectra of DDT and related compounds did not contain abundant protonated molecules, MH^+ .⁹

The heats of formation of many of the basic ionic structures needed to determine (or estimate) the heats of reaction with many reasonably complex molecules are not well known. Dissociative proton transfer from the t -butyl ions to the alcohols to produce alkyl ions, isobutene, and water is clearly endothermic. It is likely that proton transfer from the t -butyl ion to these types of compounds is endothermic and that the sample ionization is formed by X^+ abstraction,



One can show, however, a selectivity of the t -butyl ion for polar compounds over hydrocarbons, when compared with the isomeric s -butyl ion (formed from n -butane as a reagent gas), or the propyl ions (from propane as a CI reagent gas). The rate constants for reaction of propyl and butyl ions with hydrocarbons and with simple polar compounds are approximately the same (within a factor of approximately two); however, the rate constants for the reaction of the t -butyl ion with simple polar compounds are some twenty or so times larger than the rate constants for reactions with hydrocarbons containing tertiary hydrogens, and perhaps one thousand times as large as the rate constants for reactions of t -butyl ions with n -alkanes.¹⁰

Another widely used and highly selective reagent gas is ammonia. Early work indicated that strongly basic (nitrogen-containing) compounds and not non-polar hydrocarbons would be selectively ionized in ammonia CIMS.¹¹ The reactions are not exclusively proton transfer, since adduct ions, $(\text{M} + \text{NH}_3)^+$, are formed with some polar compounds. The ratio of intensities for the two major species, $(\text{M} + \text{NH}_3)^+/\text{MH}^+$, depend strongly on the experimental conditions, as well as the proton affinities of the samples being ionized.¹² The low sensitivity as adduct ions for simple polar molecules appears to result from a displacement reaction,



Another complicating factor in ammonia CI mass spectra is the variable abundance of substitution ions, $(\text{M} + \text{NH}_3 - \text{H}_2\text{O})^+$. Di-isopropyl ether (DIPE) has a proton affinity very close to that of ammonia, but DIPE should be less reactive and less strongly solvating than ammonia. A few experiments have been done using DIPE as a reagent gas which give CI spectra which contain predominantly $(\text{M} + \text{H})^+$ ions and virtually no adduct ions.¹³

Negative ions have been observed in mass spectrometers under conventional electron ionization conditions for many years, but the low sensitivities and frequently uninformative spectra for small molecules discouraged most applications.¹⁴

One of the problems with negative ion mass spectrometry under the low pressure conditions of conventional EI mass spectrometry is the low electron capture cross section for the high energy electrons. The electron energies can be reduced by collisions with molecules under CI conditions and early experiments showed significant abundances of negative ions under high pressure conditions. The mechanisms for the formation of the negative ions are not always clearly defined and frequently involve both electron capture and ion/molecule reactions. The "methane-enhanced" negative ion mass spectra of chlorinated pesticides showed multiple modes of negative ion production and the sensitivity of these negative ion spectra to oxygen as an impurity in methane (analogous to the strong sensitivity to water as an impurity in methane positive ion CI spectra).¹⁸

A very high sensitivity has been demonstrated for negative ion mass spectrometry at high pressures for compounds which have high electron capture cross sections for low energy electrons (highly electronegative compounds containing multiple functional groups with high electronegativities). These spectra are very simple, containing predominantly only the negative molecule ions, M^- . However, the relative abundances of these M^- ions are strongly temperature dependent and the experimental conditions must be carefully controlled to maintain the selectivity, sensitivity, and quantitative reproducibility.¹⁹

The selectivity of negative ion molecule reactions in CIMS was demonstrated for the analysis of TCDD (tetrachlorodibenzodioxin). Ions from the polychlorobiphenyls frequently overlap the M^- region of the dioxins; however, the negative ion spectrum of TCDD (O_2 as the reagent gas) contains characteristic fragment ions which are in an interference free region of the mass range.²⁰ These fragment ions were postulated to involve a reaction of a sample ion with the reagent gas (oxygen, in this case). Other examples have been shown for the reactions of sample negative ions with the reagent gas in the case of $(M-H)^-$ ions of alkyl aromatic molecules with nitrous oxide. However, these reactions were not sufficient to prevent the selective analysis of alkyl aromatic hydrocarbons in complex mixtures using OH^- as the negative CI reagent ion, with a relatively low pressure of a nitrous oxide/hexane mixture as the reagent gas.²¹

There are several other types of selective ionization, such as Field Ionization, Field Desorption, Photoionization, and Penning Ionization which will not be discussed at this time.

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Historical Developments in Quadrupole Mass Spectrometry

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Introduction

The three members of the quadrupole family of instruments, that is, the mass filter, the ion trap and the monopole have very different histories. The first two ideas originated in 1953 but the monopole was not described until 1963 and had a short life as a widely used instrument. In this brief history, the flow of ideas and concepts will be described with less attention to exact dates and to acknowledging all the myriad of contributions to the development of quadrupoles as the dominant class of mass spectrometers. Inevitably some individuals' contributions may not be adequately recognised in such an approach and I offer a blanket apology to anyone thus neglected. However, in classical history, I find the history of ideas more interesting than the recitation of the lists of kings and queens and the dates of their reigns!

Another difficult factor is that I have been myself a participant in the field for about 24 years and so this history naturally reveals all my personal biases and particular interests. I will therefore concentrate on instrument performance and design rather than application and I will permit myself the luxury of some reminiscence about my own early involvement. My involvement in the three types of instrument was in opposite order to their sequence of development, beginning with the monopole in 1965, the ion trap in 1966 and the mass filter not until 1971. I will describe the development in this sequence but first I will begin with a description of how the foundations for the whole field were laid by the group of workers at the University of Bonn. Rarely has a new field been so dominated by the initial concepts developed by one group. In fact, looking back with today's knowledge, the experimental achievements in the initial years were really quite limited — but almost every concept exploited later was explored conceptually.

Table I simply lists the early published works leading to the proposal of the instruments. The principles of the use of radiofrequency quadrupole electric field for separation according to M/Q ratio were recognised at about the same time by R.F. Post at the University of California and by W. Paul and H. Steinwedel at the University of Bonn. However, the first years of exploitation and the eventual successful development can clearly be attributed to the latter group. Published results were few in the early years and the Bonn group had the luxury of five years of study before publication of the now classic paper of Paul, Reinhard and von Zahn on the mass filter (see Tables II and III). In the meantime, the quadrupole ion trap was the subject of at least two theses before the publication of Fischer's work in 1959. Significant North American effort on quadrupoles only began about that time but grew to a high level in the mid-1960's driven and funded in large part by the requirements of the space program. With the invention of the monopole in 1963 and its rapid commercialisation, the mass filter and the monopole became great rivals in the residual gas analyser market. A 1971 review, could find little difference in commercial specifications although there were some indications that in practicality and ease of use, the mass filter was beginning to win out. In fact, after that time, the monopole rapidly waned in popularity and has now almost disappeared.

My own involvement began with the monopole while I was working at the General Electric R&D center in Schenectady. The company's Vacuum Products Groups had rapidly introduced a commercial instrument based on a design reported by J. Hudson¹⁴ of RPI in 1964. Von Zahn¹³ had first described the monopole only in 1963 and it appeared to have some length advantages over the quadrupole. The G.E. product was initially successful and gained customer acceptance. Then disaster struck. In a sequence since well known to all

TABLE I: IN THE BEGINNING

- Static magnetic multipole field to focus neutral atoms (Friedburg and Paul 1951)¹
- Strong-focussing alternating gradient principle of accelerator design - a quadrupole magnetic field with spatial periodicity (Courant, Livingston & Synder 1952)²
- Use of alternating electric fields for focussing (Blewitt 1952)³
 - Mass selection 1953
- R.F. Post, University of California Laboratory Report⁴
W. Paul and H. Steinwedel, Z. Naturforsch.⁵

TABLE II: CONTRIBUTIONS FROM THE BONN GROUP

- General principle - Paul & Steinwedel 1953⁶
(Mass filter first & second regions)
- U.S. Patent Application 1954⁷ (Mass filter & Ion trap)
- Experimental results on the mass filter (Paul & Raether 1955)⁸
 - Frequency scanning, Measurement of efficiency
 - Effect of field errors on peak shape
- Detailed characterisation of the mass filter plus an isotope separator (Paul Reinhard & von Zahn 1958)⁹

- Detailed report on the ion trap¹⁰ (Fischer 1959)
- Field fault analysis¹¹ (von Busch & Paul 1961)
- Large scale isotope separator¹² (von Zahn et al 1960)
- The monopole¹³ (von Zahn 1963)

TABLE III: THE MASS FILTER

- The "Bible"; Paul, Reinhard & von Zahn⁹ Z. Phys. 152, 143 (1958)
- Stability diagram with iso-beta lines using analytical calculations
 - "Acceptance ellipses" (Fischer)¹⁰ & amplitude calculations
- *Two regions of operation using parallel ion entry (a) flat-topped-relative abundance depends on source & detector not the analyser (b) triangular peaks-transmission restricted by the analyser
 - Resolution = $n^2/12.25$

- Discussed demands on accuracy & uniformity
- $r_{rod}/r_{field} = 1.16$ (from Dayton, Shoemaker & Mosley¹⁵: should have been 1.148)
- Problems of the "transition field" discussed
 - Effect of high pressures discussed
 - Source position was transversely adjustable
- Experimental result - resolution 1500 at mass 16 using 1m. quadrupole, 4MHz frequency and 75eV ions.

TABLE IV: THE "AGES" OF ION TRAPPING

- (taken from J.F.J. Todd's introduction to "Quadrupole Storage Mass Spectrometry" by R.E. March and R.J. Hughes)
- Mass selective ion detection e.g. Fischer 1959¹⁰; Rettinghaus 1967²¹
- Mass selective ion storage e.g. Dawson & Whetten 1968¹⁸; Dawson, Whetten & Hedman 1969²²
- Mass selective ion ejection e.g. *Stafford, Kelley, Reynolds & Syka 1983²³

quadrupole manufacturers, specifications could suddenly no longer be met. Deliveries were delayed. The Vacuum Products Group appealed to the R&D center mass spectrometrists for help. As an added inducement, they offered free one monopole. I accepted this gift horse (definitely the worst spectrometer I've seen before or since). In the meantime, R.P. Lever¹⁶, a customer at IBM awaiting a monopole delivery began some numerical calculations of ion trajectories in a quadrupole field. The results, published in 1966, did not explain the performance of the conventional monopole as then used but referred to a proposed high resolution monopole using simultaneous focussing in the x and y directions. Lever's calculations remain beautiful examples of trajectory simulations and set the stage for all the future computation. With the interest and encouragement of my colleague Rey Whetten, I began the then tedious and expensive process of simulating trajectories in the monopole operated conventionally. After a great deal of effort, and to the justifiable chagrin of our colleagues in the Vacuum Products Group we demonstrated that the monopole was not able to give good performance and was bound to produce poor peak shapes. The answer to this conundrum only came when the effects of fringing fields¹⁷ were taken into account in 1971. The importance of the fringing fields was also confirmed by indirect experimental evidence. However, by now, the monopole was already in decline even though the manufacturing group had long since solved their production problems.

Sensitized by our monopole experience, Rey Whetten and I stumbled across the ion trap principle in J.B. Hasted's book on the "Physics of Atomic Collisions". Intrigued by this, we examined Fischer's paper but decided that it might be superior to store the ions mass selectively and eject them into an electron multiplier to be detected as a pulse. We coupled a study by ion trajectory simulation with an experimental program.^{18,19}

In J.F.J. Todd's introduction to the recent book²⁰ "Quadrupole Storage Mass Spectrometry" by R.E. March and R.J. Hughes ion trapping has been characterised in terms of 3 "ages" as shown in Table IV. The initial "age" was one of storing a range of different ions and selectively detecting them by some electronic property related to their frequency of oscillation. The main problems were due to interference effects between species and to ion molecule reactions if storage times were too long. Mass selective ion storage was much more straightforward and the ion trap almost reached commercialisation as a residual gas analyser in a very simple form.²² However, the real breakthrough did not come until the use of nonselective storage but mass selective ion ejection by Stafford et al²³ coupled with stabilisation of ion trajectories by collision with helium. This latter factor meant an ideal application to GC/MS. The rest of the exciting story is very recent history and does not need to be repeated here.

The key point of collisional stabilisation had been known for some time (see Table V) but not exploited. Particularly intriguing is the work on producing "frozen" arrays of particles — originally macroscopic charges dust particles as early as 1957²⁴ and very recently of atomic ions.^{31,32}

The experience with the ion trap led Rey Whetten and I to study the effect of field faults in quadrupole fields. This activity has continued to the present day with increasing levels of sophistication (Table VI) with the main applications to the mass filter.

For the mass filter, the contributions of the classic paper by Paul, Reinhard and von Zahn⁹ have been listed in Table III. The theoretical work is all the more impressive when one remembers that it was all carried out by calculations using the analytic theory — numerical simulation by computer was not yet feasible. One interesting feature of the paper was the description of acceptance ellipses (based on Fischer's work). Many years later, these became the basis for the modern ion optical theory of quadrupoles (Table VII). However, the full significance does not seem to have been realised in the original work.

TABLE V: COLLISIONAL STABILISATION

- *Wuerker, Shelton & Langmuir 1959²⁴ Trapping of macroscopic charged particles. (work first reported in 1957)
 - Effect of elastic collisions²⁵ (Major & Dehmelt 1968)
- *Dawson & Whetten (1969)²⁶ Heavy ion stabilisation by a light gas
 - Collisional focussing of ions (Bonner, March & Durup 1976)²⁷
 - Phase-space Theory for Dawson (1977)²⁸

- Confirmation of (Neuhauser, Hohenstatt, Toschek & Dehmelt 1978)²⁹
 - Application to Mass Spectrometry (Stafford, Kelley, Reynolds & Syka 1983)³⁰
- *Ionic arrays and phase transitions (Diedrich, Peik, Chen³¹, Quint & Walther 1988)³²

TABLE VI: FIELD FAULTS

- von Busch & Paul 1961 General analytic theory Errors in field geometry & harmonics in the rf¹¹
 - Dawson & Whetten 1969³³
- Experimental & computer simulation for the ion trap
- Full analytical examination - Halbach (1969) for accelerators³⁴
 - Applied to quadrupoles by Pocek 1978³⁵
- Limits to performance of the mass filter, Dawson & Meunier 1979³⁶
 - Diverging, converging, bent & bowed rods, Dawson 1988³⁷
 - Electrical drive errors much less studied

TABLE VII: APPLICATION OF PHASE SPACE METHODS

- Fischer (1959) calculated ellipses for the ion trap
- Matrix methods - Richards et al (1973) for square-wave³⁸
- Phase space concept for the ion trap - Baril & Septier (1974)³⁹
 - Similar ideas - Sheretov & Kolotilin 1975⁴⁰
- Detailed application to the mass filter including fringing fields Dawson 1975⁴¹
- Direct experimental measurements, Henniquin & Inglebert 1978⁴²

TABLE VIII: THE SPREAD OF APPLICATIONS

- 1960s - The mass filter & monopole as RGAs (Isotope separation)
 - Application to upper atmosphere & space
- 1970s - The mass filter in GC/MS SIMS systems
 - 1978 The tandem quadrupole for MS/MS
 - 1983 The ion trap detector for GC/MS
 - 1983 ICP/MS & Glow-Discharge MS
 - 1986 LC/MS/MS
 - 1987 Ion Spray/Thermospray LC/MS

TABLE IX: RF-ONLY QUADRUPOLES

- 1959 The ion trap
- 1972 Weaver et al; Differentiation of Mass Filter signal⁴³
 - 1972 Brinkmann; Retarding geometry at the collector⁴⁴
 - 1978 Holme, Sayyid & Leck Improved collector⁴⁵
- 1980 Dawson, Meunier & Tam; Annular collection system⁴⁶
 - Plus various refinements since

The classic paper also described an isotope separator — a mass filter on a larger scale operated with an auxiliary rf field to remove ions at resonance. The ultimate separator was an 8.2 m long instrument (in the stairwell at the University of Bonn in a vertical position).³⁸ The instrument was vertical and the electrodes formed by a large number of weighted piano wires. A resolution of 10,000 was reported.

The performance of the mass filter gradually improved over the years. The perfection of the fields was a significant factor. Ion source design became more appropriate, allowing the use of lower energy ions. Mass range was extended and new applications opened up (Table VIII). However, there has been no significant departure in basic design — just the steady technological improvement. After the contributions of the Bonn group, the body of work of greatest significance was that of Wilson Brubaker and various colleagues. He began by exploring the application of the mass filter to direct ion sampling in the upper atmosphere and studied in detail both experimentally and theoretically³⁹ transmission of ions as a function of transverse position, transverse angle and rf frequency. This led to the first detailed understanding of the importance of fringing fields at the entrance. Eventually, they proposed the concept of the "delayed d.c. ramp" with an rf only section to mitigate fringing field effects. Another classic study was the only detailed attempt to directly compare round and hyperbolic cross-section rods in the same system.⁴⁰

Some novel developments of the mass filter still unexploited in a practical way are the use of higher stability regions (originally recognised by both Post and Paul and Steinwedel) and of the rf only concept (Table IX). Other ideas remaining unexploited are the use of exact focussing, the quadrupole time-of-flight, resonant detection, the use of gas collisions in the mass filter and ion bunching at the mass filter entrance. One would expect therefore that history is not yet concluded and the future could continue to be exciting.

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A HISTORY OF GC/MS INTERFACES

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During the initial development period of combined gas chromatography and mass spectrometry, a great deal of effort went into the design of interfaces to accommodate the drastically different pneumatics of these analytical instruments. Subsequent improvements in the technologies of each instrument removed the need for interfaces to combine them. This history covers work on molecular interfaces for the combination of gas chromatography with mass spectrometry during the period between the late 1950s and the early 1970s.

The complementary analytical capabilities of gas chromatography and mass spectrometry were noted by early users of the two instruments. For samples of identical size, mass spectrometry could provide accurate identification of compounds whereas gas chromatography could only provide approximate identification based on retention indices. On the other hand gas chromatography was clearly superior to mass spectrometry in quantitatively analyzing samples. Both techniques required the sample be in the gas phase for analysis. Combining the two was an intriguing prospect; however, the combination was thwarted by the incompatibility of the large mass-flow rate of carrier gas required by the gas chromatograph and the static vacuum systems of the mass spectrometer. Nevertheless, because the potential power of a combined instrument was viewed as limitless, a number of workers focused their attention on solving the problem of the incompatible mass-flow rate requirements of the two instruments by means of some kind of interface.

Most researchers cite the work of Gohlke (Ref. 1) as the beginning of the age of combined GC/MS, even though Holmes and Morrell (Ref. 2) reported a combined instrument using a magnetic sector machine several years earlier. The work of Holmes and Morrell was done on a Consolidated Engineering Corporation Model 21-103B magnetic sector instrument which required nearly a minute to scan the mass range from 12 to 100 daltons. Holmes and Morrell devoted the bulk of their paper to the description of an oscilloscopic display system which permitted the operator to continuously scan a 16-mass-unit-wide portion of the spectrum to ascertain that a compound had entered the ion source, thus allowing the spectrum to be recorded with an oscillograph. However, no information was given about the manner of connecting the chromatograph to the mass spectrometer! This oversight is particularly disconcerting today since the 103B instrument had a vacuum system capable of a miniscule gas load.

Gohlke devoted more attention to a description of chromatographic instrumentation than to interfacing the instruments. Towards the end of his paper, he describes how a portion of the column effluent was split to the mass spectrometer by means of a fine needle valve. This work was done with a time-of-flight mass spectrometer which had the advantage that oscilloscopic monitoring of the mass spectrum was an intrinsic feature of the instrument. It is also noteworthy that the time-of-flight had an open ion-source and that the conductance between the ion-source housing and the vacuum system was high. Thus, in 1959, Gohlke's time-of-flight mass spectrometer had one of the most dynamic vacuum systems of any such analytical instrument. Even so, Gohlke reported that "... the mass spectrometer is consuming the effluent vapor from the chromatographic column at a rate of about 0.003 cc. [-atm] per second ... " (Ref. 1).

Quite obviously, split interfaces meant diverting a large fraction of sample molecules away from the mass spectrometer. Although Gohlke does not state the carrier gas mass flow rate, conditions of pressure (10 psig) and column

*This work was conducted under the McDonnell Douglas Independent Research and Development program.

size (10 foot x 1/4 inch) suggest that it was in the tens of mL-atm/min. Thus, probably less than 1% of the analyte reached the mass spectrometer.

In the ensuing years, attempts to enrich the analyte in the carrier gas entering the mass spectrometer proceeded along two different lines: sample-trapping interfaces, and enrichment interfaces. Typical of the sample-trapping approach is the work of Ebert in 1961 (Ref. 3), and Miller in 1963 (Ref. 4). These workers attacked the problem by modifying the static inlet system of the mass spectrometer. They were quite successful in enriching the chromatographic effluent, but for only a few components in an analysis. The alternative approach, enrichment interfaces, was also being explored by other workers and this latter technique soon dominated.

A variety of techniques for enriching the chromatographic effluent in a dynamic fashion were reported in the mid-1960s. These enrichment interface techniques can be divided into three fundamental categories:

- 1) Molecular Effusion Interfaces
- 2) Molecular Jet Interfaces
- 3) Permselective Barrier Interfaces

MOLECULAR EFFUSION ENRICHMENT INTERFACES

In the molecular effusion interface (Fig. 1), the effluent from the chromatograph enters an effusion chamber having one or more porous walls with a well-defined pore size. Flow restrictions between the chromatograph and the effusion chamber, between the effusion chamber and the mass spectrometer, and a pump evacuating the envelope surrounding the chamber allow control of the pressure to create conditions for molecular effusion. Specifically, the mean free-path of the carrier-gas molecules in the effusion chamber should be ten times greater than the pore size of the porous walls (Ref. 5). Under these conditions, the lighter molecules of carrier gas, usually helium, preferentially diffuse through the porous walls thus enriching the concentration of sample gas entering the mass spectrometer.

Watson and Biemann (Ref. 6) were the first to report a molecular effusion device for interfacing the two instruments. The problem of obtaining a mass spectrum during the relatively short time the chromatographic peak is eluted was solved by using an integrating detector, the photoplate, in a high-resolution instrument of Mattauch-Herzog geometry. This elegant solution had the advantage that the detection limit for low-concentration chromatographic peaks was improved while at the same time high-resolution mass spectra could be obtained.

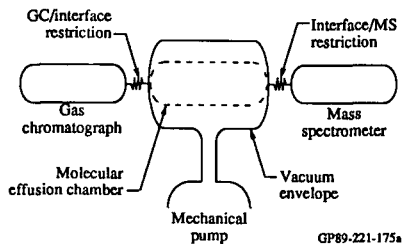


Figure 1. Molecular effusion GC/MS interface.

Immediately following this work, a number of molecular effusion devices in which different materials were used for the controlled-pore-size walls of the molecular effusion chamber were reported in the literature. Cree (Ref. 7) used porous silver membranes for the walls of the effusion chamber. His idea was followed by Blumer (Ref. 8), Grayson and Levy (Ref. 9), and a commercial company (Ref. 10). Krueger and McCloskey described an interface using a porous stainless steel tube (Ref. 11). Lipsky and co-workers (Ref. 12) reported the use of a thin-wall Teflon tube as a molecular effusion barrier. Brunée et al. (Ref. 13, 14) described a "variable slit" interface which operated on the principle of molecular effusion. Markey (Ref. 15) reported an improved glass-frit device suitable for use in biomedical applications. The performance of single- and double-stage molecular effusion interfaces was compared by ten Noever de Brauw and Brunée (Ref. 16).

Thus, in the five or six years following the introduction of the molecular effusion enrichment interface, a large number of variants on this basic concept were developed and placed at the disposal of the venturesome mass spectroscopist.

MOLECULAR JET ENRICHMENT INTERFACES

In the molecular jet interface (Fig. 2), the effluent from the chromatograph flows through a constricted orifice, thus forming an expansion plume in the evacuated envelope (Ref. 17). The core of the plume is rich in analyte; thus, a skimming orifice placed opposite the expansion orifice will extract the enriched core for sampling by the mass spectrometer. An advantage of this approach is that the analyte molecules undergo minimal contact with the surfaces of the interface and thus labile molecules have a greater chance of surviving the enrichment process without degradation--an important consideration in biological applications.

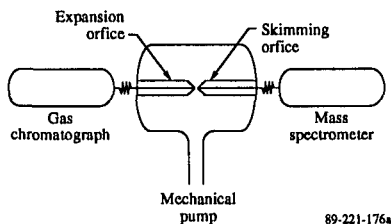


Figure 2. Molecular jet GC/MS interface.

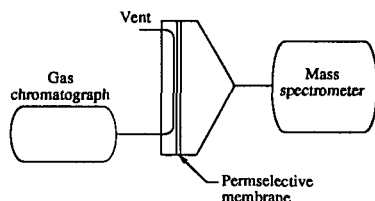
Ryhage (Ref. 18) describes the application of the molecular jet technique in a two-stage interface which was the culmination of four years of work on the enrichment problem. In this work, the scan speed of the single-stage mass spectrometer was reduced from tens of seconds to several seconds over the mass range from 12 to 500 daltons. Ryhage (Ref. 19) later described details of the optimization of the molecular jet as an enrichment device for combined GC/MS, and the comparative performance of single- and dual-stage separators.

Devices based on the molecular jet principle also flourished after Ryhage's initial work. Wikstrom and Waller collaborated with Ryhage (Ref. 20) in extending the interface to use with capillary columns. Novotny (Ref. 21) later described the use of this interface with support-coated-open-tubular columns. In 1966, a report (Ref. 22) described a commercial instrument employing the two-stage molecular jet separator. This latter instrument dominated the commercial market for combined GC/MS instruments for several years.

PERMSELECTIVE MEMBRANE ENRICHMENT INTERFACES

In this type of GC/MS interface, a barrier with widely differing permeabilities to the analyte and carrier gas is used to enrich that portion of the effluent entering the mass spectrometer. Not surprisingly, GC/MS interfaces have been developed for barriers which selectively pass analyte molecules and barriers which selectively pass carrier-gas molecules.

Analyte-permeable barriers are based on organosilicone membranes (Fig. 3) which Llewellyn and Littlejohn (Ref. 23) were the first to exploit for their permselective properties (Ref. 24) as an enrichment device. Unlike other interface technologies, which initially were developed in the academic sphere, analyte-permeable barriers were developed by an instrument manufacturer; thus most early information is found in the commercial and patent literature (Ref. 25). Commercial interfaces were originally two-stage devices because of the vacuum technology used in the mass spectrometer. However, since the membranes were readily available from other commercial sources, single-stage variations soon appeared in the literature (Refs. 26, 27, 28).



89-221-177a

Figure 3. Permselective membrane interface.
(Barrier selectively permeable to organic Cpd's)

Lucero and Haley (Ref. 29) reviewed the theory of permselective membrane devices in general and then applied it to a device based on a palladium/silver alloy as a barrier. In this device, hydrogen is used as the carrier gas. Depending upon the temperature of a palladium/silver alloy tube through which the GC effluent passes, hydrogen will preferentially diffuse out of the tube, leaving the enriched sample stream to enter the mass spectrometer. Several years later, Simmonds et al. (Ref. 30) described such a device and its operating characteristics. Catalysis of the analyte was a concern with this interface.

NON-ENRICHMENT INTERFACES

Despite developments in enrichment devices, work continued on refining and improving the utility of split-connections between the chromatograph and mass spectrometer. Lindeman and Annis (Ref. 31) extended Gohlke's work to a cycloidal mass spectrometer and reported continuous monitoring of selected ions characteristic of specific compounds in the analyte. Henneberg and co-workers (Refs. 32, 33, 34), Dorsey et al. (Ref. 35), and Brunée et al. (Ref. 36) all took advantage of chromatographic columns which used lower mass-flow rates, thus increasing the sample mass delivered to the mass spectrometer with the split-connection.

As early as 1962, other workers, notably McFadden and Teranishi (Ref. 37, 38), took advantage of the efficient pumping system of the time-of-flight mass spectrometer to directly connect the column outlet to the mass spectrometer without benefit of split or pressure reduction. Merritt and coworkers (Ref. 39) demonstrated the utility of direct connections for a variety of mass spectrometers with support-coated-open-tubular columns. Improvements in vacuum-system design (Ref. 40) made use of a direct connection practical for columns with flow rates in excess of 5 mL-atm/min.

SUMMARY

By the early 1970s, improvements in both column technology and vacuum systems for mass spectrometers set the stage for direct connection of the two instruments to dominate combined GC/MS. A polemic in *Nuclide Spectra* (Ref. 41) succinctly summarizes the arguments for direct connection of the two instruments. Today, almost all commercial instruments have direct connections for the GC/MS interface. Where higher column flow rates are required, the molecular jet is the most widely used enrichment device. Thus, a field of intense interest with many exciting developments over a short period of time was deserted when improvements in the fundamental technologies of chromatography and mass spectrometry were made. In a sense, the analytical power of combined gas chromatography/mass spectrometry was demonstrated with enrichment interfaces; these interfaces were then rendered unnecessary with the development of low mass-flow-rate chromatographic columns and dynamic mass-spectrometer vacuum systems.

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THE DEVELOPMENT OF METHANE CHEMICAL IONIZATION MASS SPECTROMETRY

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Chemical ionization mass spectrometry was an outgrowth of fundamental studies of ion/molecule reactions at increasingly higher pressures.

The first mass spectrometers which were specifically designed for high pressure operation, in the range of 0.1 torr, were reported around 1960. One of these instruments was the the Humble chemical physics mass spectrometer, designed by Frank Field to study ion/molecule reactions. The first publication from work on this instrument showed ionic products as high as C, from ion/molecule reactions in ethylene at 0.2 torr.¹ Another high pressure mass spectrometer had been developed a little earlier and had been used for the study of collisionally induced decompositions.²

Relatively minor modifications were made to the Humble (then Esso, and eventually Rockefeller) mass spectrometer in 1965 to allow operation at pressures as high as 1 - 2 torr. These modifications were the relatively minor ones of decreasing the sizes of the electron entrance and ion exit slits of the source. We re-discovered that the distributions of ions in methane at these pressures were very sensitive to very low levels of impurities and painstaking efforts were needed to remove traces of water from the reagent gas, the inlet system, the transfer lines, and the mass spectrometer. The very low levels of water as an impurity could easily be detected as ions at m/z 19. We never seriously considered CH₅⁺ as an ionic species, but it was very frustrating to observe large and variable amounts of m/z 19 in research grade methane in what was presumed to be a very clean vacuum system. It was eventually established that CH₅⁺ and C₂H₅⁺ did not react with methane to any significant extent under these experimental conditions.³

A series of experiments were then done with mixtures of methane and small amounts of various simple molecules to understand the then completely unknown ion/molecule chemistry of CH₅⁺, and to a lesser extent the ionic chemistry of C₂H₅⁺. These ions react with almost, but not quite everything, and the dominant reactions were dissociative and non-dissociative proton and hydride transfer. CH₅⁺ could be considered as a strong Bronsted acid, and C₂H₅⁺ could be considered as a Bronsted or a Lewis acid.⁴ Reactions of the methane ions, CH₅⁺ and C₂H₅⁺, with two isomeric hexanes showed ionic product distributions which were independent of pressure, similar enough that one could identify both compounds as hexanes, and sufficiently different that one could differentiate between the structures of the two isomers. The ionic distributions for mixtures of methane with small amounts of other isomeric hydrocarbons showed similar features: sufficient (M - H)⁺ ions to obtain the molecular weights of the compounds and sufficiently different distributions of fragment ions that the compounds could be distinguished. At that time, it occurred to us (to Frank Field, first, I believe) that we had a potentially useful analytical procedure.

A patent application was filed in 1965, which was disallowed because of a prior patent of Fite, et al (#3,254,209, May, 1966). There is no new thing under the sun. However, the lawyers persevered and a revised patent application was submitted in 1968 which was granted in 1971 -- my only patent and one of Frank Field's three.

The first non-company presentation on chemical ionization (not to be confused with chemi-ionization, which refers to the reactions of excited state ions with neutral molecules to form an ion and an electron) mass spectrometry (CIMS -- the initials look better in caps) was given at the Eastern Analytical Conference in New York, in November, 1965.

The first paper on CIMS was published in 1966. The review of the first version of the paper was not favorable, to say the least.

"This is a very carelessly written paper and a serious waste of a reviewer's time. ... I cannot argue with the author's [sic, there were two authors] conjectures regarding the origin of the spectra. However, despite occasional references to heats of formation and exothermic reactions, conjecture is all that it is. When some observations (e. g. p. 31 [in the original draft]) are "explained by the use of impure materials [sic, closing " and , were omitted] I think one should give serious consideration to the value of the work and the usefulness of the publication."

I thought that the originally submitted version was not that bad; however, I may be prejudiced. We persevered and the first paper was accepted, without an excessive delay.'

In the early work, comparisons were made between the methane CI mass spectra and EI mass spectra, which showed generally abundant ($M \pm H$)⁺ ions as well as structurally useful fragment ions, whose formation could be relatively easily explained by proton or hydride transfer reactions followed by simple cleavages or rearrangement decompositions from the excited ($M \pm H$)⁺ ions. Comparisons were generally made for relatively simple compounds for which the EI spectra contained little or no M^{+} ions.'

Two things greatly aided the general acceptance of methane CIMS as a routine analytical tool around 1970. One was the development by Marvin Vestal at Scientific Research Instruments of a high pressure source which could be fitted to existing mass spectrometers. The other was a series of papers by Fales and others which showed that methane CI was useful for "real" molecules of interest to organic and biomedical mass spectrometrists.' Since the 70's CIMS with methane and other reagent gases has been routinely used for mass spectrometric quantitation and characterization.

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CHEMICAL IONIZATION WITH TETRAMETHYLSILANE, PAST AND PRESENT

or

A HISTORY OF GAS PHASE TRIMETHYLSILATION

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Chemical ionization mass spectrometry (CIMS) has become a routine analytical technique which gives information complementary to that obtained by electron ionization (EI). A major advantage of CIMS is that a lower quantity of energy is imparted to the sample ions compared with EI. Because of this lower energy, CIMS frequently yields molecular weight information for compounds which give only fragment ions in EIMS. Also, an additional degree of selectivity can be added to an analysis by the use of a reagent gas which will selectively ionize only chosen classes of compounds.

In CIMS, the amount of fragmentation is dependent on the heat of the reaction, which can be altered by changing the reagent ion. Methane, the traditional and most widely used reagent gas, gives only fragment ions for several classes of compounds.¹ Because the reagent ion from iso-butane, $t\text{-C}_4\text{H}_9^+$, is a weaker acid than the reagent ions found in methane, CH_5^+ and C_2H_5^+ , this reagent increases the amount of $(\text{M}+\text{H})^+$ or $(\text{M}-\text{H})^+$ and causes a decreased extent of fragmentation when compared to methane.² Ammonia is even more basic than CH_4 , C_2H_4 , or $i\text{-C}_4\text{H}_8$; consequently, proton transfer from the ammonium ion is less energetic than proton transfer from either CH_5^+ , C_2H_5^+ , or $t\text{-C}_4\text{H}_9^+$.^{3,4} Resulting in primarily $(\text{M}+\text{H})^+$ ions being observed with very little, if any, fragmentation when NH_3 is used as a reagent gas. A second ion which is formed in NH_3 CI is the ammonium adduct ion, $(\text{M}+\text{NH}_4)^+$, which can cause problems in the molecular weight assignment of the sample. Ammonia has a very low sensitivity for compounds which are less basic than itself.^{5,6}

The lack of a reagent gas having a high sensitivity for a wide range of compounds while imparting a minimal amount of energy upon reaction has caused a search for an alternative reagent gas. One such reagent which has been used is tetramethylsilane (TMS). The major reactant ion found when TMS is used as a CI gas is the trimethylsilyl ion, $(\text{CH}_3)_3\text{Si}^+$, at $m/z = 73$. In the early work employing TMS as a CI reagent gas, the base peaks for several compounds with a variety of functional groups were the $(\text{M}+73)^+$ adduct ions, which were presumed to be formed by direct addition of the trimethylsilyl ion to the sample.⁷ Additional sample ions have been reported at $(\text{M}+131)^+$ and $(\text{M}+145)^+$, formed by the addition of other reagent ions in the high pressure mass spectrum of TMS to the sample.^{7,8,9} Mixtures of TMS and d_{12} -TMS were used with desorption CI to simplify the recognition of the trimethylsilyl adduct ions with a number of biologically important molecules.¹⁰ The formation of $(\text{M}+73)^+$ adducts has been reported recently for a large number of oxygen-, nitrogen- and sulfur-containing compounds.¹¹

The presence of $(M+H)^+$ ions were also reported in the early work with TMS as a reagent gas for a number of compounds. Because the proton affinity of 1,2-dimethylsilaethylene (the conjugate base of the trimethylsilyl ion) is much higher than the proton affinities of these compounds, proton transfer from the trimethylsilyl ion is endothermic and therefore should be very slow.¹² It has been suggested that these $(M+H)^+$ ions were formed by proton transfer from protonated trimethylsilanol, $(CH_3)_3SiOH_2^+$ at $m/z = 91$, present in the reagent spectrum from the addition of the trimethylsilyl ion to trace amounts of water, an impurity in the system.¹³

The trimethylsilyl ion acts as a Lewis acid, rapidly forming adduct ions with compounds containing non-bonding electron pairs.⁷ The gas phase Lewis basicities of ketones, alcohols, aldehydes, and esters have been studied by obtaining their relative trimethylsilyl ion affinities in an ICR spectrometer.^{14,15,16} These values were found by the bracketing technique: the presence or absence of the trimethylsilyl cation transfer reaction is observed between base pairs.

The study of trimethylsilyl ion affinities suggests the use of ternary mixtures (methane, TMS, and much smaller amounts of a Lewis base) as a CI reagent system.¹⁷ With these systems, the major reactant ion is the adduct of the Lewis base, this ion will transfer the trimethylsilyl ion to samples which are stronger Lewis bases. Because adduct ions formed by displacement reactions are expected to have lower energies than the adduct ions formed by direct addition, less decomposition of the adduct ion should be seen when ternary mixtures are used. Along with increasing the gentleness of the trimethylsilyl ion transfer, the selectivity was enhanced because only a stronger base will displace the one present in the original mixture. These mixtures can consequently be used to ionize selectively compounds based on their trimethylsilyl ion affinity.

Several kinetic studies of the gas phase reactions between the trimethylsilyl ion and oxygenated compounds have been made using an ion cyclotron resonance (ICR) spectrometer.^{18,19,20,21,22} These experiments showed adduct ion formation, $(M + 73)^+$, as well as fragmentation reactions under ICR conditions. The product ions from the reactions of $(CH_3)_3Si^+$ with alcohols under ICR conditions²⁰ were different from the ions reported in the high pressure CI mass spectra¹³. More recently, the reactions of the trimethylsilyl ion with aliphatic alcohols²³ and ethers,²⁴ have been studied under both ICR and CI conditions.

Two very different mechanisms have been found to lead to the formation of the $(M+73)^+$ adduct ions. The first involves the formation of a stable intermediate by dissociative addition, which then reacts with a second sample molecule to form the adduct ion. When this two step process is not possible because of the lack of an exothermic dissociative addition reaction, the adduct ion is formed by direct addition of the trimethylsilyl ion.

Studies of the proton affinities of trimethylsilanol, trimethylsilyl alkyl ethers, and trimethylsilyl alkyl sulfides have recently been performed.²⁵ Possibly eliminating the discrepancy in the literature values for the proton affinity of trimethylsilanol.^{17,26} Also, the value for the heat of formation of the trimethylsilyl ion, which has been disputed recently in the literature,²⁷ can be obtained from the value of the proton affinity for trimethylsilanol. The values for the proton affinity of trimethylsilanol and the trimethylsilyl alkyl ethers are of interest because of their use in determining trends in proton affinities, as well as comparisons between the silicon compounds

and their carbon analogues. Several improvements have been made in the bracketing technique which is used to determine proton affinities. These improvements have led to an increase in the accuracy of the values obtained for the proton affinities and a decrease in the time needed for these measurements. Also, with this technique, the proton affinity is determined for compounds whose neutral species are not present, allowing the determination of the proton affinity for unstable compounds.

Finally, the selectivity of the trimethylsilyl ion has been used to decrease the demands on the chromatographic separation in the analysis of complex mixtures. The selectivity of this reagent allowed the quantitation of oxygenated blending components in gasoline with relatively little sample preparation, and a simple separation.²⁸

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TANDEM MASS SPECTROMETERS FOR FUN, NOT PROFIT: Jean Futrell; Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716.

This paper attempts to review briefly the history of the development of tandem mass spectrometers from their origin as a gleam in the eye of several investigators interested in fundamentals of mass spectrometry to the present day. Emphasis is placed on three instrument configurations developed in the author's laboratories at Wright-Patterson Air Force Base, the University of Utah, and the University of Delaware and shown in Figures 1, 2, and 3. Figure 1 is immediately recognized as the tandem combination of two double-focusing mass spectrometers. Figure 2 is similarly recognizable as a triple quadrupole instrument while figure 3 is a tandem hybrid mass spectrometer. In 1989 most mass spectroscopists would grasp the significance of these instruments as powerful tools for analytical mass spectrometry. The interesting story of the evolution of MS^2 and MS^n for chemical analysis is told separately at this Symposium. This is not the thrust of this paper.

Our story traces another pathway and emphasizes the applications of tandem mass spectrometry to fundaments of ion reaction mechanisms and dynamics. The story is told from the biased perspective of the author with the intent of being historically accurate without attempting to write a comprehensive review. As a minimum it is hoped that the newest members of our Society will be intrigued by the dates associated with these instruments and their direct ancestors, cousins and descendants.

Studies of ion molecule reaction kinetics in the 1950's determined reaction mechanisms, branching ratios and rate coefficients primarily by relating appearance potential curves for reactants and products and on ion source pressure and using simple electrostatics to calculate residence times. It was always obvious that the study of elementary reactions could best be accomplished by selecting a reactant ion and colliding it with the reactant molecule under controlled conditions. Once the instrumentation for carrying out these kinds of measurements became available, it was a frequent source of astonishment to the author that the deductions for complex reaction schemes based on appearance potential measurements so often proved to be correct!

TRANSVERSE GEOMETRY INSTRUMENTS The first example of a technically sophisticated tandem mass spectrometer was the instrument designed by Einar Lindholm and constructed at Stockholm in 1954. This instrument consisted of two 180° mass spectrometers arranged so that the ion beam of the mass selector instrument was shot through a collision cell at right angles to the ion path for the second stage mass spectrometer. The focus of Lindholm's reactions at was charge exchange reactions at relatively high energy (100 eV to kilovolt energies initially, later extended to several eV). In this geometry the charge transfer product is formed at thermal energy and extracted at right angles while the primary ion projectile is shot through the cell and not observed. A relatively elaborate deceleration lens developed by Lindholm and collaborators allowed the observation of some ion molecule reaction products in addition to charge transfer product ions. This apparatus clearly pointed the way to the development of subsequent instruments designed specially for the study of ion molecule reactions.

When Professor Lindholm retired, one of his students, Professor Imre Szabo of the University of Lund, inherited the most sophisticated version of this apparatus and has continued to develop this technique. In its present three-stage configuration it provides state selection of reactant ions plus the capability of following second and higher order reactions quantitatively. The instrument is obviously capable of extension to higher order and Szabo has investigated and described mass-resolving and non-mass-resolving RF ion pipes for this purpose.

Other relatively sophisticated transverse geometry tandem spectrometers

were constructed by Tal'roze and co-workers in the Soviet Union and by Fite, Brackman and Snow at General Dynamics in the United States. [Wade Fite and Ted Brackman dropped this line of work when Wade became Professor of Physics at the University of Pittsburgh; they started Extranuclear Labs (now Extrel) shortly thereafter.] A rather different but conceptually similar system which utilized a time-of-flight second state analyzer was constructed in England about the same time. A simpler version with a 1" radius first stage mass selector was constructed by Koski and co-workers at Johns Hopkins. In Koski's apparatus the small radius and low accelerating voltage of the ion selector spectrometer facilitated turning the ion beam in the direction of the exit slit of the collision chamber for the investigation of ion molecule reactions.

COLLINEAR GEOMETRY INSTRUMENTS. Ion molecule reactions other than charge transfer (or hydride transfer) frequently impart forward momentum to product ions. This is intrinsic to proton transfer, condensation and pick-up reactions. In addition, all reactions which proceed via the formation of an intermediate complex will generate product ions which are forward scattered in the laboratory frame at 1 eV or higher ion kinetic energies. Severe discrimination effects are therefore anticipated.

The first tandem mass spectrometer specifically designed for studying ion molecule reactions involving momentum transfer was constructed by Clayton Geise when he was at the University of Chicago in 1963. This apparatus replaced the ion source of a large sector mass spectrometer with a 1" radius mass selector mass spectrometer and a complex deceleration/acceleration lens and collision chamber. This design was further elaborated by his student, William B. Meyer II who constructed an improved version at the Los Alamos Laboratory. Other instruments of in-line geometry were constructed in the 1960's in Professor Bailey's laboratory at the University of Florida, at General Dynamics by Turner, Fineman and Stebbings and at the Aerospace Research Laboratories in Dayton, OH, by the author and his collaborators.

The instrument shown in Fig. 1 was conceptually designed in 1962 and completed in 1965. It contained a number of ideas which were quite sophisticated for its day and enabled it to be applied quite extensively to the study of ion molecule reaction kinematics. It also has many features, evident in Fig. 1, which qualify it for current day MS² kinds of experiments.

The first stage reagent ion selector is a Nier-Johnson geometry double-focusing mass spectrometer consisting of a 90° electric sector and a 60° magnetic sector. An energy slit at the intermediate focal point established the energy band pass of the electric sector at 0.2 eV. It also served as an object slit for the 3" radius, 60° magnetic sector and as a detector for tuning the ion source and electric sector optics.

The acceleration voltage for the first-stage mass spectrometer is 169.8 volts and the collision chamber is at 3 KV, fixing the ion source voltage at 3.1698 K.V. The magnetic field coils of both electromagnets are at ground. Soft ion field plates for the ion gun magnetic field placed inside the flight tube were separated electrically (and physically) from field plates outside the vacuum envelope. Both the magnetic sector and ESA fields were terminated by Herzog shunts.

The ion beam at the mass resolving slit has a width of 0.003" and a half angle of divergence of 0.0152 radians. The mass resolving slit is the object for the 60° turning electric sector whose image forms the object of the deceleration lens system. This plate also serves as a beam monitor and, when the sector voltages are unbalanced, serves as a total beam detector for the first stage mass spectrometer. Einzel lenses placed between the source and electric sector and inside the electric sector provide vertical and horizontal focusing, increasing both transmission and resolution by a 0.25" gap.

The deceleration lens system is undoubtedly the most critical element of tandem mass spectrometers intended for the study of low energy ion-neutral interactions. The lens in the Fig. 1 instrument was designed in collaboration with Chris Kuyatt and J. A. Simpson of the NBS (now NIST). We called it a "slot" lens because it had slit geometry but considerable thickness. This lens design has subsequently been utilized in several ion collision apparatuses. Its currently accepted name is a "rectangular tube lens" and its optical properties have been thoroughly described. Remarkably enough, our design--based on the assumption that its strength as a focusing element was twice that of a slit lens because it is a 2D rather than a 3D lens--matches almost exactly the dimensions deduced from recent, accurate calculation. Two stages of deceleration, each with a retardation ratio of 9 and demagnification factor by 2 were used for our design.

For the reacceleration lens a standard lens originated by Soa was selected. Both the deceleration and reacceleration lens systems are high transmission devices optical elements. At the design retardation final energy of 2.1 eV and for all higher energies essentially 100% of the ion beam entering the lens is transmitted through the image slit (collision chamber). The (space-charge-limited) transmission was of the order of 70% at 1 eV and 30% at 0.3 eV, making the instrument a useful tool for investigating low energy ion-molecule reactions. These characteristics constituted a significant "breakthrough" in beam techniques for studying ion-neutral collision phenomena, decreasing the energy range accessible to quantitative measurements by nearly two orders of magnitude.

The second stage mass spectrometer was constructed from components of a Mattauch-Herzog geometry Consolidated model 21-110 mass spectrometer. To make the two mass spectrometers compatible, the object slit and ESA slits were enlarged. A quadrupole lens scaled down by a factor of 0.8 from the design of Giese was placed approximately mid-way between the object slit and the entrance slit of the electric sector to convert a tall, narrow beam of product ions to a square cross-section beam which is transmitted with high efficiency by the electric sector and the rather narrow magnet gap. Beam deflectors and intermediate ion collectors were installed at several points to monitor the primary ion beam.

As a parenthetical note, this second stage instrument was used to demonstrate the "decoupling" (our word) or "defocusing" (everybody else) technique for studying metastable ion decompositions in double-focusing mass spectrometers. This technique was developed independently and contemporaneously by Jennings and Elliott utilizing an AEI MS-9 spectrometer. This utilized the second state ESA as an energy selector for the magnet mass analyzer. It was intended, but never implemented, that this energy analysis capability be utilized to measure the energy distribution of product ions from ion molecule reactions. Analogously, the planned feature of comparing product distributions at 60° and 0° by locating a second collision chamber at the focal point of the first stage spectrometer (e.g., replacing the 60° turning sector by a collision chamber) was never implemented.

A most important collaborator in developing the Fig. 1 instrument was Dean Miller, coauthor on the Review of Scientific Instruments paper that described the characteristics of the instrument. Miller was an electronics technician who worked with the author during the frantic days of testing components, modifying designs, rebuilding and converging on the final configuration of the instrument. His many ingenious contributions made it possible for us to learn together enough about ion optics, vacuum techniques, and signal processing to exceed a signal-to-noise ratio of unity within about six months of our initial assault on the machine. Standing in the wings and eager to carry out measurements on the instrument as soon as it became available was Postdoctoral Fellow Fred Abramson, whose chemical insight and ambition moved the early experiments forward quite rapidly. Lieutenant Wayne Sieck, Ph.D., joined the research team shortly thereafter and also made major contributions in the early measurements.

Captain Larry Bone carried out his Ph.D. thesis research on this apparatus. Tom Tiernan, already a research collaborator for a few years, was away at graduate school completing his Ph.D. at the time that the instrument came on stream. He joined the tandem mass spectrometer research team on his return from Carnegie-Mellon University. Postdoctoral Fellow Mason Hughes joined the team shortly thereafter. To my regret, his arrival preceded by only a few months my becoming the team dropout when I left Air Force employment to join the University of Utah.

Having deserted this elegant tandem mass spectrometer and the research team that used it effectively for many years for a lower budget environment at the University of Utah in 1967, I turned my attention to a new technique for the study of ion-molecule reactions. This technique was ion cyclotron resonance mass spectrometry developed especially by Peter Llewellyn and John BaldeSchweiler. It eventually assumed the central role in the investigation of ion molecule reaction kinetics. Developments in this field, under its current designation as Fourier Transform mass spectrometry (FTMS), continues to evolve as one of the key developments of mass spectrometry. Our contributions to ICR emphasized mechanisms of ion molecule reactions and their dependence on ion kinetic energy. Not surprisingly, we soon turned our attention to the development of the tandem ion cyclotron resonance mass spectrometer. We utilized a 180° mass analyzer as the ion injector for this new tandem replacing the ion source of a standard drift cell ICR. This was based on the principle discussed by Dempster years earlier that the ion source and detector occupy symmetric positions at 180° within a uniform magnetic field. Consequently provided the optics are equivalent, the properties of the ion beam presented to the detector are identical to those presented at the ion source end. Mass selection of low energy ion beams with a resolution of several hundred is trivially simple. The injection of mass-selected ions also proved to be rather simple in that the ion projectories curl up on themselves as the ion is slowed to near thermal energies. We demonstrated that moderate mass resolution and low energy ion selection were simultaneously achieved using a relatively simple retardation lens geometry. Use for about seven years at Utah, this spectrometer has recently been rejuvenated by Professor John Wronka at Northeastern University. A similar version is currently a primary research tool in Professor Mike Bowers' laboratory at the University of California at Santa Barbara.

ANGLE RESOLVED INSTRUMENTS. In 1989 most mass spectroscopists have some familiarity with the topic of angle resolved mass spectrometry (ARMS). Our colleagues who care about the details of disposal of energy and the dynamics of ion reactions--as distinguished from reaction kinetics and mechanisms--have long understood that the precise description of how these reactions occur requires a knowledge of both scattering angles and the post-collision kinetic energy of product ions. In fact, the velocity vectors of the reactants and products before and after collisions and the internal energy states need to be measured to achieve a full description of ion reactions at the most fundamental level. From an instrumentalist's perspective this requires that the experiment be carried out using the technique of crossed molecular beams. This technology was first developed for neutral beams and only a few laboratories have developed the technology for working with low velocity (and variable velocity) ion beams, crossing them with molecular beams and analyzing the products with respect to energy, identity, and scattering angle.

The grandfather of all such apparatuses was developed by Zdenek Herman and Richard Wolfgang at Yale University. This instrument provided the first definitive information on the detailed mechanism of proton stripping reactions. It consisted of a permanent magnet first stage mass analyzer which mass selected (with resolution of about 50) ions generated by electron impact. The entire first stage apparatus--ion source, mass selector and neutral beam source--was mounted on the lid of a scattering chamber which is sealed with a rubber "O" ring. This lid and the attached apparatus are mechanically rotated about the object slit of a sector mass

analyzer. A more elaborate version of the same apparatus is in Dr. Herman's laboratory at the Heyrovsky Institute in Prague, Czechoslovakia.

The coincidence in time that a remarkable graduate student named Marvin Vestal arrived in Salt Lake City, that the author was awarded an Alfred P. Sloan Fellowship which provided enough money to construct a new instrument and the availability of Postdoctoral Fellow Calvin Blakley encouraged us to proceed with the construction at Utah of a rather more elaborate version of a tandem crossed beam apparatus. A talented graduate student, Philip Ryan, also appeared in my office one day to say that his current thesis problem was too easy and he would like to join the Vestal and Blakley team. Together they managed to construct a fairly formidable research instrument.

The new features of this tandem crossed beam apparatus were the inclusion of a high pressure, or chemical ionization, ion source which permitted (in favorable cases) the specification of the internal energy state distribution for the reactant ion, a higher resolution (to m/m 200) first stage mass analyzer, a new design for the deceleration lens which permits the deceleration of ions from 750 eV to below 1 eV laboratory energy, the inclusion of a supersonic jet which provides accurate specification of the neutral reactant velocity vector, and the addition of a high resolution energy analyzer for product ions. With moderate improvement in most of these features, refurbishment of components, and several subsequent generations of computer equipment for data acquisition and data reduction, this instrument is currently in use in the author's laboratory at the University of Delaware. A contributed paper at this Conference presents new data on quantum-state specific angular scattering in charge transfer reactions.

A frequent visitor at Utah during this period was Professor James D. Morrison of LaTrobe University. We engaged in many discussions of quadrupole mass filters and various experiments that could be designed if several of these instruments could be stacked in series (Morrison is now up to five). An apparatus which was constructed in our laboratory by Marvin Vestal and Gerard Mauclair (an exchange visitor from Orsay) is shown in Fig. 2. It was designed for and utilized in laser photolysis of ions. The first stage quadrupole was used as a mass selector, the second stage quadrupole was used as a strong focusing device to hold the ions in the light path of the laser, and the third quadrupole was used as a mass selector to distinguish the photolysis products from that experiment.

One of the major experimental artifacts plaguing the laser photolysis experiment was "ion noise" caused by metastable ions and collision induced dissociation (plus the collisional loss of electrons in negative ion experiments) which occurred in the central quadrupole of the apparatus. This clearly suggested that the apparatus might be useful for studying ion molecule reactions and collision induced dissociations. This was independently discovered and exploited very effectively by Enke and Yost and not exploited at all by us. A similar triple quadrupole constructed in Jim Morrison's laboratory served as the prototype for the triple quadrupole at Michigan State which now has many descendants. This story is told elsewhere at this Symposium.

The author's love affair with tandem mass spectrometers is far from over. Our most recent entry is shown schematically in figure 3, which utilizes a commercial double focusing mass spectrometer as the ion gun. An elaborate lensing system, which is a lineal descendant of the lenses first conceived for the Fig. 1 apparatus, serves to decelerate positive or negative ion beams generated in the first stage mass spectrometer to energies as low as 1 eV. The upper energy limit is currently 3000 eV. This ion beam is crossed at right angles by a supersonic jet molecular beam. Products are analyzed by a hemispherical energy analyzer and quadrupole mass filter which rotate about the scattering center. A full description of the apparatus and its characteristics has recently been

published and three contributed papers describing some of our first exciting results were presented elsewhere at this Conference. The key collaborators who have contributed to the development of this machine are the listed coauthors on these papers.

This apparatus was designed explicitly for the investigation of the reaction dynamics of collision-induced dissociation of polyatomic ions--e.g., the fundamental mechanism of MS^2 and MS^n types of experiments. This contemporary instrument utilizes a number of technologies developed for other purposes during the last 25 years. These include the use of lasers as aligning devices, the use of optical tables and rotators and choppers, digitally driven power supplies throughout, computer control of experiments and much more sophisticated signal averaging techniques than were possible in the Fig. 1 device. It also has many of the characteristics that have been exploited in earlier instruments developed in the author's laboratories. The motivation is unchanged and these remarkable devices still have a lot to teach us about the properties of ions in low, moderate and high energy collisions.

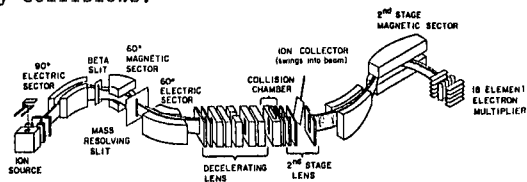


Fig. 1. Tandem Five Sector Mass Spectrometer built at the Aerospace Research Laboratories, Wright-Patterson Air Force Base in 1963-65.

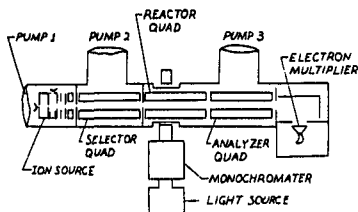


Fig. 2. Triple Quadrupole prototype built at University of Utah in 1974-75.

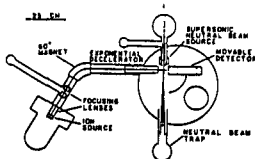


Fig. 3. Utah crossed beam apparatus constructed at University of Utah in 1972-74.

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P. J. Ausloos, Ion Molecule Reactions in the Gas Phase, Advances in Chemistry Series, 58, American Chemical Society (1966).
Fig. 1. Tandem Five Sector Mass Spectrometer built at the Aerospace

Tracing the History
of Analytical MS/MS

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Mass spectrometry/mass spectrometry (MS/MS) as an analytical tool developed almost inevitably from the extensive work of thirty years on the origins and reactions of metastable ions. The border between the study of metastable ions occurring as a result of unimolecular dissociations of gas-phase ions in transit through a mass spectrometer, and the explicit collection of daughter ions formed as a result of purposeful collision-induced dissociations is a nebulous one. No singular new experiment or instrument heralded the beginning of analytical MS/MS. Rather, the development of analytical MS/MS was essentially a conceptual one; the instruments for MS/MS experiments were completed long before chemists realized the extent of the information that could be drawn from them. The development of the intellectual framework of MS/MS continues even today. Analytical chemists who use MS/MS today should by all rights appreciate the efforts (often unheralded) expended in the development of the technique, and should conscientiously explore both its advantages as well as its shortcomings.

Mass spectrometry/mass spectrometry involves the measurement of the mass-to-charge ratios of ions before and after some reaction that occurs within the confines of the instrument. Such reactions have been known since the earliest mass spectrometers were constructed; the poor vacuums of the time made the occurrence of ion/molecule reactions inevitable. In retrospect, recognition of the true natures of these reactions was an extraordinary achievement. An equally important achievement was the recognition of the analytical value of MS/MS reactions. Experiments in analytical MS/MS were initially described about 1976, given the definition of analytical MS/MS as the use of MS/MS for determination of the presence or levels of particular compounds in a mixture of compounds. After several prototype instruments were fielded by manufacturers, MS/MS with commercially available hardware was introduced in the early 1980s. Widespread availability of the commercial instruments, coupled with the intrinsic analytical capabilities of the MS/MS method and ten years of continued interest and development, has culminated in today's breadth of MS/MS research and applications. This presentation provides a retrospective on some of the significant instrumental and experimental developments in analytical MS/MS.

Instrumentation

Multi-sector instruments were first developed for other purposes, but were also one of the first instrumental configurations to be used for analytical MS/MS. Inghram and Gomer assembled a BB instrument in 1954, and White et al. developed a number of multi-sector instruments from 1954 through 1972, including BB, BBB, BBE, and EBBE. These instruments were used for isotope measurements in which high abundance sensitivities were required, in the generation of high purity atomic ion beams, and in energy loss and charge exchange experiments. Molecular dissociation experiments were carried out for small molecular ions. Investigators at Oak Ridge National Laboratories assembled a number of multi-sector instruments used for isotope work. The construction of multiple sector instruments by Futrell and Todd and McLafferty, among others, was pursued in increasingly sophisticated studies of fundamentals in ion chemistry.

Both EB and BE geometry sector instruments can provide solutions to the double focussing equations, but the BE instrument offers the ability to mass select parent ions by momentum analysis through the magnet, and to use the electric sector for kinetic energy (and therefore mass) analysis of daughter ions formed in an inter-sector collision cell. Beynon, Cooks, Amy, Baitinger, and Ridley at Purdue University described the reverse-geometry MIKES instrument in the A pages of Analytical Chemistry in 1976, with the express purpose of using the instrument for mixture analysis with analytical MS/MS experiments. Construction of the instrument literally involved reversing the normal order of sectors in an MS9 instrument. Such a project could only be possible with the involvement of an excellent machinist and instrument builder, and Tom Ridley's involvement with mass spectrometric instrumentation was both long and productive. The

MIKES instrument survived several source, vacuum, and electronic updates, and several generations of graduate students, and as of this date, is still producing data. The popularity of ZAB instruments (VG Instruments) is derived in no small part from the capability of 'reverse' geometry instruments for MS/MS. Triple- and four-sector instruments of various geometries were developed to provide higher resolution for either the parent or the daughter ions, at the expense of added size, cost, and complexity.

The first multi-quadrupole instruments were described by Vestal and Futrell, and by McGilvery and Morrison, and were used for photodissociation research. A multi-quadrupole instrument developed expressly for MS/MS applications was described in 1978 by Yost and Enke at Michigan State University with the contributions of machinists Russ Geyer and Len Eisele. A patent in 1980 described the basic attributes of a 'tandem quadrupole' instrument for CID MS/MS. Early prototypes of triple quadrupole MS/MS systems were placed in research laboratories around the country. The research group of Hunt at the University of Virginia was especially active in demonstrating the power of the triple quadrupole instrument for analytical MS/MS. By 1982, sales for commercial triple quadrupole MS/MS instruments had entered the high growth period that continues today. Combination of relatively low price with relative ease of operation provided the impetus for the success of the triple quadrupole instrument. These attributes are exactly those that characterize the use of ion trapping spectrometers for MS/MS as demonstrated in the last few years.

Triple quadrupole instruments provided unit mass resolution for both parent and daughter ions in MS/MS experiments. BE sector instruments could provide better than unit resolution for selection of parent ions, but less-than-unit resolution of daughter ions due to the kinetic energy release measured by the electric sector. The stage was set for hybrid instruments (combinations of sectors and mass-analyzing quadrupoles) to be developed. A BQ was first described in 1974 for studies of the structures of organic ions, and a BQ combination instrument assembled by Armentrout and Beauchamp was used in 1980 for studies in studies of organic ion/molecule reactions and metal ion/organic ion interactions. A hybrid BQQ instrument for analytical MS/MS was first described by Glush et al. in 1982. This work led to a prototype BEQQ instrument in the same laboratory, and then to commercial EBQQ and BEQQ instruments.

Experiments

Studies of metastable ions are studies of daughter ions formed by unimolecular dissociations of parent ions. With the purposeful introduction of collision gas into a reaction region of a mass spectrometer, either through a leak valve or through the simple expedient of loosening a bolt on a vacuum flange, product ions resulting from collision-induced dissociations could be increased in relative abundances. As such studies continued on multi-sector instruments, equations that described the masses of the parent and the daughter ions reacting in various regions of the mass spectrometer were derived. A conceptual advance occurred when the study of metastable ions and CID products drifted from careful studies of ion structure to the examination of ions from various component molecules present in the source of the mass spectrometer at the same time, and then moved to studies in which the presence or absence of a particular metastable or daughter ion MS/MS spectrum was interpreted to reflect the presence or absence of a particular compound in a mixture of components in the source.

The study of daughter ions produced by collision induced dissociation has a long history in the study of ion structure, drawing upon the extensive studies in metastable ion dissociations. Careful studies by a number of workers, including McLafferty, Levsen, Beynon, and others, showed that the daughter ion MS/MS spectrum could be interpreted to yield structural information just as could the electron ionization mass spectrum, and that the method could be extended to the study of components in unseparated mixtures. The early work extending the concept to the determination of components in mixtures appeared in 1976 and 1977. Levsen and Schulzen in 1976 reported the use of daughter ion MS/MS spectra to identify six major components in the mixture of pyrolysis products of DNA. Using the MIKES instrument, analysis of simple mixtures of organic compounds was demonstrated by Cooks et al. in 1976. A series of papers starting in 1977 from Cooks' laboratory highlighted the use of daughter ion MS/MS for the direct analysis of trace components in crude extracts derived from natural products. It was the figures that accompanied these papers that captured the interest of those who saw the possibility of

direct mixture analysis without separation. The work could be extended to levels of trace components at a few percent in the original sample, and 0.1 to several micrograms in the source of the mass spectrometer. Matrix effects exert significant effects below this level, and are a complex function of the nature of the sample and the chemical matrix. Retention of at least a rudimentary sample clean up or chromatography step is used to minimize these matrix effects.

Linked field scanning of sector instruments for the acquisition of parent ion MS/MS spectra was described by Boyd and Beynon, and Evers et al. in 1977. It was the introduction of the triple quadrupole mass spectrometer, with its ease of calibration and scanning, that made both the parent ion and the neutral loss experiment more accessible. The parent ion scan was not mentioned in the first Yost and Enke paper on the triple quadrupole mass spectrometer, but appears as part of the three modes of operation (daughter, parent, and constant neutral loss scans) in the 1979 review in the A pages of Analytical Chemistry. The use of parent ion scans highlights the ability of MS/MS to provide different sorts of information than normal mass spectrometry. One of the more common uses of parent ion MS/MS is in the determination of the number of structurally related compounds in a mixture. In the case of drug metabolites, for example, most of the metabolites retain the bulk of the structure of the original drug. If a daughter ion characteristic of that original structure is established, then a parent ion MS/MS spectrum can be used to establish the number of precursors to that ion. Similarly, classes of related natural compounds can be linked via the appearance of a common ion in each of their daughter ion MS/MS spectra.

In 1979, neutral loss scans (in which the selected masses of parent and daughter ions are simultaneously varied to maintain a constant mass offset between them) were described for sector instruments (Zakett et al.) and for triple quadrupole MS/MS instruments (Yost and Enke). The neutral loss scan provides specific information on the chemical reactivity of the ions studied. In one of the first examples, it was shown that anions formed from carboxylic acids typically undergo loss of 44 daltons (neutral carbon dioxide) in the CID daughter ion MS/MS spectrum. Other functional groups also exhibit their characteristic neutral losses, so that the MS/MS experiment can provide functional group information on the various components in a mixture. More importantly, as demonstrated originally by Hunt in his work on environmental MS/MS, derivatizations of the mixture can provide both functional group specificity in the derivatization reaction, with the ability to design an MS/MS constant neutral loss experiment tied to that derivatization label. The use of derivatization labels is all the more significant since compounds of higher mass tend to contain several different functional groups, and their gas phase CID fragmentations are dominated by one functional group over the other. Derivatization occurs in solution under conditions designed for more complete reaction, and the gas phase dissociations of the labels are more independent of the remainder of the ionic structure. In the past decade, neutral loss scans specific for sulfur-containing aromatics, sulfonated dyes, and nitro-group-containing compounds, among many others, have been described in the literature.

MS/MS instruments can be manipulated to provide information other than the usual daughter ion, parent ion, and neutral loss MS/MS spectra. Energy-resolved MS/MS experiments were introduced in 1981 by McLuckey et al. These have mostly been used for ion structural studies, but a few analytical applications have been reported. Despite the 'nodes' in transmission through the rf-only collision quadrupole, the collision energy can be related to the internal energy deposited in the parent ion. Charge permutation reactions, once thought to be entirely within the domain of sector instruments and high collision energies, have been demonstrated on multi-quadrupole instruments. With low collision energies in the triple quadrupole instrument, ion/molecule association reactions can be studied; the first reports in 1980 have been followed by many other examples, but the intrinsic selectivity of the associative reaction has not yet been exploited in analytical applications. Consecutive reactions (MS/MS/MS) on a triple sector instrument were reported by Burinsky et al. in 1982. A few pentaquadrupoles have been assembled in research laboratories, and MS/MS/MS for studies of ion structure have been reported. Trapping mass spectrometers excel at MS/MS/MS/... within the limits of ion trapping and dissociation efficiencies; whether analytical problems exist to justify the regular use of these extended sequences is still unknown. Finally, the conjunction of MS scans of various types to yield new variants such as the

'familial' scans and the 'reaction intermediate' scans, is, in concept, already three years old, but these new means of extracting information about ion chemistry through MS/MS are just now being explored.

The initial excitement for MS/MS focussed on its abilities to identify components in complex mixtures without separation. Debate then, as now, pitted a separations method that could resolve any component of any mixture against a detection method so selective that no separation of the mixture would be necessary. The development of the selective detection of MS/MS came at a time when new developments in separations methods were sparse, adding to the debate. The early discussions of MS/MS were somewhat mis-directed, the result of errors in judging the long range potential and areas of strength of the analytical method. The broad scope of uses of MS/MS today were not envisioned fifteen years ago when analytical MS/MS was first demonstrated, not by the initial workers in the field, nor by those who stood up to declare MS/MS to be without merit, but who are now practitioners. The best insurance against the inability to judge strengths and potentials is a willingness to investigate high risk but high return research areas, and to support those who do the same. Almost all of the researchers involved in the original developments of MS/MS are still active in the field, and have been joined by those who use the approximately 500 instruments worldwide. The outlook for continued development is secure.

THE DETECTION AND RECORDING OF ION BEAMS: FROM THE GOLD-LEAF ELECTROSCOPE TO THE INTERACTIVE GRAPHICS WORKSTATION.

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T6G 2G

Objective

The mass spectrometer consists of many parts all of which must work correctly and simultaneously if useful information is to be obtained. Achieving this is not as difficult now as it was in the early days.

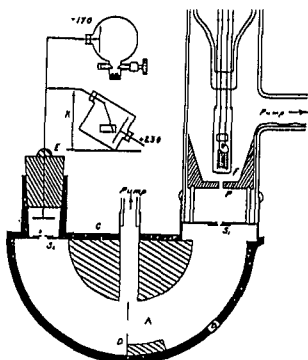
Much of our present concern is to produce ions in the most appropriate way and to separate them with the best compromise between sensitivity and resolution, but we tend to take for granted that they can be detected and measured and the output displayed in an easily handled format. The earliest pioneers in the field did not have even basic electronics available to them, let alone computers, and detecting and recording the ions they produced was a far from simple task

This poster reviews some of the important developments which led to our present demand for ever faster and more intelligent workstations to examine the vast quantities of data routinely produced by modern mass spectrometers.

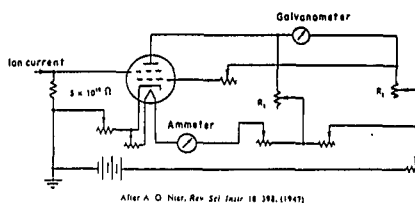
Historical Development

Mass spectrometry predates the science of electronics and the first methods for the detection and recording of ion beams used such devices as scintillators, photographic plates and mechanical electroscopes. J.J. Thompson's spectrograph produced parabolas on a scintillating screen or a photographic plate, but no useful intensity measurements were possible until he replaced the plate with a parabolic slit and collected the beam on a Wilson gold-leaf electroscope. Dempster achieved the first really quantitative recording of a mass spectrum also using a gold leaf electroscope. In his apparatus the positive charge deposited by the ion beam caused the leaf to deflect and this could be balanced by allowing beta particles from a radioactive source to fall on another detector attached to the electroscope. A mechanical shutter with a graduated scale controlled the amount of beta particles reaching the collector and so the intensity of the original ion beam could be quantified in units of the graduated scale.

The ability of ion beams to darken photographic emulsion was used from the earliest days as a means of both detecting and recording for mass spectrometry and this has only recently disappeared from use. There are no longer any commercial mass spectrometers available with photoplate detection although there may still be laboratories which use the technique on older instruments. The biggest drawback of photoplate recording was the nonlinearity of the darkening process and its lack of dynamic range but this could be overcome with multiple exposures and by making plates of uniform quality which were carefully calibrated with a good microdensitometer. The biggest advantage was the ability to integrate weak or unstable signals for as long as necessary to obtain a good record. Thus photoplate recording reached its greatest popularity in recording complete high resolution spectra and also the fluctuating output of spark sources.



MASS SPECTROMETRY PREDATES ELECTRONICS. DEMPSTER'S INSTRUMENT WITH CALIBRATED ELECTROSCOPE.



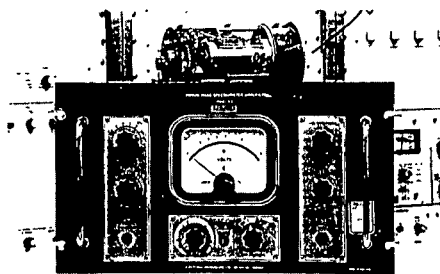
NIER'S WHEATSTONE BRIDGE CIRCUIT UTILISING A VACUUM TETRODE.

The advent of the thermionic valve, or vacuum tube, made the first electronic detection of ion beams possible. If the beam was allowed to hit a Faraday cup which was attached to ground via a high value resistor, a voltage could be generated which was proportional to the ion current and this could be applied to the grid of a vacuum tube. In an early circuit described by Nier a tetrode is used in a battery operated Wheatstone bridge network where the filament to screen and filament to cathode spaces form two segments of the bridge. A galvanometer measures the unbalance bridge current which is proportional to the original ion current. Such circuits were capable of measuring beams as low as 10^{-15} A but were very unstable, slow reacting and had no means of recording other than noting the galvanometer deflection by hand.

As electronics developed and negative feedback circuitry was invented, tube type amplifiers became common and the first commercial mass spectrometers used them typically with a 954 or ME1403 electrometer tube followed by a multi-tube d.c. amplifier. Great pains had to be taken to reduce noise and drift and the electrometer tube was enclosed in an evacuated, light-tight and magnetically shielded enclosure. Some of the d.c. amplifiers used dry cell battery coupling between stages and, if the batteries were not enclosed in thermally insulated boxes, they were a major source of drift at high gains.

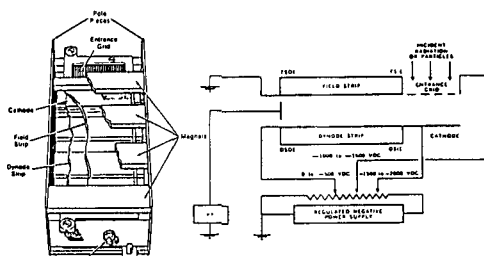


VACUUM PENTODES. THE 954 "ACORN" HAD AN INDIRECTLY HEATED CATHODE. THE ME1403 WAS DIRECTLY HEATED.

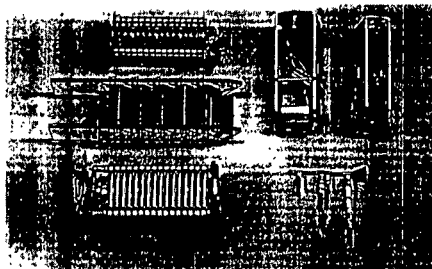


THE VIBRON VIBRATING REED ELECTROMETER. THE REED IS IN THE ALUMINUM CYLINDER.

It was known that a.c. amplifiers were inherently drift free and in the 1940s vibrating reed electrometers were developed which incorporated a capacitor with one plate fixed and the other, the reed, connected to a stable mechanical oscillator run at a frequency of about 1000Hz. The input from a Faraday cup was attached to the reed and also via a high megohm resistor to ground. This produced an ac signal which could be stably amplified with little drift. These electrometers found use in isotope ratio instruments but were not widely used in more conventional mass spectrometers because electron multipliers were developed around the same time. These had many advantages. Because high drift-free gain could be achieved in the multiplier, a dc electrometer using a much smaller resistor could be employed and this minimised drift in the amplifier and also increased its speed. The drawbacks of the early multipliers were unstable gain (which made them unsuitable for isotope ratio work) and susceptibility to magnetic fields. These problems were solved and a number of different dynode structures were developed, each with its proponents and claimed advantages.

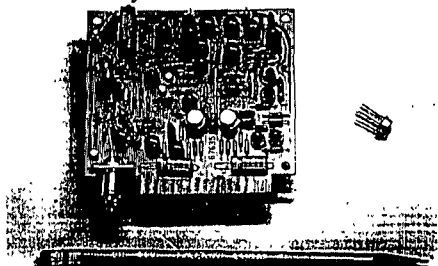


THE BENDIX CONTINUOUS DYNODE MULTIPLIER USED A MAGNETIC FIELD TO CAUSE ELECTRONS TO STRIKE THE DYNODE REPEATEDLY.

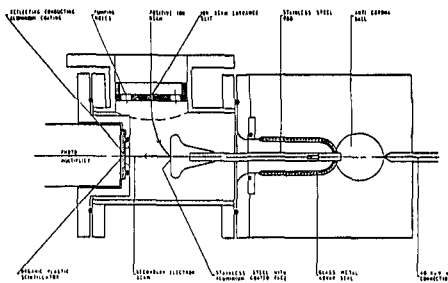


ELECTRON MULTIPLIERS OF BOTH DISCRETE DYNODE AND CHANNEL DESIGN IN A VARIETY OF CONFIGURATIONS.

When solid state electronics began to replace vacuum tubes in the 1960s some hybrid electrometers were used where the very high input impedance of the vacuum pentode was combined with the stability of a transistorized amplifier but eventually the high impedance MOSFET (metal oxide silicon field effect transistor) replaced even the electrometer tube and totally solid state electrometers became standard on all mass spectrometers. This progressed to the FET input integrated circuit amplifiers which we use today.

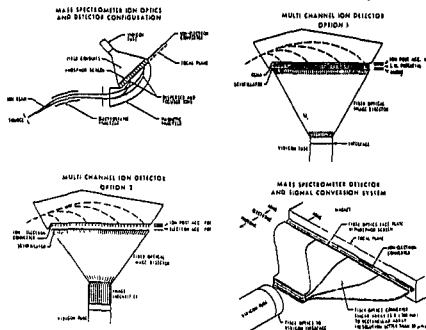


AN EARLY KEITHLEY DISCRETE COMPONENT FET INPUT ELECTROMETER. BESIDE IT IS A MODERN IC FET INPUT OPERATIONAL AMPLIFIER IN A TO99 CAN.



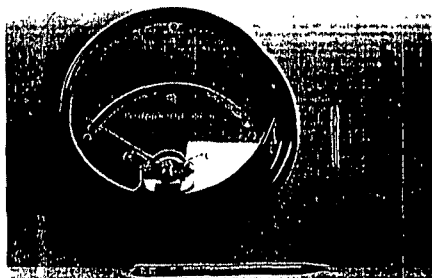
THE SCINTILLATOR USED BY J.J. THOMPSON REAPPEARED IN 1960 IN THE DALY DETECTOR AND IS ALSO USED IN SOME MODERN SYSTEMS.

For some special low signal applications the electron multiplier, usually of the channel type, was run in the saturated mode so that pulses produced by individual ions could be counted using fast digital electronics thus preserving the inherently digital nature of the ion beam rather than converting it to an analog signal.



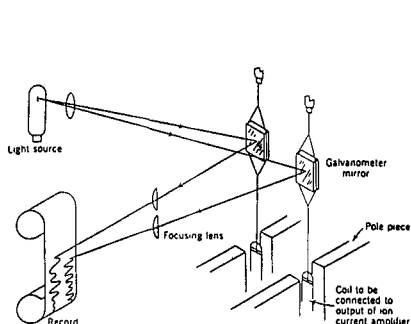
HEINTZ BOETTGER DESCRIBED AN "ELECTRIC PHOTOPATE" AT THE 1973 ASMS CONFERENCE. MODERN MICROCHANNEL ARRAYS DEVELOPED FROM THIS.

The Daly detector and some later developments of it returned almost to Thompson's original design by using a scintillator which was followed by a photomultiplier although in this modern system the original ions strike a conversion dynode to produce secondary electrons and these strike the scintillator rather than the ions. The multichannel array detector, which is one of the most promising new devices in our field was first discussed in the early 1970s when it was expected to combine the merits of electrical and photographic recording and was dubbed "The Electric Photoplate".



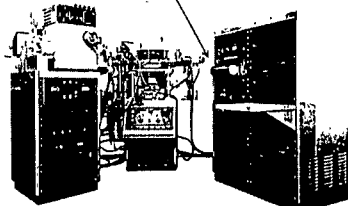
LARGE MOVING-COIL GALVANOMETERS DISPLAYED ELECTROMETER OUTPUT ON EARLY COMMERCIAL INSTRUMENTS LIKE THE METROPOLITAN-VICKERS MS2. THE MINIATURE MIRRORED GALVO WAS USED IN A UV OSCILLOGRAPHIC RECORDER.

Recording the output of electrometer amplifiers began with moving coil galvanometers whose deflection could be noted, but for a continuous record either long arm recording galvanometers or self balancing potentiometric recorders writing with ink on paper were necessary. The long arm galvanometers did not give a very linear response but had the advantage that several overlapping arms with different attenuations could be used to extend the dynamic range. They were not used to a great extent in mass spectrometry. Potentiometric recorders were accurate and reliable (except for the ink feed which was always the weakest point) but to provide good dynamic range an automatic range changer was desirable. Some imaginative designs evolved which sometimes required repeatedly backstepping the scan generator until a peak could be recorded on scale while the range was reset to maximum sensitivity when the signal dropped back to baseline. These recorders had a response time of about one second at best but were very widely used until electron multipliers became common.



OPERATING PRINCIPLE OF THE OSCILLOGRAPHIC RECORDER.

6. A high speed MULTI-CHANNEL OSCILLOGRAPH and its 36" scanning automatically driven wide chart strip - allows continuous recording after calibration and allows the operator to mark any mass peak with a recording spectrum



PHOTOGRAPHIC DARK ROOM

Learn to those who make use of a dark room: a dark room should be provided for processing the oscillograph chart. Even with a direct writing oscillograph recorder like the Haverhill 1000, the glass protection is still required for permanent display. For the Haverhill 1000, the light source should be dark red. Connections may vary, see 10" x 14" for details and 10" x 14" for the dark room. A quartz type oscillograph recorder and a dark room safety light 10" x 14" are recommended.

THE EARLIEST UV OSCILLOGRAPHS USED SEALED CASSETTES AND REQUIRED A DARK ROOM FOR PROCESSING.

Because a multiplier detector can operate at both high gain and high bandwidth, recording systems with bandwidths in the kilohertz range became necessary. Oscilloscopes were suitable but had to be photographed for a permanent record. High speed miniature mirrored galvanometers could be used to reflect a beam of UV light onto photographic chart paper and multiple channels could be used to provide dynamic range. The first systems used paper which moved in a light-tight cassette and the record had to be developed in a dark room. This was far from satisfactory but soon direct print paper became available which developed in room light and was reasonably permanent in the absence of UV. These oscillographic recorders were very widely used and are still available today although the cost of paper can be daunting. They remain one of the few ways of examining the analog output from a scan complete with "metastable" peaks, noise spike and assorted glitches - features which are usually lost when the signal is digitized, massaged and replotted through a computer. Some ink-jet and electrostatic recorders could also operate in the high bandwidth range but never became common in mass spectrometry.

The Present Day

In the early 1970s, dedicated minicomputers became generally available for use on mass spectrometers. Since then the recording systems previously used were rapidly displaced by the analog to digital converter, on-line minicomputer and printer/plotter. Electron multipliers, scintillator/photomultipliers and multichannel arrays are still the state of the art but, with the current trend towards closed-loop design where detectors are controlled by software or even by feedback from the output, the operator may be unaware of precisely what components are being used. This was never the case with earlier mass spectrometrists.

References & Acknowledgements

The most complete source of references to all of the earlier instrumental developments is:

J.H.Beynon, "Mass Spectrometry and its Application to Organic Chemistry", 1960, Elsevier.

Valuable chapters can also be found in:

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Some of the illustrations for this poster are taken from the original literature, the above texts and manufacturers brochures. I would like to acknowledge all of these sources.

ARTIFICIAL INTELLIGENCE IN MASS SPECTROMETRY

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This presentation outlines some important milestones in the history of the applications of mass spectrometry in artificial intelligence, and of artificial intelligence in mass spectrometry, for the 50 year period 1939-1989.



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For half a century - from the conceptualization and construction of the first electronic digital computer, the Atanasoff-Berry Computer of 1939-1941, to the present microcomputers, minicomputers, mainframes, and supercomputers - mass spectrometry has been intimately intertwined with computers in serving as a focus for a wide variety of applications. One of the first applications in what today might be regarded as artificial intelligence was in mass spectrometry - Berry's CEC MASCOT for multicomponent analysis based on mass spectral data.

Definitions of artificial intelligence, such as Minsky's "the science of making machines do things that would require intelligence if done by men," Rich's "the study of how to make computers do things at which, at the moment, people are better," Winston's "the study of ideas which enable computers to do the things that make people seem intelligent," and Boden's "the use of computer programs and programming techniques to cast light on the principles of intelligence in general and human thought in particular," are appropriate to the applications of artificial intelligence in mass spectrometry.

Expert systems, robotics, planning and decision-making, and other component areas of artificial intelligence have been and are directly involved in the development of mass spectrometry; still other areas, such as natural language processing, automatic programming, and intelligent computer-assisted instruction also impact our efforts in mass spectrometry.

It is significant, of course, that some schools recognized as leaders in artificial intelligence also are recognized as leaders in mass spectrometry; possibly the most important of these schools are MIT and Stanford. Some early Biemann papers (with Hertz, Hites, and others) considered photoplate data conversion, data acquisition systems, and the determination of amino acid sequences in small peptides in computer-aided interpretation of high resolution mass spectra. And the Stanford DENDRAL program (Feigenbaum,

Lederberg, Djerassi, Buchanan, Smith, *et al.*) for computing structural descriptions of complex organic compounds from their mass spectra and related data, together with related contributions, continue to be cited in nearly all artificial intelligence publications.

Some of the visionary early work of the ASTM Committee E-14 in inaugurating a collection of mass spectra, the establishment of the Uncertified Mass Spectra, and the early work of Grotch and others in encoding mass spectral data, provided one of the best scientific databases to use in searches, and to apply logic and heuristics to in the development of expert systems. The interpretation of mass spectra to provide identification and library searches, such as done by McLafferty, Venkataraghavan, and others (NIST, NIH, NBS, etc.) with PBM and STIRS, etc., and the early work of Jurs, Isehour, and Kowalski in artificial intelligence, particularly pattern recognition, factor analysis, and graph theoretical methods, have all had an important effect upon mass spectrometry.

It is clear that a statement made in 1953, "It would not be practicable at the present time to associate such a machine [computer] with *each* mass spectrometer, and the necessity for speed in analysis may prohibit the transmission of data and answers to and from a central organization," indicated much foresight for the needs that were to be experienced and the development that was to come.

The following provides a chronological synopsis, albeit likely incomplete, of some of the major developments in artificial intelligence and mass spectrometry in the past fifty years.

- 1939 Atanasoff and Berry develop the first automatic electronic digital computer.
- 1942 First commercial mass spectrometer delivered by CEC to Atlantic Refining.
- 1943 British Colossus used to decipher the German Enigma code.
- 1944 Aiken and IBM develop ASCC, an electromechanical machine to solve polynomial equations.
- 1945 Von Neumann conceives "stored program" design for serial digital computers.
- 1946 Berry describes a computer (early MASCOT) for solving linear simultaneous equations for mass spectrometric analyses.
- 1946 ENIAC dedicated at the University of Pennsylvania.
- 1950 Turing describes his test for machine intelligence.
- 1950 Shannon speculates on the prospects for computer chess.

- 1952 Formation of ASTM Committee E-14 on Mass Spectrometry.
- 1953 First ASTM Committee E-14 meeting (jointly with Pittsburgh Analytical Chemistry Conference).
- 1954 Production of FORTRAN by team headed by John W. Backus initiated.
- 1955 Bernstein develops the first working chess program using heuristic techniques.
- 1956 McCarthy, Minsky, Rochester, and Shannon organize the Dartmouth Conference.
- 1956 McCarthy coins the term Artificial Intelligence.
- 1956 Newell, Shaw, and Simon develop the *Logic Theorist*, the first successful AI program.
- 1957 McCarthy invents LISP, the first popular AI programming language.
- 1957 Samuel develops a champion-play checkers program using learning routines.
- 1958 FORTRAN-II, the first widely-used scientific programming language is made available.
- 1962 Quasi-Equilibrium Theory computer calculations by Vestal, Wahrhaftig and Johnston.
- 1962 FORTRAN-IV becomes available.
- 1964 Abrahamsson, Stenhagen-Stallberg, and Stenhagen publish proposal to use a computer to search mass spectra.
- 1965 Feigenbaum develops heuristic *DENDRAL*, the first expert system, for analysis based on mass spectral data and predictions of molecular structure.
- 1965 BASIC created by Kemeny and Kurtz at Dartmouth College.
- 1966 Biemann, *et al.*, use computer to determine amino acid sequence in oligopeptides.
- 1966 Weizenbaum develops ELIZA at MIT.
- 1966 Quillian invents the semantic network.
- 1967 Hites and Biemann describe completely automated data collection system for mass spectra.
- 1967 Greenblatt develops MacHack, the first competent chess program.
- 1968 Crawford and Morrison identify unknowns via catalog search.
- 1969 Charter and formation of ASMS.

- 1969 Jurs, Kowalksi and Isenhour apply pattern recognition to mass spectrometric analyses.
- 1969 Extensive DENDRAL applications begun by Djerassi, *et al.*
- 1970 Computer-operated mass spectrometer systems developed by Lederberg, *et al.*, and by Sweeley, *et al.*
- 1970 Colmerauer and Roussel at the University of Marseille begin development of the PROLOG language.
- 1971 Graph theoretical applications to mass spectra made by Jurs.
- 1972 Computer identification of mass spectra by Grotch using highly compressed code.
- 1972 MYCIN, first practical expert system to use production rules, developed by Buchanan and Shortliffe.
- 1972 Winograd completes SHRDLU, a natural language processing program.
- 1973 Self-training interpretative and retrieval system (STIRS) developed by McLafferty, *et al.*, for interpretation of mass spectra.
- 1974 Minsky publishes "A Framework for Representing Knowledge" paper.
- 1975 McLafferty, *et al.*, establish network availability for mass spectral interpretation.
- 1975 Factor analysis applied to mass spectral analyses by Isenhour and Rozett.
- 1975 The Lisp Machine, the first specialized AI computer, invented at MIT.
- 1975 The first personal computers begin to become available.
- 1976 Automatic rule formation applied to mass spectral data using meta-DENDRAL.
- 1976 McLafferty, *et al.*, develop probability-based matching (PBM) system for mass spectral identification.
- 1977 FORTRAN-77 standards are defined.
- 1981 Japanese launch "Fifth Generation" AI research effort.
- 1982 Clark and McCabe argue for PROLOG use in building expert systems.
- 1986 Berliner's HiTech is first chess computer to receive Senior Master title.

Most of the above interfaces of mass spectrometry and artificial intelligence has involved electron impact mass spectral data. It is expected that LV/MS, FAB/MS, CA/MS, FI/FD/MS, *etc.*, will play a significant role in the future as the databases for these and other (new) mass spectrometric techniques are established more fully.

From the above digest, it is observed that in the past decade relatively few significant innovations have occurred. Interestingly, contributions to the area of artificial intelligence in mass spectrometry from scientists in China and the Soviet Union appears to be on the increase. These features provide a hint that this interesting area in mass spectrometry may now be poised on the threshold for further significant advances.

Dare we envision mass spectrometer systems with fully-developed robotic features and planning/decision support, augmented with expert systems for analysis and interpretation, that will replace (some/many/most/all) human mass spectrometrists in the next fifty years ?

A RESOURCE FOR BIOMEDICAL MASS SPECTROMETRY

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The Washington University Mass Spectrometry Facility began with the acquisition of an LKB-9000 in 1969. The decision to obtain a GC/MS instrument at Washington University at the School of Medicine was largely based on the report, in 1968 by Hites and Biemann (1), that using an IBM 1800 it was possible to acquire and process mass spectra in times compatible with the elution periods of gas chromatography and store them at disk for later retrieval. The decision to buy the LKB was made following experiments performed with the help of Chuck Sweeley on the University of Pittsburgh LKB-9000 and because of the outpouring of results on the LKB instrument from the laboratories of Elliot, Holmstedt, the Hornings, Ryhage, Sweely, Waller and others. The instrument was received in 1968.

William F. Holmes, then a joint appointee in the Departments of Biological Chemistry and the University Biomedical Computer Laboratory, agreed to design a data system for the LKB. Bill was experienced with the LINC, a "small" (refrigerator-size) display-oriented computer. At that time DEC was manufacturing the LINC as their PDP-12. As we obtained the system it had two LINC-tapes as storage devices. These were ~4 inch diameter, large-hub 3/4 inch wide magnetic tapes each of which could store 225,000 12-bit words. The system used a 10-bit analog to digital converter and 8K (!) of magnetic core memory. The PDP-12 cost \$36,500 and the plotter \$3500, (cf. the ratio of cost of the mass spectrometer, for the LKB about \$60,000, to the computer then and now as well as today's prices for a PC with 80 times the computer memory). During this period several other GC/MS-computer systems were under development around the world (see references in [1] and [3] which include then-current developments in the Sweeley/Jack Holland lab and in Sandy Markey's lab). Of these the PDP-12 approach was unique with respect to the large display which was the user interface of the system.

During the computer development period the LKB was used by a small group of individuals that were willing to work with the light beam oscillographic output. Among the users was Denny Bier, a pediatrician, who performed his first experiments using stable isotope-labeled substrates as metabolic tracers in patients for whom exposure to radiotracers was unacceptable. While far from optimal analytical conditions, without the advantages of signal averaging that later became available with ion monitoring, these early studies showed the feasibility of doing the experiments using doses of the labeled species that were small enough so as to not disturb the pool size of the metabolite and thus complicate the interpretation of the results. It was also shown that blood samples for these studies could be small enough to carry out experiments on newborns.

Using the techniques developed on the LKB-9000, Denny performed the first measurements of glucose production and alanine kinetics in young children in 1972 and subsequently described the developmental changes in glucose production rates throughout childhood (14). Perhaps the ultimate flattery is that these numbers are now incorporated into pediatric textbooks as established fact without refering the source. Subsequently, using similar GC/MS techniques and stable isotope tracer infusions, Denny described the onset of gluconeogenesis in the human infant immediately after birth (15), the source of gluconeogenic carbons in these infants (15,16), and the turnover and uptake of fatty acid and ketone body fuels in human neonates (16,17).

Another early user was Bill Sherman who began cataloging spectra of carbohydrates and other biological substrates and their phosphate esters as the trimethylsilyl and boronate derivatives. Some of these spectra became part of the catalog of "Mass Spectra of Compounds of Biological Interest" compiled by Sandy Markey and others and printed by the, then, Atomic Energy Commission. Another early published effort done without the data system was a study which showed that, in the enzymatic conversion of glucose-6-phosphate to *myo*-inositol-1-phosphate, the intermediate molecular species never left the enzyme molecule. This was done by carrying the transformation out with an equimolar mixture of perdeuterated

and undeuterated glucose-6-phosphate. The inositol-forming process occurs by transfer of a hydrogen atom from glucose-6-P to the cofactor NAD^+ . This same hydrogen was postulated to be returned to the incipient inositol-1-P. If the NADH (or the hydrogen abstracted by the NAD^+), or the oxidized glucose-6-P, or the incipient inositol-1-P, dissociated from the enzyme complex, any of these intermediates could carry a hydrogen to a deuterium-labeled species (or vice versa) which would be evident from the isotopic composition of the product. The only products that were formed were found to be the fully deuterium-labeled and completely unlabeled inositol phosphate proving that the hydrogen removed was the same one returned and that all of the intermediates in the reaction remained enzyme-bound until the product dissociated from the enzyme (2). This kind of experiment could not have been performed with radioisotope-labeled substrates, it uniquely requires a mass spectrometric solution.

The LKB-9000/PDP-12 system was described in 1971 (3). The system acquired data and presented mass calibrated spectra in bar graph form on the PDP-12's 12 inch display oscilloscope in GC real time. The mass marker used was the Hall effect device designed by Ryhage, the squared analog output of which, when digitized, was the equivalent of a 14-bit ADC. Maximum scan rates were from 20-500 u in 4.2 seconds, 20-1000 u in 12 seconds, mainly slowed by the magnetic field collapse time. These rates were seldom incompatible with packed column gas chromatography. The data were stored on LINC magnetic tape for review, listing or plotting. The maximum size spectrum was limited to 850 peaks by core size. Over 200 spectra of 200 peaks each could be stored on one tape, while the second tape carried most of the programs. Sequential spectra could be recovered from tape and displayed at 2 second intervals.

A useful feature of the PDP-12 system was a variable mass range scan which resembled oscillographic output in that the actual peak profiles including metastable ions were displayed in real time. Another feature was a display that used data that could be stored with every spectrum that gave the deviation of an assigned mass from the nearest mass calibration value. For example a spectrum that was acquired and stored with a user-assigned mass defect per 1000 u, and containing mass assignment data in 0.1 u increments, could be displayed with the stored mass assignments as made with perfluorokerosene. Any incorrectly assigned peaks (e.g. misassigned because they fell close to 0.5 u from a nominal mass) were evident by their deviation from continuity within a 1 u window established by the PFK. Thus each spectrum could be viewed retrospectively to verify the mass assignment for any ion.

The choice of the PDP-12 computer was based on the philosophy of the University Biomedical Computer Laboratory that the user interface with the computer should be what is termed today as "friendly". The interactive display has, of course, now become standard but then it was common to enter instructions on many computers by switches and obtain results via printer. The alphanumeric and graphic displays of the PDP-12, as used with Bill Holmes' system, allowed users to set up experimental conditions by filling out questionnaires and presented the mass spectral data in an essentially "publishable" format that was intuitively understandable. This philosophy became one of the underpinnings of the "hands-on" aspect of the Facility. Most individuals learned how to use the LKB for their own experiments thus freeing the Facility staff for other activities.

By early 1973 Bill Holmes had developed computer controlled ion monitoring programs and hardware (4). This and J. T. Watson's PDP-12 based system (5) were the first fully digitally-controlled ion monitoring hardware and programs, precursors to what are today routinely employed techniques for quantitative analysis. These devices were a logical extension of the relay-switched three channel Accelerating Voltage Alternator (AVA) of Sweeley et al., the first practical device for selected ion monitoring of gas chromatographic effluents (6) which was improved on by Holland et al. (7) by interfacing the AVA to a computer. At about the same time Klein et al. (8) had designed a two channel AVA that used a multichannel analyzer for storage. The Holmes device generated a variable accelerating voltage that was calculated by the computer and added to the accelerating voltage to bring up to eight ions into focus with a fixed magnetic field. A feature of the system was a 0.5 u scan around any ion selected. This ion profile was used with PFK for source tuning and slit adjustment to establish the desired ion peak shape, resolution and maximum intensity. Since this display was available in what is now referred to foreground-background mode it was also used during data collection to insure that the analyte ion was in mass focus (i.e., was the correct mass defect chosen? had the magnetic field drifted?). This was done in real time using 0.2 seconds of the total

1 second of sampling time, with the rest of the time divided between the other ions selected, which could be eight in number. Thus the system could be monitored while data was collected and stored and the mass focus could be corrected at any time by adjusting the magnetic field. This monitoring feature was of especially great importance to us when we began to use quadrupole instruments which are subject to mass drift. The complete PDP-12 system with ion monitoring capabilities was duplicated and used successfully in 8 different locations including the Karolinska Institute.

The ion monitoring capabilities opened many doors for us as it did for many other laboratories. Since there was a strong focus at Washington University on stable isotope tracer studies in humans, and because the precision of isotope enrichment measurements with the new equipment was so great, this area literally took off.

An illustration of the power of the system was the ability to combine ion monitoring GC/MS with analyses of tissue samples obtained using techniques for dissecting and weighing single cells or extremely small and anatomically well-defined tissue sections. In collaboration with a group which practiced that art we obtained, and analyzed for myo-inositol, tissues weighing as little as 3 nanograms dry weight (9). The weighing technique used "fishpole" balances: made from a quartz fiber drawn and fixed in a horizontal position and calibrated with quinine crystals which are placed on the tip of the balance and the amount of deflection measured. The quinine is then dissolved in water and the fluorescence of the solution measured and thus the mass of the weighed material determined. The balance used had a deflection of 1 mm with 33 picograms. For analysis the entire dried and weighed tissue was immersed in 10 of trimethylsilylating reagent. When derivatization was complete the entire sample was injected onto a packed GC column for analysis. Only myo-inositol was evident using m/z 305, a three carbon fragment ion characteristic of TMS-inoitol, and m/z 318 for validation.

Another "first" for the Facility was the development of an electron ionization GC/MS method for measuring all of the then-used tricyclic antidepressants in plasma samples from patients under treatment (10). The need for the method was, and is, due to the extremely variable blood levels achieved by patients on a given dose, leading to a lack of therapeutic effectiveness in patients with high metabolic rates. Because of the fragmentation characteristics of some of the drugs, which produces mainly an ion at m/z 58, the method was criticized as impractical because drug levels are so low (25 to 300 ng/ml) and because interferences would probably interfere with the analyses. Nevertheless, the method is still in use and is still run on a quad that is shared by Facility users.

The success of the PDP-12, and especially the power of the ion monitoring method, increased the demand for instrument time as individuals recognized the potential of GC/MS. Increased demand was met by the purchase of a quadrupole instrument in 1975 and by the development of a less expensive (~\$20,000) and smaller (file-cabinet-size) data system by Bill Holmes, Phil Berger and Peter Norberg (the latter two individuals subsequently formed the Teknivent Corporation). This quad data system centered on an extremely fast, flicker-free display generator that they designed and a Computer Automation LSI-2 CPU for which mass spectrometer control and data processing programs were written (11, 12). Those programs followed the general philosophy of the PDP-12 software and added extended capabilities that resulted from the ability to digitally control all aspects of the scanning and data collection as well as processing.

The precision with which ion monitoring data could be collected was improved by the design and construction of digitally coupled wide dynamic range device consisting of an electrometer, an integrator, an autoranging amplifier (gain X1, X4, X16, X64) and an ADC optically coupled to the computer. This device, known with affection as "the Rempel", (13) allowed measurements of 0.3 - 0.5% precision to be made at 1/256 of full scale if necessary.

During 1975 and 1976 we added another EI quadrupole and two CI quads to the Facility. The "user-friendliness" of the computer and a few safeguards on the instruments made the Facility almost completely into a hands-on operation.

While the Facility had been heavily involved in stable isotope tracer studies using GC/MS to follow isotopes in amino acids and in glucose from patient blood samples there was a need for analysis of ^{15}N and ^{13}C in the metabolic end products CO_2 and urea. In 1979 Dwight Matthews joined the Facility and shortly began to build an automated isotope ratio instrument using a commercial 3 inch 60° sector magnetic mass spectrometer, a home-built gas sampling and handling apparatus and a DEC PDP 11/23-based home-built and programmed system for controlling the analytical process and handling the data. When completed, the automated IRMS was completely under computer control, sampling breath samples from blood collection tubes (Vacutainer tubes) in which samples from investigators all over the world were sent. Tubes set in a fraction collector were sampled in sequence, processed and transferred to the mass spectrometer where they were analyzed and the results automatically printed out. Since being built the system has analyzed over 10^4 samples, quantifying $^{13}\text{CO}_2$ generated from the oxidation of amino acid, lipid and carbohydrate tracers of substrate inter-organ fuel transport in man. Also capable of analyzing $^{15}\text{N}/^{14}\text{N}$ ratios by the hypobromite method, the system remains in active use today for measuring body protein nitrogen turnover using various ^{15}N -labelled amino acid tracers. Working together, Denny Bier and Dwight Matthews developed many of the stable isotope tracer techniques now regularly used throughout the biomedical field to determine amino acid kinetics and oxidation and to estimate body protein flux, synthesis and breakdown. In this regard, Dr. Matthews published two seminal papers (18,19) describing the use of $[1\text{-}^{13}\text{C}]$ leucine and $[1\text{-}^{13}\text{C},^{15}\text{N}]$ leucine to measure body protein/amino acid dynamics which are now classics in the field and widespread in their application. Using approaches developed in the resource, Drs. Bier and Matthews in collaboration with many resource users, but in particular with Dr. Vernon Young's group at MIT, have published a long series of investigations on the role of dietary protein and energy intake on amino acid and body protein kinetics in man (20-45). These studies form a significant fraction of the currently available information on essential and non-essential amino acid kinetics in the human.

When FAB came on the scene we found that either a saddle field or a capillaritron gas gun could be used with our CI GC/MS systems by directing the beam at right angles to the analyzer axis through the high pressure reagent gas exhaust of the source thus allowing us to use the instrument for GC/MS or FAB without any mechanical changes. This system served us until 1985 when the Facility received funds from the University and from the NIH Shared Instrument Program to purchase an extended mass range high resolution instrument which was acquired and installed in 1986.

Gradually, by user preference, the LKB was used less and less and ultimately was replaced, in 1987, with heavy hearts, by a thermospray quad. As the original GC/MS quads have aged they have become greater maintenance problems. Recently, through the NIH Shared Instrument Program, we were able to replace two of the originals with two new EI/CI quads. It is interesting to note that, in positive ion CI mode, the original dedicated CI systems are more sensitive, and retain that sensitivity longer, than the new quadrupole instruments.

The Facility now has 10 instruments that provide a full range of services. A measure of the usefulness of the Facility to the scientific community is that over 10,000 hours of instrument time were logged by over 100 investigators in 1988. Much of the growth, development, research and sustenance of the Facility has been supported by NIH through two grants, RR-00954 and AM-20579 as well as by University support and by grants from the NIH Shared Instrument Program.

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MASS SPECTROMETERS IN SPACE. ROCKETS TO SATELLITES TO
MARS 1960-1976

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Mass spectrometry has played an important role in our investigation of space. A first detailed picture of the chemistry in the earth's atmosphere was revealed by mass spectrometers flown on both rockets and high altitude balloons. Often these instruments were fitted with ion sources that could measure both existing ionic species and neutral atoms and molecules along the flight path. Data from these flights have provided atmospheric scientists with the insight they needed to formulate a unified explanation of the complex chemical and physical interactions of earth's atmosphere today (Nier, 1985). It was a natural extension of the investigation of the earth's atmosphere that has led to the use of mass spectrometry in our planetary probes.

The Viking mission to Mars carried two mass spectrometers on each of two spacecrafts. The aeroshell which was used to protect each spacecraft during entry into the atmosphere of the planet had an instrument in its outer surface. The sensitivity of this instrument was enhanced by the ram effect produced as a result of the high speed of the spacecraft. The instrument was of the Mattauch-Herzog geometry and had two collectors. The design was very similar to the instrument flown on the Atmospheric Explorer C, D and E (Nier, 1973; see Figure 1 for schematic drawing). This instrument had two collectors in the focal plane. One covered a mass range of m/z 1-7 and the other m/z 7-49. Before the entry vehicle encountered the more dense portion of the Martian atmosphere (above 100 km) this "neutral mass spectrometer" was able to make some important measurements of the planet's temperature and atmospheric mixing (Nier, 1977).

Using data taken from the height of 200 km down to 100 km, the atmospheric science team was able to report that: the Martian atmosphere consists mainly of CO_2 , with traces of N_2 , Ar, O_2 , CO and O. Some evidence for NO in the upper atmosphere was also observed (Nier, 1977). A more detailed study of the singly charged CO_2 peak cluster at m/z 44-46 indicated an $^{18}\text{O}/^{16}\text{O}$ isotope ratio of 0.0020 ± 0.0001 . The doubly charged CO_2 peaks gave a very similar ratio (e.g. 0.0021). Using the peak heights at m/z 12 and 13 and m/z 44 and 45, a $^{13}\text{C}/^{12}\text{C}$ isotope ratio of 0.0115 ± 0.0004 was determined. These values for carbon and oxygen are very close to values for terrestrial measurements. In contrast, the isotope ratio for $^{15}\text{N}/^{14}\text{N}$ was found to be 75% greater on Mars 0.0064 ± 0.001 . This data has been used to estimate the nitrogen content of the Martian atmosphere over the planet's geological past (McElroy, 1976).

Each of the Viking landers was fitted with a gas chromatograph/mass spectrometer (GC/MS) that had been designed to study both the soil and the atmosphere at the surface of the planet. The mass spectrometer (see Figure 2) was a Nier-Johnson configuration fitted with a permanent magnet (6326 G). The ion source was differentially pumped with selectable 45 or 70 eV energies for EI ionization. Individual mass spectra could be obtained at the rate of 1 every 10 sec by scanning the accelerating potential from 2350 to 125 volts.

FIGURE 1

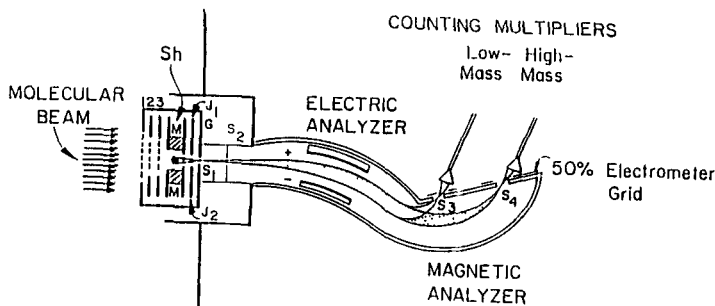
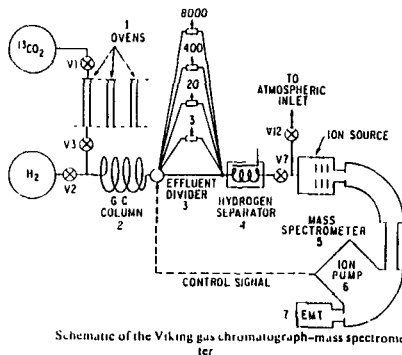


FIGURE 2



This covered a mass range of 12 to 220 amu. Vacuum was maintained by a sputter ion pump (500 ml/sec) that shared the magnetic sector magnet.

Atmospheric measurements were made through a molecular leak into the ion source. Since CO_2 was expected to be the most abundant molecular species in the atmosphere, an additional system was added to the inlet before the molecular leak. This chemical absorption unit was attached to the volume adjacent to the molecular leak through a series of valves. In one chamber, CO was oxidized to CO_2 by silver oxide and all the CO_2 was removed by reaction with LiOH . This process removed 99.98% of the CO_2 and 99% of the CO and released H_2O . In a second chamber, all atmospheric H_2O including the H_2O produced during

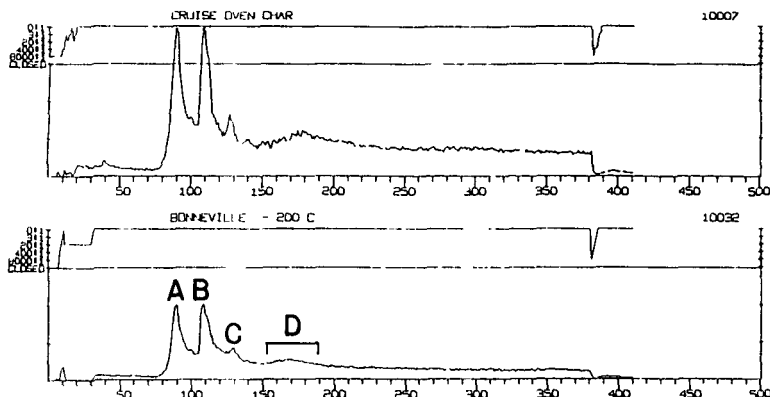
the removal of CO_2 was absorbed onto $\text{Mg}(\text{ClO}_4)_2$. One cycle of chemical absorption took 1 hour. By repeated cycles, one could "enrich" the atmosphere in the chamber with those natural constituents that were unaffected by the chemical absorption system (e.g. N_2 , Ar, Xe etc.).

The GC/MS system on each lander presented some interesting engineering problems. Not that it wasn't enough to manufacture a complete gas chromatograph/mass spectrometer and data system that would fit into one cubic foot (excluding size consideration for the soil processor) a system had to be designed that would maintain a sufficient low pressure in the mass spectrometer while sampling the pyrolysis products of a soil sample. Power and weight were also extremely limited. These requirements led to the development of a unique system capable of withstanding large overloads of H_2O and CO_2 , while detecting part per billion (ppb) levels of organic molecules released from heated soil samples. The solution to this problem was a five stage effluent divider, controlled by the mass spectrometer's ion pump current, that would open or close a parallel series of valves and their associated flow restrictors (Rushneck, 1978). A second unique feature of the GC/MS system was the use of a pressure reduction GC interface that did not require a vacuum pump. Hydrogen gas was used as a carrier gas and was removed with a heated palladium alloy separator (Dencker, 1972).

Results from the lander atmospheric experiments confirmed the isotopic ratios reported during the entry phase of the mission. Namely, the abundance of ^{18}O and ^{13}C were similar to those found on earth but ^{15}N was between 50 & 75% more abundant compared to earth's atmosphere. In addition, argon isotopes at m/z 36, 38 and 40 were detected and their ratios measured. Using the chemical absorption unit to remove CO_2 and CO and repeating the enrichment cycles 15 and 30 times, krypton and xenon were detected and their isotope ratios measured. Krypton ratios were very similar to terrestrial values but the xenon 129 to 132 ratio is enhanced (Owen, 1976). The noble gas abundances indicate that at least 10 times the present atmosphere amount of N_2 and 20 times the CO_2 abundance were released by the planet during geologic time; the outgassing of a large amount of water must also have taken place (Owen, 1977; McElroy, 1976).

Martian soil samples released only a small amount of water and carbon dioxide when heated to 350 or 500°C. The water was thought to be present in the form of mineral hydrates, from which it is released at these temperatures. The total ion chromatogram shown in Figure 3, for a complete experiment sequence carried out with an empty oven during flight to Mars, indicates a small amount of terrestrial contamination. The presence of these contaminants was a fortunate circumstance because they proved that the system operated reliably. The same signal was obtained when heating soil taken from the Martian surface (peaks A -> D are identified as acetone, methyl fluorosiloxane, benzene and C_1 to C_3 alkylbenzenes respectively). A detailed inspection of individual mass chromatograms from each soil sample at 200, 350 and 500°C from m/z 12 thru m/z 220 did not reveal additional organic constituents in these samples. The limit of detection for organic compounds in soil was in the 0.01-1 ppb range. The absence of organic molecules at these levels significantly reduced the possibility that life exists or existed on Mars (Biemann, 1977).

FIGURE 3



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POTASSIUM ION IONIZATION OF DESORBED SPECIES (K^+ IDS)-BY-FAB:
A USEFUL METHOD FOR PROBING THE SELVEDGE REGION, AND FOR
OBTAINING MOLECULAR WEIGHT INFORMATION

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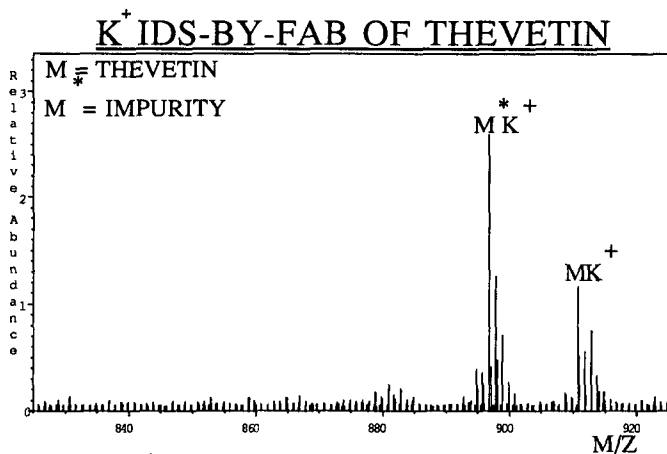
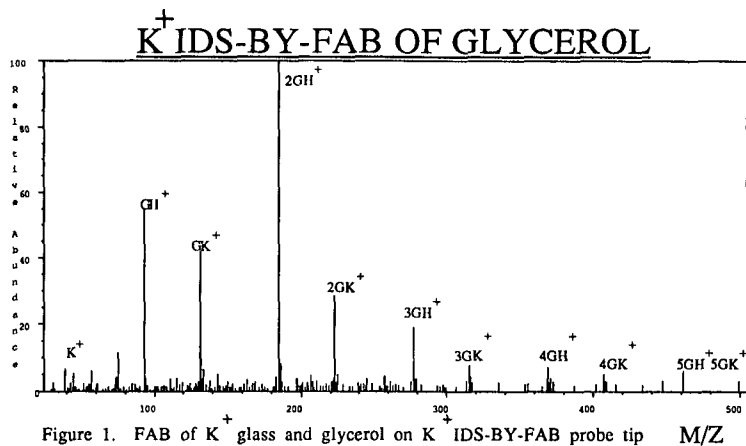
We have reported previously a desorption/ionization technique that we call K^+ IDS, K^+ ionization of Desorbed Species (1). It utilizes rapid heating, which generates intact molecules and simple thermal degradation products in the gas phase from a thermally labile/nonvolatile analyte, followed by K^+ attachment to the desorbed neutrals. The K^+ ions are formed by thermionic emission from a heated filament coated with a silico-aluminate glass containing K_2O (" K^+ -glass"). We investigate here a split FAB probe that supports the conventional glycerol matrix/analyte mixture, and K^+ glass, the two being physically separated to prohibit interaction. Upon fast atom bombardment, glycerol and analyte molecules are desorbed, presumably creating a high pressure "selvedge" region above the target. Fast atom bombardment of the glass yields copious amounts of K^+ ions, which are "injected" into the selvedge region. The goal is to use K^+ ions, which attach to neutral molecules without inducing fragmentation, to "sample" the neutrals formed in FAB. Figure 1 shows the results when glycerol and the K^+ glass are subjected to FAB. If the ions from glycerol - fragment ions and proton-bound clusters (G_nH^+) - were desorbed directly from the matrix, one might expect to see only one additional ion in this experiment at m/z 39, K^+ . However, a G_nK^+ series is observed. The similarities between the proton-bound clusters and K^+ -bound clusters suggest that they are both formed in similar processes, i.e., by a gas phase mechanism. The spectrum also suggests that FAB leads to desorption of the intact glycerol molecules, with no significant amount of neutral degradation products formed (such as $[G-H_2O]$).

In the FAB spectrum of cholic acid in a glycerol matrix, no M^+ or MH^+ ion is observed. The ion of highest m/z due to this analyte is an $[M-H_2O]H^+$ ion at m/z 391. One could envisage two possible scenarios. In the first, the cholic acid is desorbed intact, and protonation results in complete fragmentation via water loss. In the second, fast atom bombardment induces dehydration, the molecule desorbs as $[M-H_2O]$, and is then protonated. K^+ IDS-by-FAB can be used to investigate these possibilities. When K^+ ions are injected into the selvedge region, only the MK^+ ion for cholic acid is observed, indicating that the desorption of the intact molecule occurs, and protonation leads to essentially complete fragmentation. This also points out the analytical utility of this method. The formation of the MK^+ ion confirms the molecular weight, and is useful when the analyte is an unknown.

In our laboratory, the FAB mass spectra of cardiac glycosides is under study. The FAB spectrum of a commercially available sample of thevetin, $C_{42}H_{64}O_{19}$, showed an MH^+ ion at m/z 873, and an intense ion 14 amu lower. Before attempting to interpret the mass spectrum, there must be a determination of whether the spectrum was for a pure compound. the K^+ IDS-by-FAB spectrum of thevetin in glycerol is shown in Figure 2. K^+ attachment to two compounds, separated in mass by 14 amu, shows that the original sample was a mixture and the lower mass compound was the major component.

These examples, demonstrate that injection of K^+ ions into the selvedge region of a FAB experiment is useful in providing insights into the nature of FAB ionization, and has applications in molecular weight confirmation and purity analysis. The spectra are cleaner, and less complex, than if KCl were introduced into the matrix. We present this as a method which can be extended to other ions in addition to K^+ , and demonstrates the basic approach to a new FAB-based method.

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THE CHARGE STRIPPING OF ALKALINE EARTH SPECIES GENERATED BY SOLID FAB

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INTRODUCTION

The study of trends within a group is of fundamental importance in chemistry. Series of diatomic molecules composed of elements from two different families provide a simple system for studying these trends.

Under solid FAB (matrixless) conditions interesting ions can be desorbed and studied by FAB MS/MS.¹ In addition to the species reported earlier, we have observed MX^+ and MY^+ ions ($M = \text{Mg, Ca, Sr, Ba}$), ($X = \text{F, Cl, Br, I}$), and ($Y = \text{O, OH, MO}$).

The CAD/CS spectra of MX^+ reveals a trend in the relative ion intensities of M^+ and X^+ . The translational energy loss of MX^+ accompanying charge stripping yields the second ionization energy of MX , which can be seen to vary periodically.

DISCUSSION

M^+ , MX^+ , and MY^+ are among the major ions produced by solid FAB of the alkaline earth halides. Figures 1 & 2 contain the CAD/CS spectra (air as collision gas) of the CaX^+ and MBr^+ series. CaX^+ and MBr^+ represent series of diatomic species in which one of the elements is a different member of a group. For example, in the CaX^+ series the Ca is bonded to different members of the halogen family.

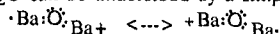
The abundance of X^+ increases monotonically with decreasing ionization potential of X. (See Figure 1.) There is an analogous trend for the abundance of M^+ . (See Figure 2.) Also, the abundance of M^{++} increases as the electron affinity of the halogen to which it is associated in MX^+ increases.

The MX^{++} or MY^{++} formed by charge stripping is a prominent ion in the CAD/CS MIKES spectrum of MX^+ and MY^+ . By measuring the translational energy loss of these ions upon charge stripping, one can obtain the energy required to remove an e^- from MX^+ or MY^+ , the second ionization energy (IP2) of MX or MY . These values, along with the experimental second IP's of M are listed in Figures 3 & 4. The alkaline earth metals can be viewed as standards that validate the experimental procedure since their second IP's are well known.

For a given metal bonded to a different halogen, IP2 increases as the halogen becomes more electronegative. Conversely, for a given halogen bonded to a different metal, IP2 decreases as one proceeds down the alkaline earth metal group. The values for MOH^+ parallel the trend for MX^+ .

These trends can be understood by considering a molecular orbital approach. As the separate M^+ and X (or Y) are brought together from infinity, a bond is formed. Using CaX^+ as an example, the energy of the HOMO formed upon bonding with a halogen lower in that family is expected to be higher. The valence electrons of a lower member in the halogen family are higher in energy than a previous member. Also the orbital overlap can be expected to decrease as the halogen atomic number increases. The lowest IP2 would be expected for BaI and the greatest for MgF , as is observed.

The low IP2 for M_2O can be understood by a simple picture of the electronic structure:



The e^- on the uncharged metal resides in a nonbonding, slightly antibonding, σ orbital. This view has been presented in the discussion of the bonding in MX .² Indeed, the first ionization of, for example, $BaCl$ is analogous to the second ionization of Ba_2O^+ , each involving the removal of an e^- from a nonbonding orbital located on Ba. The energy required to remove an e^- is relatively low: 5.0 eV for $BaCl_2^+$ and ca. 9 eV for Ba_2O^+ .

The closed shell MX^+ species are isoelectronic with neutral alkali halides and have high ionization energies.

EXPERIMENTAL

Experiments were carried out on a Kratos MS50 TA (EBE) equipped with a Kratos FAB source operated at 8 KV and an Ion Tech FAB gun. Aqueous solutions of MX_2 were produced on the probe tip by adding $M(OH)_2$ to a drop of diluted HX . This solution was dried in vacuo.

The MS/MS spectra are CAD MIKES spectra obtained by scanning ESA2. Air was admitted to the second collision cell, until the main beam intensity was reduced by ca. 20%. The use of air or O_2 as the collision gas increases the abundance of the MX^{++} charge stripped product ion.

The data in Figures 3 & 4 were obtained by measuring the translational energy loss upon CS of the parent ion. The experiment is performed by focusing the parent on the detector with ESA2 slaved to ESA1. Using a MID built in this lab, ESA2 is unlinked from ESA1 and scanned in peak switch fashion. The voltage to ESA2 can be precisely divided, and variable width proportional energy scans were made over both the parent and charge stripped ions. For CS, the ESA voltage that transmits the parent ion is reduced by 1/2, because the product ion is 1/2 the energy to charge ratio-both being nominally 8 KV ions. The data are collected and signal averaged on a Nicolet 1170 ADC and transferred to a Data General Nova 4X computer for processing.³

The peaks are fit by Gaussians and the position of the center and the width of the Gaussian used to obtain the intercept of a line tangent to the curve with the abscissa. The tangents on the high energy side of the peaks yield a minimum or onset value for the energy required to remove an e^- from the parent ion.

ACKNOWLEDGMENTS

We are grateful to Enrico Davoli* for stimulating discussions about solid FAB. In addition, appreciation is extended to Darrell Giblin of the Midwest Center for Mass Spectrometry for pointing out that the intercept of the tangent line to the Gaussian peak could be used for consistent, unbiased treatment of the data, and for helpful discussions.

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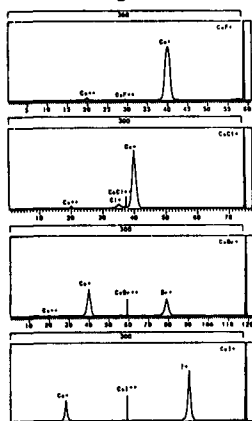


Figure 1. The air CAD/CS spectra of the CaX^+ series

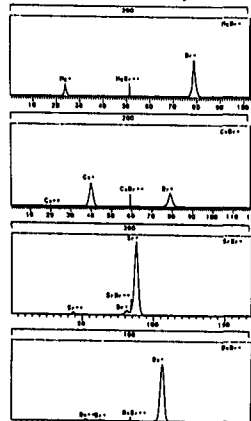


Figure 2. The air CAD/CS spectra of the MBr^+ series

SECOND IONIZATION POTENTIALS OF THE
ALKALINE EARTH METALS (eV)

	stable work	others ^a	spectroscopic
Mg	15.2	*	15.83
Ca	11.4	11.6	11.87
Sr	10.9	11.1	11.83
Ba	10.5	10.3	10.80

a. Hildenbrand, M., Aue, T., Hergen, J., *International Journal of Mass Spectrometry and Ion Processes*, 1984, 61, 21
* Mg must be obtained after calibration

Figure 3.

SECOND IONIZATION POTENTIALS OF SOME
ALKALINE EARTH SPECIES (eV)

	F	Cl	Br	I	O	OH	MO
Mg	16.9	16.3	15.9			16.0	
Ca	15.4	14.7	14.8	14.0	13.4	14.9	
Sr	15.5	14.4	14.0	13.2		14.7	8.6
Ba	14.7	14.5	13.7	13.1		14.5	8.8

Figure 4.

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ON PREFORMED IONS AND ION-MOLECULE REACTIONS IN FAB
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INTRODUCTION

The ionization processes in Fast Atom Bombardment are not well understood. Two major mechanisms have been considered - ion/molecule reactions and precursor ion formation.

When a FAB mass spectrum is very different from the ionic composition in the matrix prior to the impact of the fast atom, ion molecule reactions (IMR) must occur during the desorption event. Several observations support that IMR's are indeed important in FAB. The observed preference for analytes with higher gas-phase basicities (GB) is quite consistent and kinetic models based on IMR has been constructed that well reproduces the FAB spectra of selected systems¹.

When the DI spectrum reflects (changes in) the ionic composition of the liquid matrix prior to the impact of the fast atom, it is reasonable to assume that desorption of the solvated ions is an efficient process. Several experimental observations support such precursor ion formation. For example, it is often found that analyte signals increase substantially when acids are added to the matrix - "the acid effect". The object of the present work was to investigate the reasons for this "acid effect".

RESULTS

It has been found that the "acid-effect" has several different explanations: A) Acids frequently improve the solvation of analytes. Thus, Figure 1 illustrates the dramatically improved solvation of adenosine in glycerol as HCl is added.

B) Protonation of the analyte frequently inhibit the destruction of the analyte by radiation induced chemistry. This is illustrated in Figure 2, which shows the effect for diethanolamine in glycerol as a function of matrix viscosity (temperature). This effect is expected to be particularly important for larger molecules with many reactive sites.

C) Protonation of a relatively volatile (non-polar) analyte increases analyte signal by suppressing the selective evaporation of the analyte. An example is tetramethylethylenediamine.

D) Protonation of the analyte usually effects the desorption energetics, such that the extent of fragmentation decreases. The effect is very common but small.

E) Ionization of an analyte effects its surface activity. Thus, when two analytes are present, the suppression of one by the other may be overcome, leading to increased analyte signals.

F) We have not found strong evidence for efficient precursor ion formation for the systems that we have studied with the exception of very high viscosity matrices. This is illustrated in Figure 3, which shows that analyte to matrix ion ratios for DEA glycerol solutions increases strongly above 10,000 cP.

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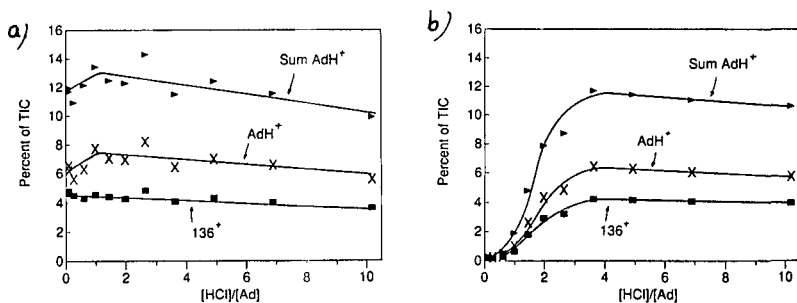


Figure 1. Effect of addition of conc. HCl to a) a 1.7 mol% supersaturated solution of adenosine in glycerol and b) a suspension of adenosine in a saturated glycerol solution with the same total amount of adenosine; Ad=adenosine; 130⁺ is the major fragment ion.

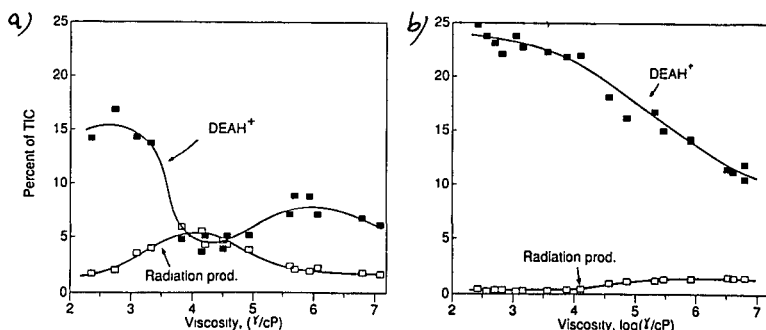
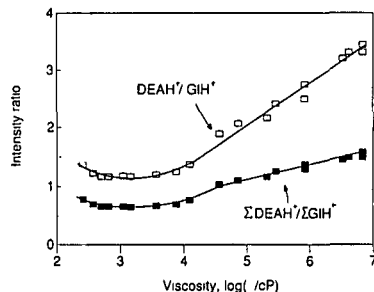


Figure 2. Intensity of DEAH⁺ and of "radiation products" in FAB spectra of a) 2 mol% DEA in glycerol and b) 2 mol% DEAHCl in glycerol as a function of matrix viscosity; the viscosity was changed by varying the matrix temperature; m/e=118, 130, 148 and 160 are included in "radiation products".

Figure 3. Analyte to matrix ion ratios in FAB spectra of 2 mol% DEAHCl in glycerol as a function of matrix viscosity, changed by varying the matrix temperature.



MITIGATION OF BEAM-INDUCED DAMAGE IN DESORPTION IONIZATION MASS SPECTROMETRY:

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Optimally, desorption ionization generates molecular or quasi-molecule ions as well as structurally significant fragments. Beam-induced reactions can, however, alter the structure of the analyte, resulting in the appearance of ions not directly related to the undamaged analyte. For example, FAB beam-induced reduction frequently results in the addition of one or more electron(s) and/or hydrogen atom(s). As a result, ions at $[M+1]$ and $[M+2]$ can have intensities greater than would be expected based upon isotopic abundances. Primary beam flux (1-2) and energy (3-4), and matrix composition (5-6), all have been shown to affect the appearance of desorption spectra. This study assesses the relative importance of these factors in controlling damage, and considers how the parameters interact with each other.

To assess the extent of beam damage, FAB spectra were obtained for a series of organic dyes in glycerol under standard conditions (8 kV Xe primary atom, emission current (i_e) = 1 mA). In all such spectra (e.g., methylene blue in Figure 1), the abundance of ions at $M+1$ and $M+2$ clearly exceeds expected isotopic contributions. Electrohydrodynamic (EH) MS confirmed that the samples were not contaminated with reduced species, since the predicted isotopic ratios were reproduced to within experimental error ($\pm 5\%$) (EH spectra are free of beam-induced reduction artifacts).

In order to assess the dependence of beam damage on the primary atom flux (i_p), it was necessary to establish the relationship between the measurable emission current (i_e) and i_p . Because secondary ion yields are known to be linearly dependent upon i_p , the observed linear dependence of Cs^+ intensity on i_p at various beam energies confirmed that i_e is a reasonable measure of i_p .

The most notable change in the dye spectra with increasing flux was an increase in absolute signal. This more than offset the rise in noise (RSD of signal) so that better signal/noise (S/N) could be obtained at high flux. More importantly, the signal/background (S/B) and signal/matrix (S/M) ratios decreased with increasing flux, so that better limits of detection (LOD) should be obtained at low flux.

Two measures of beam damage were considered: the extent of reduction ($X = \{\text{measured intensity-isotopic contribution}\}/\text{isotopic contribution}\}$) and extent of fragmentation ($F = E_{\text{fragment ions}}/E_{\text{molecular ions}}\}$). Both were smallest at low emission current (e.g., data for methylene blue in Figure 2). Reduction damage was more sensitive to changes in i_p than was fragmentation.

In contrast with the flux data, noise increased faster than signal as primary atom energy increased, so that better S/N was obtained at lower energies. Better S/B, S/M and LOD were also obtained at lower energies. Beam damage, however, was least at higher energies, despite the higher background (e.g., data for methylene blue in Figure 3). Plots like Figure 4 demonstrate that flux and energy interact. However, S/B and X were more sensitive to changes in emission current than energy, suggesting that i_p is more important in controlling damage and sensitivity.

Another factor that affects beam-induced reactions is choice of matrix. Thioglycerol (T) and m-nitrobenzyl alcohol (M) are better radical scavengers than glycerol (G). The effectiveness of these matrices in controlling damage is $M > T > G$ (e.g., data for methylene blue in Figure 5). Comparing the data for methylene blue in the three matrices, the importance of the parameters in controlling damage is matrix $>$ flux $>$ energy.

Finally, the nonlinearity of a plot of $\ln ([M+H]^+/M^+)$ vs E^+ for the dyes suggests that simple thermodynamic (Nernst) behavior is not the only factor involved in determining the relative extent of dye reduction (Figure 6). There must be some degree of kinetic control of the reactions involved.

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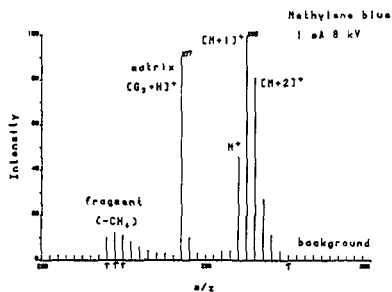


Fig. 1 FAB of Methylene Blue

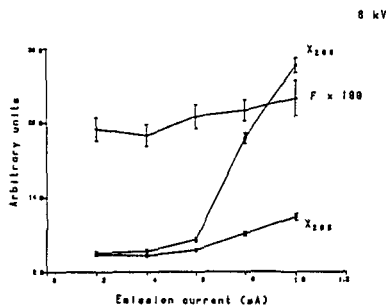


Fig. 2 Flux Dependence: Reduction (X) and Fragmentation (F)

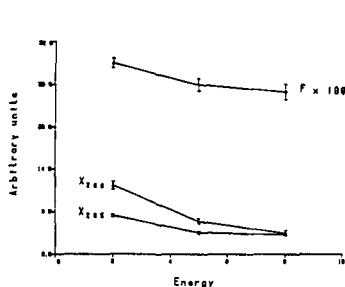


Fig. 3 Energy Dependence: Reduction (X) and Fragmentation (F)

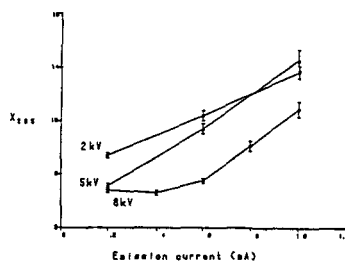


Fig. 4 Flux Dependence of Damage at Various Beam Energies

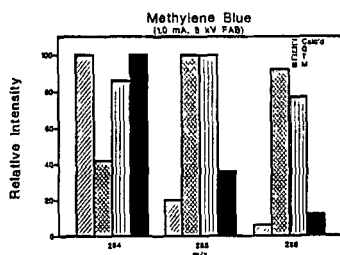


Fig. 5 Reduction in Glycerol (G); Thio-glycerol (T); m-nitrobenzyl alcohol (M)

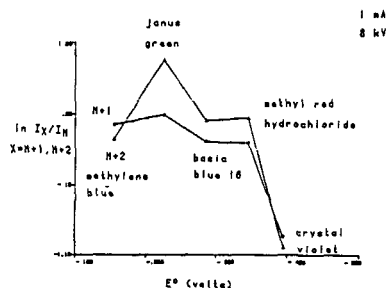


Fig. 6 Dependence of Reduction on Dye E°

EFFECT OF SORBITOL CRITICAL BEHAVIOR ON FAB TOTAL ION CURRENT

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The temperature dependence of secondary ion intensity was studied for a sorbitol matrix. The FAB total ion current of the supercooled melt of sorbitol decreased dramatically with increasing temperature (fig. 1). This drop in total ion current begins at 55° C.

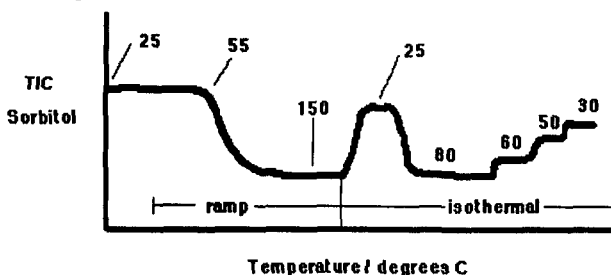


Figure 1. Total ion current (m/z 70-300) of sorbitol as a function of target temperature.

The rapid decrease in total ion current correlates with a phase change. The supercooled melt of sorbitol undergoes an endothermic change at 55° C when analyzed by differential scanning calorimetry (DSC).¹ Rheologically, this transition has been described as softening of the supercooled sorbitol melt with increasing temperature. The effect of this transition on FAB total ion current may be a function of the increased heat capacity of the softened sorbitol melt. The increase in internal degrees of freedom generally associated with increased heat capacity allows for transfer of the incident atom energy to modes which do not give rise to secondary ion formation and ejection. The supercooled melt of sorbitol is a glass forming system. Glass forming compounds are characterized by their reluctance to crystallize once they have been melted. Its choice as a FAB matrix lies in the fact that exposure to high vacuum and elevated temperatures does not lead to recrystallization and loss of fluidity.² The supercooled melt was formed by extensively heating a supersaturated sorbitol solution. Water was removed by further heating until a brown caramel was formed. Upon cooling to room temperature spontaneous crystallization does not occur and a solid is formed. This solid can be described essentially as sorbitol hydrate, however the actual water content is unknown. True sorbitol glass is formed by melting crystalline sorbitol until an opaque phase clears and then cooling to room temperature. The term supercooled melt used here refers to melted sorbitol which has been cooled to a temperature which lies between its glass transition temperature ($T_g = -80^\circ\text{C}$) and the melting temperature of pure crystalline sorbitol. The supercooled melt of sorbitol used in this study does not recrystallize under any of the experimental conditions.

In our hands saturated solutions of sorbitol invariably recrystallize under high vacuum in the ion source at 80° C. When a saturated sorbitol solution is used as the supporting matrix for an analyte, secondary ion emission for that analyte is lost upon crystallization. Ackerman et. al.³ have studied a variety of analytes by FAB using saturated sugar solutions. The secondary ion emission for each compound studied was very temperature dependent. Each had a distinct temperature at which secondary ion emission was maximized. This temperature is known as the BMT (best matrix temperature). The BMT ranged between 80 and 100° C for all compounds studied. When the temperature rose past this range secondary ion emission for analyte return to baseline values. This effect was ascribed to depletion of water. They propose that analyte partitions into water. As water ebulliates from the matrix analyte follows, thus presenting analyte on the surface to incident atoms. Therefore, when water is depleted this

mechanism for analyte transport would be gone. However, the loss of analyte ion emission could be due to crystallization of the sugar from the saturated solution.

Maintenance of matrix fluidity may be a key factor in sustained secondary ion emission in FAB. Fluids, as opposed to solids, can absorb incident atom energy in a way which prevents sample damage. Also, if transport of analyte is required, adequate mobility is necessary. Certainly crystallization of the supporting matrix would impede these mechanisms.

The secondary ion emission of leu-enkephalin and tetramethylammonium hydroxide in supercooled sorbitol melt was studied as a function of temperature. These samples were prepared by adding aqueous solutions to the sorbitol melt on the target which was maintained at 100° C. Excess water was removed with a heat gun. Once in the source compartment a conditioning temperature was used to remove water. The ramp was slow to avoid excessive sample loss due to splashing and held at 150° C for 2 minutes. No visible signs of water ebullition were evident. The temperature could be lowered or raised without any sign of crystallization. After conditioning the sample was run on a Finnigan-MAT TSQ-70 using the liquid cooled solids probe to apply heat to the copper FAB target. The Ion Tech saddle field gun was operated at a potential of 7 KeV and 1mA emission current with xenon gas. Various temperature ramps were used as well as selected isothermal settings.

The effect of temperature on leu-enkephalin secondary ion emission is very similar to that observed for various analytes in saturated sugar solutions. In general, total ion current for sugar ions decreases with increasing temperature and ions due to analyte go through a maximum at relatively high temperature. As seen for the supercooled sorbitol (neat), a sharp decrease in total ion current for sorbitol ions occurs at 55° C. The maximum yield of leu-enkephalin pseudo-molecular ions occurs at 100° C.

The behavior of TMAOH is much different. At 55° C a sharp increase in molecular ion yield is seen. This increase occurs when the temperature is increased or decreased past the ion current-temperature maximum. Interestingly, the supercooled melt at 55° C is essentially solid. There probably is not a discreet decrease in viscosity decrease since supercooled sorbitol melts follow Vogel-Tammann-Fulcher behavior (viscosity vs. temperature is logarithmic with no discontinuities). Therefore the overall mobility of TMAOH in supercooled sorbitol does not change significantly i.e. transport does not play a role in this increase. This increase must be associated with the phase change. The TMA⁺ ion emission does not increase as temperature is raised to 150° C where fluidity has been increased dramatically. Thermal decomposition could not be significant since the maximum occurs again as the sample is cooled to ambient.

The secondary ion emission of TMAOH as a function of temperature indicates that mobility of TMAOH in supercooled sorbitol is not an important factor in its desorption. Also, Ligon⁴ has shown that mobility cannot account for transport of analyte to the surface of glycerol within the time frame of the FAB experiment. Further, the flux of the beam is not high enough to mix the sample, at least on the molecular scale. If a substance is to be sputtered at all it must be in or near the surface system prior to the FAB experiment. For hydrophobic compounds a surface excess is most likely obtained before the FAB experiment.

It should be noted that supercooled sorbitol is considered to be a fluid. Melted sorbitol must be supercooled below its glass transition temperature before it acquires thermodynamic properties of a solid (crystal). Even as a glass, sorbitol retains the structural characteristics of a fluid (i.e. the liquid structure is frozen in.). It has been noted that incident atoms or ions at high flux decompose solids. Supercooled sorbitol at room temperature is solid, but on the molecular scale it behaves much more like a fluid. This may be why the incident atoms energy can be transferred to internal degrees of freedom which give rise to ion formation rather than decomposition.

¹Sztatisz, J., Gal, S., and Pungor, E. *Proceedings of the First European Symposium on Thermal Analysis [ESTA 1]*, David Dollimore, ed., Heyden, New York, NY., 1976; pg. 205

²Jayasihulu, K., Day, R. A., Metral, C. J., Wermeling, J., and Madis, M. E. (1985) 510, *Proc. 35th ASMS Conf.*

Metral, C. J. and Day, R. A., *Anal. Letters* **19**: 217 (1986)

³Ackerman, B. L., Watson, J. T., and Holland, J.F. *Anal. Chem.* **57**: 2656 (1985)

Ackerman, B. L., Watson, J. T., and Holland, J.F. (1985) 968, *Proc. 35th ASMS Conf.*

⁴Ligon, W. V. *Int. J. Mass Spectrom. Ion Proc.* **55**: 1157 (1983)

EFFECTS OF SAMPLE PROBE SURFACE TEMPERATURE ON THE DETECTION OF PEPTIDES BY FAB/MS

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A thermoelectrically cooled sample probe, which was developed in our laboratory and allows reliable reproduction of desired probe surface temperatures (1), was used to investigate its effects on the detection of peptides by FAB/MS. Renin substrate, angiotensin I, and analogs thereof suppress the ionization of ACTH (1-10) when the latter is mixed with one or more of the former peptides in a 1:1 (or 2:1) ratio and the FAB mass spectra are recorded at the probe surface temperatures at which a mass spectrometer ion source usually operates. Upon cooling the probe surface, the total ion current decreases but the ionization suppression is removed (Figure 1). Suppression of the ionization of components in peptide mixtures from enzyme digests of proteins may often be removed in this manner, allowing for more complete detection of the various components of such mixtures (Figure 2). Because such suppression effects are not always removed by cooling the probe surface (Figure 3), FAB mass spectra should be recorded at probe surface temperatures in the range 4°C-10°C in addition to those recorded at higher temperatures in order to achieve more complete detection of all components of peptide mixtures.

- (1) Martin, S.A., Hill, J.A., and Biemann, K., *Proceedings of the 36th ASMS Conference on Mass Spectrometry and Allied Topics*, 795-796.

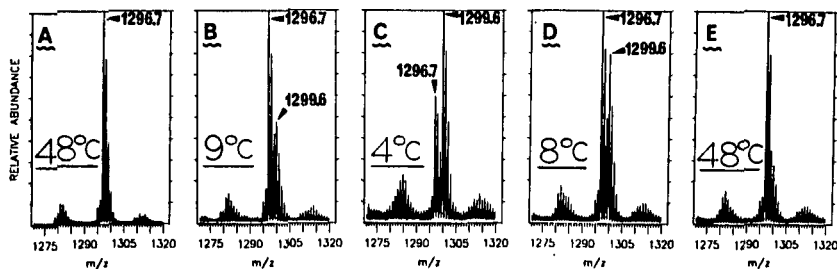


Figure 1. The protonated peptide molecule region of an equimolar mixture of angiotensin I and ACTH (1-10). The protonated angiotensin I molecule ($m/z=1296.7$) totally suppresses the ionization of ACTH (1-10) at higher temperature (A). As the probe surface temperature is lowered, the protonated ACTH (1-10) molecule ($m/z=1299.6$) begins to appear (B), until its intensity becomes greater than that of angiotensin I (C). Reduction of cooling begins to reverse the process (D) until, at the initial probe surface temperature, the ionization of the protonated ACTH (1-10) molecule is suppressed by angiotensin I (E). The sample was dissolved in a 1:1 mixture of glycerol and thioglycerol.

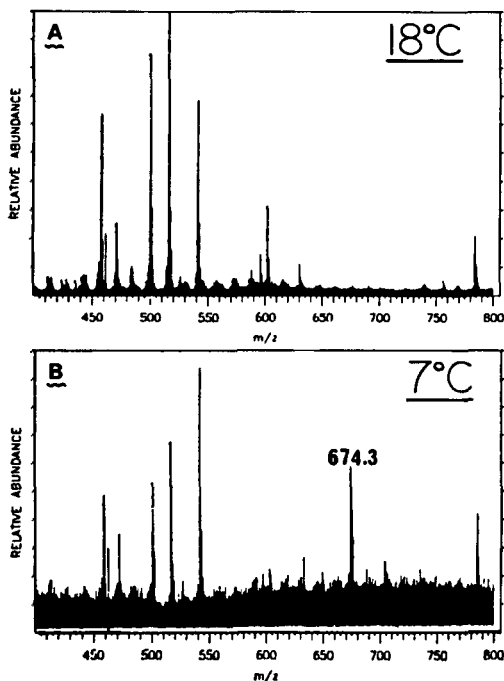


Figure 2. Partial FAB mass spectra (in glycerol) of an HPLC fraction from a chymotryptic digest of calf thymus glutaredoxin. By lowering the probe surface temperature (B) the signal-to-noise ratio decreases and two ions *ca.* $m/z=600$ disappear; however, the protonated peptide molecule at $m/z=674.3$, which is not present in the FAB mass spectrum acquired at higher temperature (A), is detected.

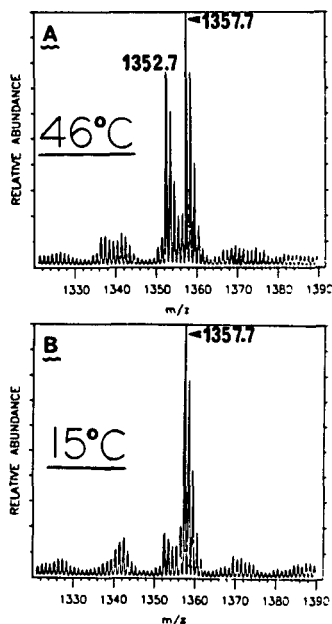


Figure 3. The protonated peptide molecule region of two peptides from a tryptic digest of glucagon. Both are easily detected at higher probe surface temperature (A), in contrast to a lower temperature, at which the ionization of the protonated peptide molecule at $m/z=1352.7$ is almost totally suppressed by the protonated peptide molecule at $m/z=1357.7$.

The Use of Non-Ionic Surfactants as Internal Mass Standards
for Exact Mass Measurements in FAB Mass Spectrometry

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Techniques reported for exact mass measurements in FAB mass spectrometry have included the successive insertion of a reference and a sample and the use of a rotatable or wobble probe with split targets for sample and reference. Because the sample and reference materials are analyzed separately the mass measurement accuracy is not optimal. More accurate mass measurements are obtained when the reference material is used as an internal mass standard when mixed together with the sample. Exact mass measurements in FAB mass spectrometry utilizing PEG's (polyethylene glycols) [1,2] and PPG's (polypropylene glycols) [3] as standards have been demonstrated for manual peak matching and for computerized exact mass measurements. The PEG's and PPG's produce reference peaks 44 and 58 daltons apart, respectively.

In our hands, the PEG's are useful up to ~700 daltons in either MB ("magic bullet", dithiotreitol/dithioerythritol 4/1) or MNBA (meta-nitrobenzyl alcohol) matrices for producing $[M+H]^+$ and/or $[M+Metal]^+$ ions, at resolutions >6,000. Above ~700 daltons, the ion abundances of the PEG's are low. To remedy this situation, PPG's were proposed as high mass standards [3]. PPG's work best with MNBA as a matrix but tend to suppress the sample intensity. Also, the reference peaks are further apart than the PEG's by 14 daltons, increasing the likelihood for errors in the exact mass measurements.

The low mass oligomeric mixtures of PEG's up to ~700 daltons serve as excellent internal calibration standards. When these mixtures are reacted with alkyl phenols (with masses from ~200 - 350 daltons), mono-substituted PEG surfactants are produced which are useful as internal mass standards up to ~1400 daltons. The structures of these surfactants are illustrated and their useful ranges are listed in Table. (These non-ionic surfactants are available from GAF Corp., Wayne, NJ.) These surfactants are most sensitive with MB as the matrix and most useful adulterated with KCl to produce $[M+K]^+$ ions. When neat or mixed with MB, no $[M+H]^+$ ions are produced. The relative sensitivities of the metal-adduct surfactant molecular ions (for the most abundant metal isotopes) are: $[M+Rb]/[M+K]/[M+Na]/[M+Cs]/[M+Ag]/[M+NH_4]/[M+Li] = 12.0/10.0/6.7/6.0/1.3/1.1/1.0$.

When oligomeric fractions of the alkylphenoxy PEG derivatives were isolated by HPLC, the mass range of the internal standards was extended to ~2,000 daltons. (HPLC conditions: Zorbax CN column, Heptane/50% Isopropanol-50% 2-Methoxy Ethanol 80%/20% -> 20%/80%, 2.0 ml/min.) Figure 1 illustrates the FAB mass spectrum of an isolated HPLC cut and the original surfactant mixture as $[M+K]^+$ ions. The cuts of higher mass oligomers tend to have two oligomeric components. For computerized peak matching two reference peaks are necessary. Therefore, sets of lower mass cuts have to be mixed while the high mass fractions can be directly used. The advantages of the isolated surfactants versus the surfactant mixtures for exact mass measurements are: 1- Less background interference from other surfactant components, 2- The sample signal can be optimized by minimizing the amount of surfactant necessary for the reference signal. Typically, sample sensitivity enhancements of 2 to 10 times are observed in the isolated surfactant because excess surfactant in the surfactant mixture suppresses the sample signal, and, 3- By reducing the amount of surfactant needed for the measurement, the likelihood of source contamination is reduced. Examples of computerized peak matching using isolated surfactant fractions for the 1,300 to 2,000 dalton mass range at 10,000 resolution are listed in Table II and one example is illustrated in Figure 2.

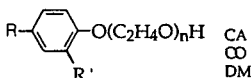
[1] J.M. Gilliam, P.W. Landis, J.L. Occolowitz Anal. Chem. 56, 2285 (1984).

[2] L.J. Goad, C.M. Prescott, M.E. Rose Org. Mass Spectrom. 19, 101 (1984).

[3] K.L. Rinehart, Jr. in Mass Spectrometry in the Health and Life Sciences, Eds. A.L. Burlingame, N. Castagnoli, Jr., pp. 119 - 148, Elsevier, Amsterdam, 1985.

NON-IONIC SURFACTANT STRUCTURES

PEG $\text{HO}(\text{C}_2\text{H}_4\text{O})_n\text{H}$
PPG $\text{HO}(\text{C}_3\text{H}_6\text{O})_n\text{H}$
P-30 $\text{C}_{16}\text{H}_{33}\text{O}(\text{C}_3\text{H}_6\text{O})_n\text{H}$



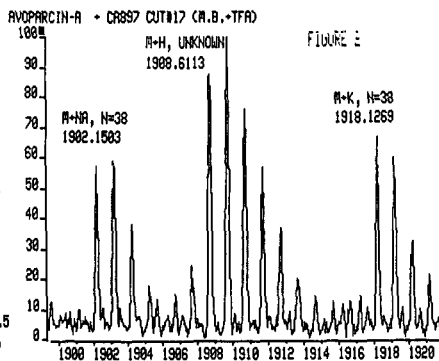
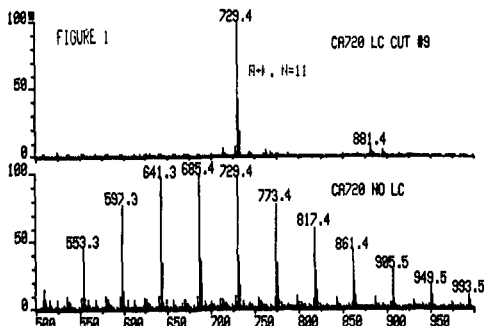
R C_8H_{17} -
 C_9H_{19} -
 C_9H_{19} -
R' H-
H-
 C_9H_{19} -

TABLE I
USEFUL SURFACTANT MASS RANGES FOR
EXACT MASS FAB MEASUREMENTS

Surfactant (n value)	Useful Mass Range (daltons)
PEG 200 - 600	200 - 700
PPG 1750	1566 - 2262
PPG 2000	1624 - 2321
CA 520 (5)	377 - 597
CA 720 (12.5)	597 - 905
CA 887 (30)	1043 - 1573
CA 897 (40)	~ 1100 - ~ 2500
CO 850 (20)	699 - 1007
CO 887 (30)	~ 1000 - ~ 1700
CO 897 (40)	~ 1100 - ~ 2500
DM 430 (7)	516 - 869
DM 710 (15)	605 - 1011
DM 730 (24)	737 - 1221
P-30 (30)	1209 - 1907

TABLE II
TYPICAL HIGH MASS COMPUTERIZED PEAK MATCHING RESULTS OBTAINED
UTILIZING ISOLATED SURFACTANT OLIGOMERS AS INTERNAL REFERENCE
MASS STANDARDS IN FAB MASS SPECTROMETRY

Reference Compound/ FAB Matrix	Low Mass Reference (Oligomer)	High Mass Reference (Oligomer)	Experimental Mass of "Unknown" (Oligomer)	Δ (amu)	"Unknown" Elemental (M + H or M + K)	"Unknown" Compound Name
CA 887, Cut #15/ MS + KCl	1345 7861 (n = 25 + K)	1389 8124 (n = 26 + K)	1348 5945 (M + K)	-0.1	$\text{C}_{26}\text{H}_{52}\text{N}_2\text{O}_{12}\text{K}$	Decapeptyl
CA 887, Cut #16/ MS + KCl	1433 8386 (n = 27 + K)	1434 8419 (n = 27["C"] + K)	1434 2310 (M + K)	-1.9	$\text{C}_{26}\text{H}_{52}\text{N}_2\text{O}_{12}\text{S}_2\text{K}$	N Acetyl Callichemichin B ₁
CA 887, Cuts #17 (n = 28) and #18 (n = 29)/ MS + KCl	1477 8648 (n = 28 + K)	1521 8910 (n = 29 + K)	1485 3922 (M + K)	-1.7	$\text{C}_{26}\text{H}_{52}\text{Cl}_2\text{N}_2\text{O}_{12}\text{K}$	Vancomycin
CA 897 Cut #17/ MS + TFA (1 μl)	1902 1503 (n = 38 + Na)	1918 1269 (n = 38 + K)	1908 6133 (M + H)	-6.1	$\text{C}_{38}\text{H}_{76}\text{Cl}_2\text{N}_2\text{O}_{12}$	Avoparcin α



LIQUID CHROMATOGRAPHY/HIGH PRESSURE FAST-ATOM BOMBARDMENT MASS SPECTROMETRY: CHEMICAL IONIZATION OF SPUTTERED SPECIES

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INTRODUCTION: Fast-atom bombardment (FAB) and high-pressure fast-atom bombardment (HPFAB) are soft ionization techniques that can produce spectra with abundant molecular ions but little structural information. Liquid chromatography/high-pressure fast-atom bombardment (LC/HPFAB) uses the recently developed liquid chromatography/fast-atom bombardment (LC/FAB) technique under HPFAB conditions. The HPFAB technique has been shown to enhance the abundance of molecular ions compared to that observed in conventional FAB ionization by collisionally stabilizing the molecular ions and ionizing the sputtered neutral species.¹

EXPERIMENTAL: A hybrid tandem mass spectrometer (VG 70-SEQ) with an EBQQ geometry (5 keV Xe atoms) was used for these studies. A continuous flow FAB probe was built for use in the LC/FAB and LC/HPFAB studies. The liquid was introduced into the mass spectrometer at a flow rate of approximately 6 $\mu\text{L}/\text{min}$ through a 75 μm i.d. fused silica capillary column contained within this probe. The FAB source was modified in three ways for this study. The first modification was to reduce the ion volume of the source block to increase the ion extraction efficiency. The second modification was the use of a movable slit to change the size of the ion exit aperture. The small aperture allows us to conduct HPFAB studies while a large aperture was used for FAB studies. The third modification was the addition of an ionizing filament to the source block. Polyethylene glycol (PEG) was used to analyze the effects of high pressures, matrices, buffers, and reduced ionization chamber size over a wide mass range. The PEG was made of equimolar concentrations of average molecular weights of 200, 300 and 600 in solution. The matrices were varied between 10% and 30% glycerol and 5% and 20% ethylene glycol to obtain the best spectra and the greatest pressure stability in the mass spectrometer. Methane and isobutane were used in the LC/HPFAB studies. During the buffer studies, 0.1M ammonium acetate was added to the mobile phase.

RESULTS: The effect of reducing the ion chamber size on ion abundance was investigated. Pressures in the source housing were more stable (i.e. less ion gauge fluctuation) using the reduced ion volume source block or closed source. The spectrum of positive ion abundances using the reduced ion volume source shows the same molecular ions but at less abundances than with the open source block. The reduction in abundances is approximately 1 order of magnitude.

Fast-atom bombardment mass spectrometry uses a larger ion exit aperture than HPFAB. This larger aperture creates a lower pressure within the ionization chamber. In HPFAB, the sample is enclosed in a high-pressure region. The reagent gas, added to the ionization chamber, can react with the sputtered molecules via ion/molecule reactions to enhance the ion abundances.

In LC/HPFAB, the solvent can serve as the reagent gas. We have investigated the use of an LC mobile phase as a reagent gas in LC/HPFAB. Research has shown HPFAB enhances the molecular ion abundances of neat polar compounds of MW 500u by three orders of magnitude compared to FAB.¹ The abundances of molecular ions of carbonyl-containing organic compounds has been shown to increase with the use of water as a CI reagent gas.² Figure 1 is the spectrum of PEG in 20% ethylene glycol/80% water using LC/FAB. Figure 2 shows the spectrum of the same solution using LC/HPFAB in which the mobile phase functions as the reagent gas. Figure 2 shows an increase in ion abundances of greater than two orders of magnitude compared to Fig. 1. These increased abundances are a result of ion/molecule reactions including proton-transfer occurring in the source.

Figure 3 shows the spectrum of PEG in 20% ethylene glycol/80% water with approximately 0.1 M ammonium acetate. Both the protonated molecule and the ammonium adduct ion ($[\text{M} + \text{NH}_4]^+$) can be observed. However, the ammonium adduct ion is in lesser abundance than the protonated molecule. This is in direct contrast to the abundances observed in thermospray mass spectrometry for PEG with approximately 0.1 M ammonium acetate added to the mobile phase.

CONCLUSIONS: LC/HPFAB has been shown to increase the abundances of protonated PEG over LC/FAB. This increase is due to ion/molecule reactions occurring within the source. Since the mobile phase in LC analyses usually contain water, the ability to use water as a buffer/reagent gas in LC/HPFAB is ideal. Water as a CI reagent gas increases the abundance of protonated molecules by 100-200 fold. Preliminary results show ammonium acetate, added to the mobile phase, produces ammonium adduct ions but in lower abundance than protonated molecules.

ACKNOWLEDGEMENTS: The National Aeronautics and Space Administration/Johnson Space Center provided financial support for this research.

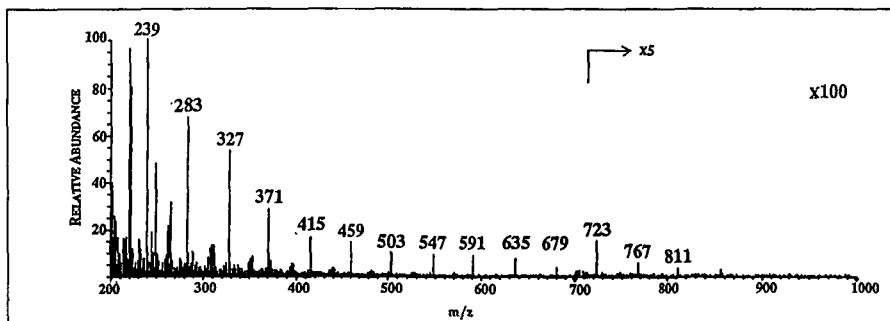


Figure 1: Spectrum of PEG using LC/FAB (no ionizing filament)

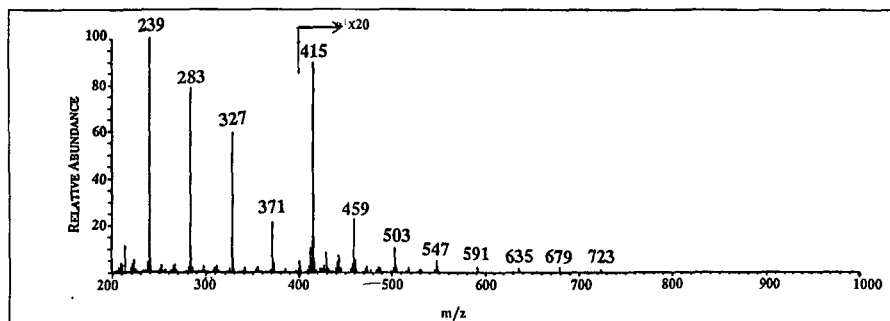


Figure 2: Spectrum of PEG using an ionizing filament and water as a CI reagent gas.

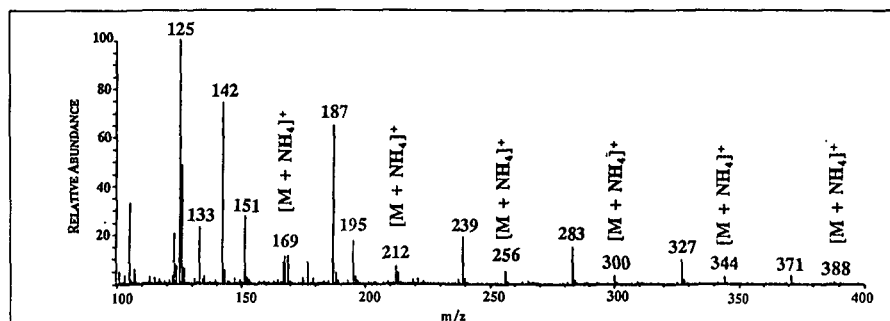


Figure 3: Spectrum of PEG in 20% ethylene glycol/80% water with 0.1 M ammonium acetate added as a buffer.

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2. Hawthorne, S.B.; Miller, D.J. *J. Appl. Spectr.* 1986, 40, 1200-1211.

PEPTIDE DIMERIZATION REACTIONS IN THERMOSPRAY AND FAB IONIZATION

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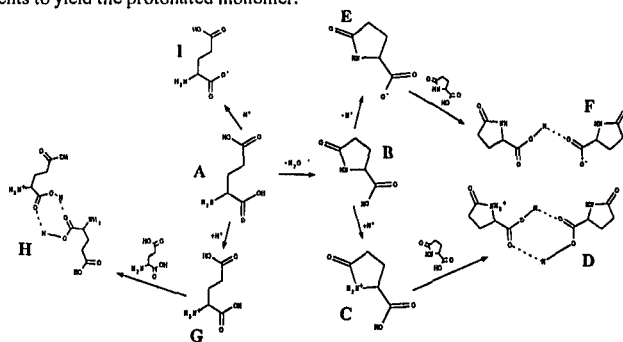
INTRODUCTION: Thermospray and fast-atom bombardment (FAB) are "soft" ionization techniques that have been used to characterize peptide and protein samples.^{1,2} Tandem mass spectrometry (MS/MS) has been used to obtain additional structural information for biological samples. We have used thermospray, FAB, and FAB MS/MS to study the formation of dimers in peptide samples containing one or more glutamic acid residues.

EXPERIMENTAL:

THERMOSPRAY LC/MS: A quadrupole mass spectrometer (HP Model 5988A) and a gradient HPLC system (SSI Model 230) were interfaced with two Vestec ThermosprayTM ion sources. Compared to the old ion source, the new version (model 721) has a smaller desolvation chamber, an auxiliary tip heater, and a needle-tip repeller. Two vaporizer probes used were the standard probe with a tip i.d. of approximately 150 μ and the restricted probe which has a stainless steel capillary tip with an i.d. of 100 μ . Glutamic acid was prepared at a concentration of 1×10^{-4} M in 0.1M ammonium acetate. The peptide samples were introduced by direct injection in a mobile phase of 0.1M ammonium acetate.

FAST-ATOM BOMBARDMENT MS/MS: A hybrid, tandem mass spectrometer with an EBQQ geometry (VG Analytical, 70-SEQ) was fitted with a saddle-field FAB gun (8 keV Xe atoms). The samples were dissolved in 0.5M HCl and placed in the matrix, m-nitrobenzyl alcohol, on the FAB probe tip. Collision-induced dissociation (CID) spectra were obtained with argon as the collision gas and a collision cell pressure maintained at 1×10^{-6} mbar. The collision energies ranged from 20 to 30 eV.

RESULTS: Glutamic acid (GLU) was chosen as a model compound to study the formation dimers in peptides. Glutamic acid was seen to lose water, A in Scheme I, in the thermospray ion source. The protonated pyrroglutamic acid $[M-H_2O+H]^+$, C in Scheme I, is less abundant in the mass spectra than $[M-H_2O-H]^+$, E in Scheme I. This follows solution phase acidities in which the negative ion pyrroglutamic acid ($pK_a=10$) is more stable than the positive ion ($pK_a=0$). The proton-bound dimer of the positive ion pyrroglutamic acid, $[2M-2H_2O+H]^+$, and the negative ion, $[2M-2H_2O-H]^-$, D and F in Scheme I, were observed using thermospray. In FAB, the GLU residue does not form pyrroglutamic acid. The CID spectrum of the proton-bound dimer, m/z 295, fragments to yield the protonated monomer.



Scheme I: A postulated mechanism for dimer formation from glutamic acid in thermospray and FAB ionization.

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We have studied peptides containing one or more glutamic acid residues to study the formation of dimer ions. VAL-GLY-ASP-GLU (VGDE) is a peptide that incorporates a C-terminal GLU. Another peptide, PHE-LEU-GLU-GLU-ILE (FLEEI), has two adjacent internal GLU residues.

The thermospray negative ion mass spectrum (Fig. 1) of VGDE produced using the new source and restricted probe displays ions at m/z 417, $[M-H]^-$, a proton-bound dimer at m/z 835 and a sodium-bound dimer at m/z 857. The FAB positive ion mass spectrum (Fig. 2) of VGDE displays the protonated molecular ion at m/z 419 and the proton-bound dimer at m/z 837. The structure of the dimer was confirmed using CID of m/z 837 which fragmented to yield the protonated monomer at m/z 419.

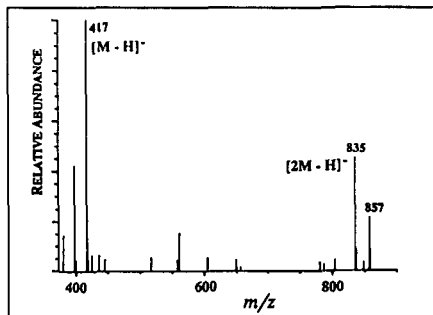


Figure 1: The thermospray negative ion mass spectrum of VGDE (new source/restricted probe).

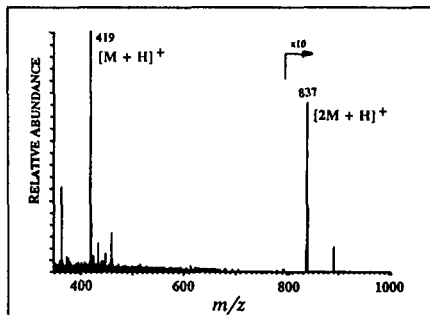


Figure 2: The FAB positive ion mass spectrum of VGDE.

The thermospray negative ion mass spectrum of FLEEI produced using the new source and restricted probe (Fig. 3) displays the molecular ion and a proton-bound dimer at m/z 1297. The spectrum also shows a sodium and potassium-bound dimer at m/z 1319, and m/z 1335, respectively. The FAB positive ion mass spectrum (Fig. 4) shows the protonated molecular ion at m/z 650 and a proton-bound dimer at m/z 1299 which was confirmed by CID of m/z 1299, which fragmented to give the protonated monomer.

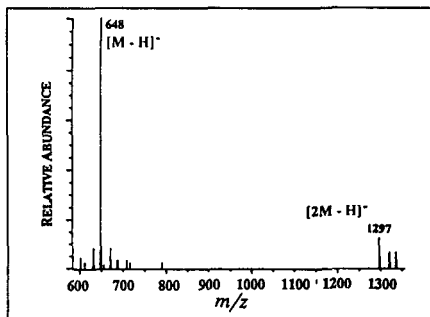


Figure 3: The thermospray negative ion mass spectrum of FLEEI (new source/restricted probe).

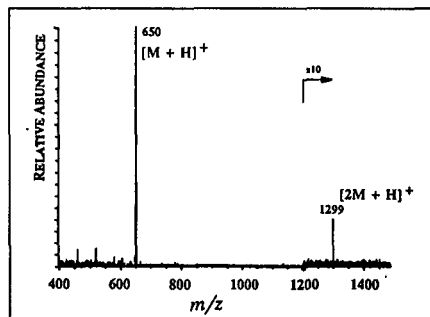


Figure 4: The FAB positive ion mass spectrum of FLEEI

CONCLUSIONS: Different dimer ions are produced in thermospray and FAB for glutamic acid. Thermospray produces a proton-bound dimer from pyroglutamic acid, $(M-H_2O)$. The positive ion proton-bound dimer produced using FAB is $[2M+H]^+$. The peptides studied produced an abundance of the molecular ion with less relative abundance of the dimers.

ACKNOWLEDGEMENTS: We wish to thank Dr. Marvin L. Vestal for his many invaluable discussions. We thank the National Institute of General Medical Sciences Health for providing financial support.

EFFECT OF PEPTIDE HYDROPHOBICITY ON MOLECULAR ION YIELDS IN NITROCELLULOSE ADSORPTION, PLASMA DESORPTION MASS SPECTROMETRY

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INTRODUCTION

Plasma desorption mass spectrometry (PDMS) has been widely used in the analysis of polypeptides and proteins [1]. Direct analysis by PDMS combined with enzymatic digestion provides molecular weight information for each peptide fragment with high accuracy. This is called PD protein mapping. In these studies, nitrocellulose membrane-protein adsorption is the most commonly used sample preparation. However, we have noted that different peptides or proteins have quite different intensities, especially in PD protein mapping while some of the expected peptide fragments are not be observed in spectra. Some peptides or contaminants appear to quench (or suppress) others, either in positive or in negative PDMS. Nielsen and Roepstorff have observed that suppression effects were only related to the potential net charge but not to the average hydrophobicity of the individual peptide [2]. On the other hand, our previous study on tryptic digest of recombinant human growth hormone has shown that the hydrophobicity of peptide fragments does appear to play a significant role in suppression [3]. Thus we were interested in the role of nitrocellulose membrane in these phenomena and what causes the different behavior of peptides.

In this poster, we considered the interactions between peptide and nitrocellulose, and peptide with peptide. Four sets of peptides with a fixed number of amino acids but different hydrophobicity indexes (H.I.) were chosen as subjects to demonstrate the effect of peptide hydrophobicity. Mixtures of peptides from the same set were also studied to characterize the suppression effect in protein mapping analysis using nitrocellulose as matrix.

EXPERIMENTAL

All data were obtained on a Bio-ion (Uppsala, Sweden) Bin 10K plasma desorption mass spectrometer. The acceleration voltage was 18 KV for positive spectra and -16 KV for negative spectra. All peptides were purchased from Sigma Chemical Co. 1 nmole/ul peptide solution was prepared in 0.1% TFA aqueous solution. 5 nmoles were applied as sample size. Spectra of samples treated with (procedure I and II) or without rinsing (procedure I only) were both recorded for comparison.

(I). Equilibrium adsorption Once 5 ul of peptide solution was added onto the nitrocellulose surface, the sample foil was covered with a microscope cover-glass for 3 min. During this period of time, equilibrium of the peptide between solvent and nitrocellulose surface was established. The glass was then removed as well as most of the extra solution.

(II). Rinsing After procedure I, the sample foil was rinsed with 1 ml 0.1% TFA (aqueous) to remove Na, K contaminants and the weakly adsorbed peptides.

RESULTS AND DISCUSSIONS

-In the comparison of spectral quality of individual peptides, the more hydrophobic peptides appear to give higher molecular ion yield and better signal to noise ratio (Fig.1).

-Rinsing preferentially removes smaller peptides. There is no discrimination against more hydrophilic peptides.

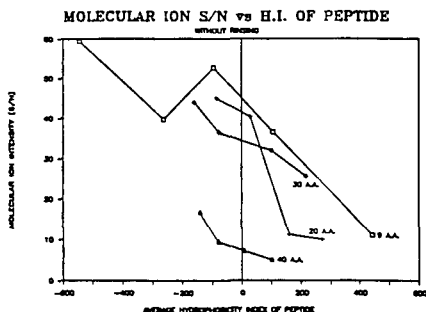


Figure 1

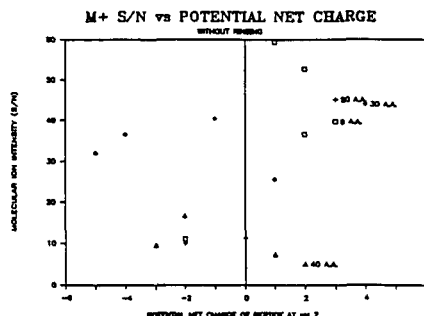


Figure 2

-In our study of pure peptides, molecular ion intensities show no significant correlation with the charge state of peptides, either at pH 7 (Fig 2) or pH 1.9 (data not shown).

-In our study of suppression effects among peptides of the same size (same number of amino acid), all factors including hydrophathy, charge state, and solubility seem to have different degrees of effects. The most hydrophobic peptides tend to suppress others. However, when mixing only the three less hydrophobic peptides, the more positively charged peptide dominates the positive-ion spectra. In the process of mixing 0.1% TFA aqueous solutions of large peptides (more than 40 amino acids), the hydrophobic peptides precipitated in the presence of hydrophilic peptides which results in the mass spectrum of mostly hydrophilic components.

-The average hydrophobicity index (H.I.) of peptide or protein was calculated based on Bull and Breese's measurement of free energy to transfer each individual amino acid from aqueous solution to the air/water interface. It can be used to represent whether the peptide (or protein) is hydrophilic (positive value) or hydrophobic (negative value). H.I. can be successfully used to explain the behaviour of peptide or protein in fast atom bombardment mass spectrometry (FAB-MS), where peptide molecules are distributed between bulk liquid phase and its surface. Although average H.I. has significant correlation with molecular ion intensity in our study, it may not be a reliable parameter to examine the interaction between peptide and the nitrocellulose surface since no liquid phase is present in PDMS. Some discrepancy in our study of molecular ion intensity vs hydrophobicity index can be explained by also considering proportion of hydrophobic amino acid residues in the peptide. Perhaps a new parameter should be used to explain the behaviour of peptides on a nitrocellulose surface.

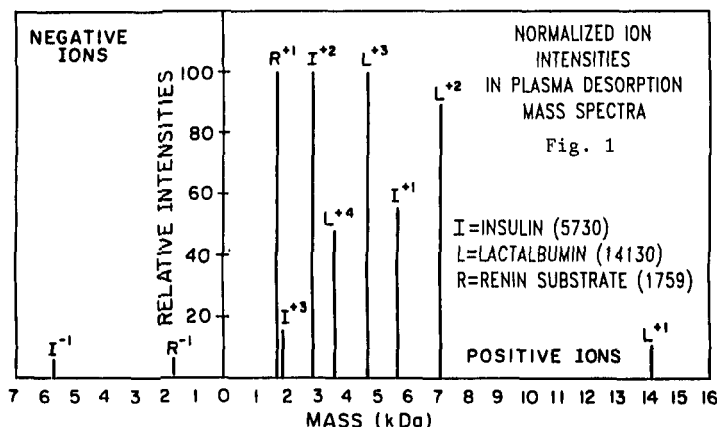
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SINGLY-CHARGED IONS, MULTIPLY-CHARGED IONS, NEUTRALS AND MASS LIMITS IN PLASMA DESORPTION MASS SPECTROMETRY

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The plasma desorption mass spectra of large peptides are generally characterized by an abundance of multiply-charged as well as singly-charged molecular ion species. It has been noted by us and by others that the intensities of multiply-charged molecular ions, as well as the average charge, generally increases with molecular size (Figure 1). In part this may be accounted for by the increase in number of basic sites resulting in multiple protonation. On the other hand, it may simply be that the charge density of these massive ions is sufficiently low that they appear "nearly neutral" in the desorption process. Finally, the fact that the charges may be widely separated in the molecule may result in ions which are stable in the gas phase to decomposition resulting from charge repulsion.



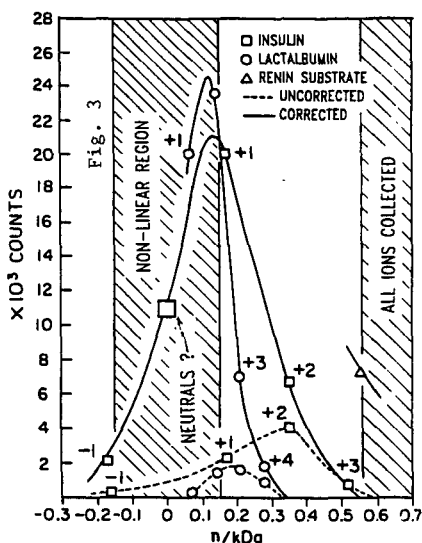
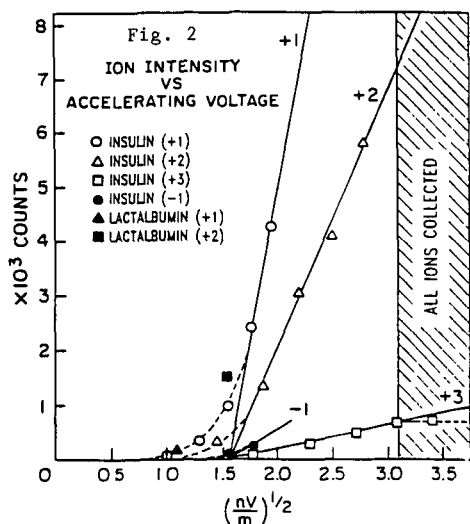
Thus, in this study we attempt to characterize the charge distribution of desorbed ions over a range of molecular weights by considering the effects of both ion transmission and ion detection on the initial distribution of ions desorbed in the source. Corrections to the "observed" (Figure 1) distribution produced some interesting results, with implications on the ultimate mass range of plasma desorption techniques.

Mass spectra were obtained on a BIO-ION (Uppsala, Sweden) model BIN-10K time-of-flight mass spectrometer with a 1 μ Ci Cf-252 ion source.

If an ion with initial kinetic energy U_0 traverses the drift region (D) in a time: $t = D(m/2neV)^{1/2}$, then the maximum radial drift (L) in that time (which may cause it to miss the detector) will be: $L = (2U_0/m)^{1/2}t$ or $L = (U_0/neV)^{1/2}$. Thus transmission is improved by higher accelerating voltages (V) and higher charge states (n), but is independent of mass (m). Detection efficiency is dependent upon ion velocity: $v = (2neV/m)^{1/2}$, and therefore upon mass, as well as charge state and accelerating voltage. Thus, in Figure 2 we determined the ion intensities of the +1, +2, +3 and -1 molecular ions of insulin as a function of

accelerating voltage (5KV to 22KV) and plotted as a function of ion velocity.

Because the ion signal from the fast-moving +3 ion did not increase between 18 and 22KV, we were able to determine the velocity at which all ions are detected. Linear extrapolation of all other ions to that velocity then provides a correction for the observed distribution of multiply charged ions as shown in Figure 3. Plotted as a function of charge/mass, it is possible to obtain an estimate of the neutral species. In addition, it appears that a large number of singly-charged ions are desorbed (but not detected) and that the average charge state falls around 1.3/10KDa.



The singly charged ions from large peptides are observed below "threshold," but are the prominent ions observed in UV-assisted laser desorption (1). It is suggested, from these experiments, that analog recording of a large group of ions of the same mass impinging on the detector at the same time would improve detection below "threshold" over detection of single ions by time-to-digital conversion.

- (1) M. Karas and F. Hillenkamp, Paper presented at the 11th International Mass Spectrometry Conference, Bordeaux 1988.

FACTORS AFFECTING EH SOURCE FLOW AND EMISSION STABILITY: RELEVANCE TO MASS SPECTROMETRY

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Electrohydrodynamic (EH) ionization is currently being pursued as an alternative method for producing gas phase, high mass biomolecular ions. Additionally, EH ionization may have other applications for LCMS and polymer characterization¹. Historically, EH emission and capillary flow stability were adequate when using high viscosity, low vapor pressure solutions e.g., glycerol. For the most part, mass analysis of peptides and proteins require solvents having low viscosity and high vapor pressures. Unless proper precautions are taken, atomization of aqueous solvents in vacuum can be counterproductive for the acquisition of stable, repetitive mass spectra. EH ion source instabilities arise from two sources: flow instabilities influenced by the geometry and material of the capillary inlet system and secondly, emission instabilities inherent in the electrostatic atomization process influenced by the solvent chemistry. The present research has addressed these instability problems resulting in improved performance for EH emission of aqueous solvents in vacuum.

Flow modeling based on the idea that Hagan-Poiseuille flow rates should exceed the rates of solvent loss by evaporation from the capillary led to the following stability criterion for a two component mixture:

$$r^2/\ell > K\eta(p_1M_1^{\frac{1}{2}} + p_2M_2^{\frac{1}{2}})/\rho T^{\frac{1}{2}}\Delta P \quad (1)$$

where r and ℓ are the radius and length of the flow capillary respectively, K has the value 6.98×10^{-4} , M_1 and M_2 are the molecular weights of the solvent components with partial vapor pressures p_1 and p_2 , η and ρ are the solution viscosity and density, T is the absolute temperature and ΔP is the pressure difference across the capillary. The quantities in Eq. 1 are expressed in cgs units with the exception of the pressure terms expressed in units of torr. To eliminate flow instabilities induced by geometry, capillary inlet dimensions must be chosen so that the quantity r^2/ℓ is larger than the right-hand side of Eq. 1. Other flow instabilities are attributed to the existence of microbubbles which form at regenerative nucleation sites on the surface of capillary flow passages. Growth and expansion of microbubbles lead to discontinuous flow resulting in aperiodic current emission. This type of instability can be prevented by using non-metallic, nucleation-free surfaces (fused silica) and flow passages uninterrupted by couplings or tube-to-tube transitions of unequal diameters.

The effects of solvent chemistry on emission stability were empirically studied with the experimental arrangement shown in Fig. 1. After passing a focused EH ion/droplet beam through an unpressurized desolvation cell, the transmitted beam current vs. time profile was mapped using an x-y recorder connected to an electrometer displaying the Faraday cup signal. Variations in the amplitude of the current-time trace are a direct measure of EH emission stability. Emission instabilities are compared in Fig. 2 for several molar concentrations in 60:40 (by volume) water-methanol mixtures. Repeated experiments reveal that solutions near 1.5M ammonium acetate show good emission stability. Similarly, experiments varying the solvent composition keeping a fixed molarity (1.5M ammonium acetate) were conducted.

An EH source, using a solvent composition characterized by good stability performance, was installed on a JEOL HX100 HF mass spectrometer. Fig. 3 shows ten consecutive scans (43 s/scan) characterizing the typical scan-to-scan repeatability measured using a 1.5M 60:40 water-methanol mixture. The stability displayed by this series of scans is essential for continuing mass spectrometric investigations with dissolved biological samples.

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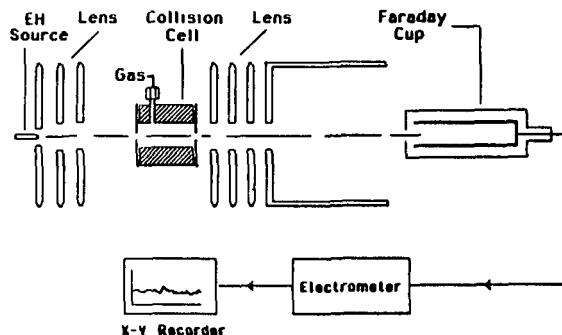


Fig. 1. Experimental Arrangement for Measuring EH Source Stability

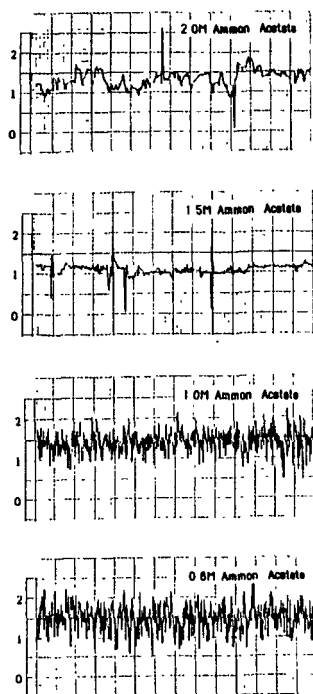


Fig. 2. Effect of Molarity on Current Stability. Vertical Axis = Faraday Cup Current, nA Horizontal Axis = Time, 10cm/min (scale: 1cm/maj.div.) Solvent: water-methanol

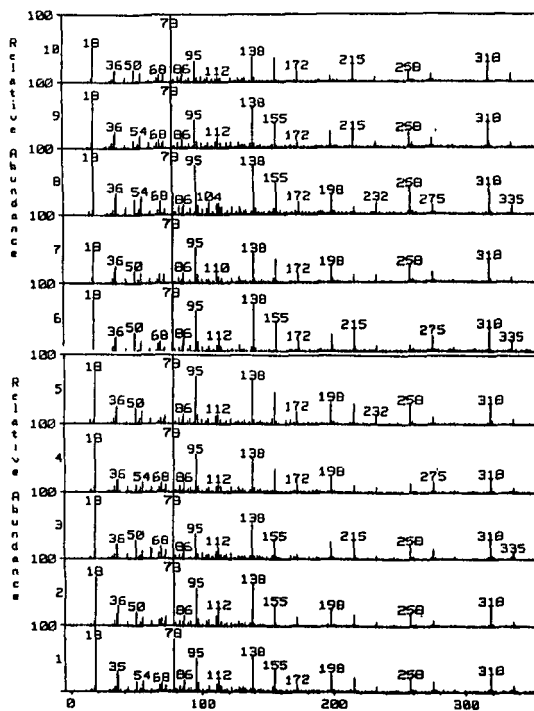


Fig. 3. Spectra of 1.5M Ammonium Acetate; 60:40 water-methanol

DISTINGUISHING ISOMERIC AND ISOBARIC IONS BY THERMOSPRAY MS PRECEDED BY ELECTROCHEMICAL ELECTROLYSIS

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Introduction: Thermospray mass spectrometry (TSMS) has proven to be an excellent interface for high-performance liquid chromatography (HPLC). One of the drawbacks, however, of TSMS is that in the majority of cases only the protonated molecule, $[M+H]^+$, is observed with very little fragmentation. In order to increase fragmentation for purposes of structural elucidation, "repeller" voltages were employed, as well as extending the temperature range of the thermospray (TS) effect (Plasmaspray™). Tandem TSMS is an expensive alternative for augmenting fragmentation for identification purposes. Because TSMS utilizes polar solvents with a volatile salt at an usual concentration of 0.1 M, interfacing an electrochemical cell on-line prior to TSMS is easily accommodated.

For electrochemically active compounds, the products of electrolysis may be sufficiently distinct so as to allow for discrimination of isomeric and isobaric (same nominal mass) compounds as observed via TSMS. This combination of electrochemistry (EC) with TSMS has been given the name "solution electrochemistry mass spectrometry" (SECYMS).

Experimental: A Delsi R-1010-C quadrupole with Nermag-Delsi TS source and Vestec TS controller were used. A coulometric ESA guard cell, 5020, was placed in series between the TS quadrupole and HPLC pump. SECYMS conditions for direct injections of aminophenols (AMP) were 90/10 H₂O/MeOH at 0.1 M ammonium acetate (AA) at a flow rate of 1.0 ml/min. HPLC/TSMS of AMP used a mobile phase of 90/10 0.1 M AA at pH 4.8/0.1 M AA in MeOH at a flow rate of 1.0 mL/min on a reversed-phase ODS-II column. Acetamidophenols (ACP) and guanine were continuously pumped into the TS source at $ca. 1 \times 10^{-4}$ M ACP and guanine in 90/10 H₂O/MeOH at 0.1 M AA at a flow rate of 1.2 mL/min. In order to solvate guanine, the AA was adjusted to pH 4.5.

Results: For AMP, TSMS detected only a $[M+H]^+$ ion at m/z 110 for each isomer; however, upon oxidation of each isomer a distinctive ion was observed as seen in Fig. 1: m/z 108 for *p*-AMP; m/z 217 for *m*-AMP; m/z 213 for *o*-AMP. In this way, the isomer of AMP was determined by direct injections.

The HPLC/TSMS of AMP generated three chromatographic peaks with the base peak at m/z 110. By using a combination of HPLC/EC/TSMS at a potential of +0.2 V (Fig. 2), a distinction between the isomers of AMP was possible *without* injecting the isomers of AMP separately to determine their retention times. At +0.2 V, only *p*-AMP and *o*-AMP were oxidized as shown by the hydrodynamic voltammograms in Fig. 3. The distinguishing ions, as seen in Fig. 2, were: m/z 108 for *p*-AMP (I); m/z 110 for *m*-AMP (II) (no change); m/z 124 and 213 for *o*-AMP (III). Because concentrations of AMP were low in the HPLC/EC/TSMS analysis, dimerization was not as evident for *o*-AMP compared to the direct injection analysis.

For ACP and guanine, the $[M+H]^+$ ion at m/z 152 was the base peak. A $[M+NH_4]^+$ ion and $[M_2H]^+$ ion was also detected for ACP by TSMS. Upon oxidation as shown in Fig. 4, distinctive mass spectral peaks were observed for each isomeric and isobaric compound: m/z 108 and 150 for *p*-ACP; m/z 152 and m/z 168 for *m*-ACP (very little change); m/z 167 for *o*-ACP; m/z 166 and 113 for guanine.

Conclusions: The method of SECYMS enables the mass spectrometrists to determine on-line the isomers of AMP and ACP, as well as isobaric compounds. The ease of use and the inexpensive cost of the electrochemical cell makes SECYMS a viable technique for determining structural isomers of electroactive compounds by TSMS.

Acknowledgements: One of us (TAG) was supported in part by an appointment to the ORAU postgraduate research program at the National Center for Toxicological Research, which is administered by the Oak Ridge Associated Universities through an interagency agreement between the US Department of Energy, and the US Food and Drug Administration. We like to thank C. Hartwick, R. Levine, and NCTR Photo Lab for their help with poster.

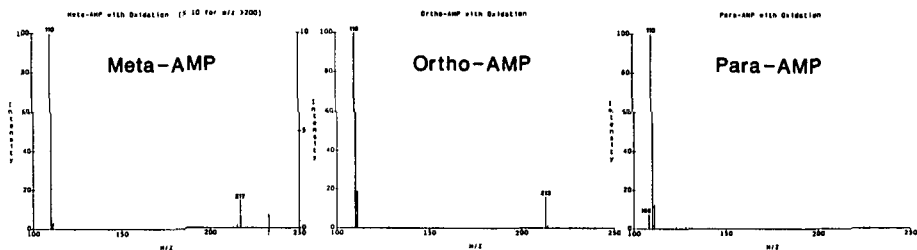


Figure 1

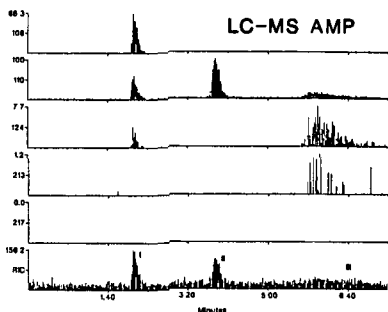


Figure 2

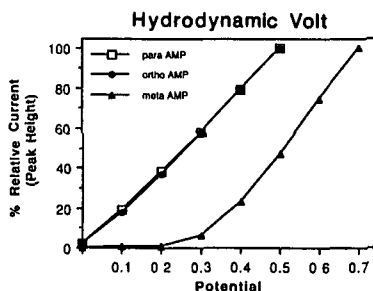


Figure 3

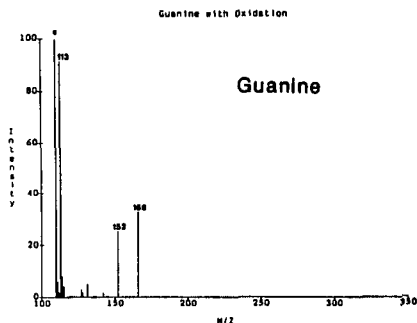
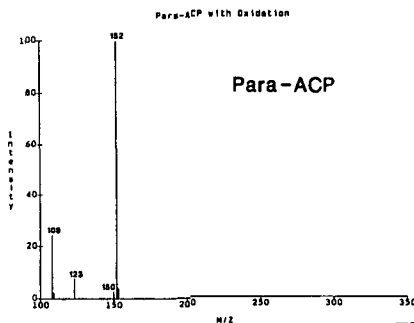
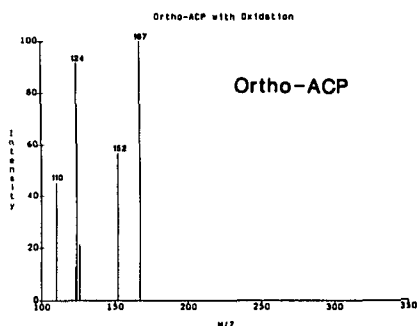
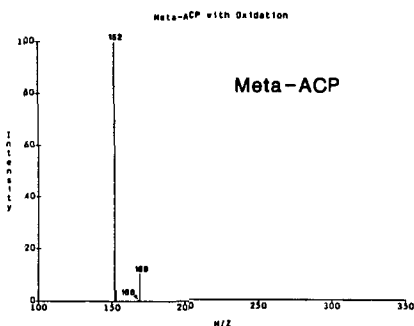


Figure 4

CO₂ AS A MODERATING GAS IN ELECTRON CAPTURE
CHEMICAL IONIZATION

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The use of CO₂ as a moderating gas in electron capture chemical ionization has been explored. All chemical ionization studies were carried out using a ZAB-2FQ mass spectrometer operating with a source temperature of 250°C.

The negative ion mass spectrum of CO₂ at high pressures shows signals for O⁻ and CO₃⁻; in addition, near-thermal energy electrons are present. The CO₃⁻ ion is essentially unreactive since CO₃ has an electron affinity of 3.3 eV [1] and CO₃⁻ has a proton affinity of approximately 324 kcal mol⁻¹ (measured by reaction bracketing using the FA-SIFT at the University of Colorado). The reactions of O⁻ have been documented [2].

With compounds of reasonable electron affinity, such as nitroarenes, polyfluoroaromatics and 1,2-diketones, negative ion mass spectra are obtained using CO₂ moderating gas which are very similar to the spectra obtained using CH₄ moderating gas; presumably in these cases the spectra are produced primarily by electron capture. However, compounds of low electron affinity show a reduced sensitivity and, in most cases, show spectra more closely resembling those produced by O⁻ CI. In addition, in many cases, these spectra are made more complex by the presence of substantial artifact peaks. Thus, aliphatic ketones show substantial [M+14]⁻ and [M+12]⁻ ion signals. These products are not enhanced by addition of O₂ to the reagent gas and do not arise by ion/molecule reactions of CO₃⁻ with M or by reactions of [M-H]⁻ or [M-2H]⁻ with CO₂; rather they appear to be the result of unidentified surface reactions in the ion source. The spectra also are rather non-reproducible from day to day. The use of CH₃CD₂COCDC₂CH₃ showed that, for this compound at least, the [M+14]⁻ ion corresponded to [CH₃CH₂COCOCCH₃]⁻ while the [M+12]⁻ ion corresponded to [CH₂=CHCOCOCCH₃]⁻. With acetophenones, in addition to an [M+14]⁻ artifact peak, an [M+28]⁻ artifact peak is observed as the result of a surface reaction. Experiments with C₆H₅COCDC₃ showed that the CD₃ group was lost in forming this product, which we believe corresponds to the molecular anion of phthalic anhydride.

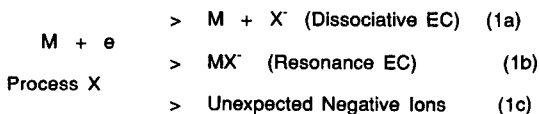
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USE OF CO₂ AS A BUFFER GAS IN HIGH PRESSURE ELECTRON CAPTURE MASS SPECTROMETRY. THE ELIMINATION OF UNUSUAL NEGATIVE IONS.

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High Pressure Electron Capture Mass Spectrometry (HPECMS) involves the capture of thermalized electrons by an analyte, MX. This process of electron capture (EC), conducted at 0.1 to 1.0 Torr, may occur through dissociative (1a) or resonance (1b) capture of the thermal electron. Thermalized electrons are generally produced by the interaction of a high energy electron beam with the buffer gas.



It has been demonstrated that exceptionally high chemical specificities and sensitivities are often obtainable with EC reactions¹. This is the direct result of the nature of the reaction, shown in expressions 1a and 1b, which can have a rate coefficient 2 orders of magnitude larger than those of ion-molecule reactions.

Since the EC reaction can often proceed with very fast rates, certain chemical events (the results of which may not be observed in the more general field of Chemical Ionization Mass Spectrometry) may occur leading to the production of unusual or unexpected negative ions. Process X (1c) provides a generalized route by which unusual negative ions may be produced in HPECMS. Figure 1 shows four of these routes which we have found to be kinetically favorable, with respect to reactions 1a and 1b, for the production of negative ions under HPECMS conditions².

Reactive products generated by the interaction of the electron beam with methane are often involved in the formation of unexpected negative ions. As seen in Figure 1, which has been described in greater detail elsewhere², the various products of irradiated methane may be associated with any one, or all four of the Routes toward generation of unusual negative ions. A logical step toward the control or elimination of these unusual ions in HPECMS spectra is to simply alter the buffer gas used for thermalization of the electrons.

We have tried several gases and have found that CO₂ consistently provides EC spectra which are free of negative ions that arise from secondary processes. Additionally, the use of CO₂ as a buffer gas provides equivalent or lower levels of detection for most analytes as compared to sensitivities when using CH₄ as the buffer gas. Although other buffer gases did provide spectra which were represented only by ions from the primary EC reaction, they lacked the desirable feature of sensitivity for which HPECMS is so often used.

Figures 2, 3, and 4 show a comparison of EC spectra from CH₄ and CO₂ buffer gases of various compounds which are representative of unusual negative ion formation by Routes A, B, and C from Figure 1. In all spectra, the buffer gas pressure is 0.5 Torr, the source temperature is 150°C, the electron energy is 150eV, and the emission current is 0.2mA. Figure 2 provides examples of spectra of fluoranil, chloranil, and Pentac. In CH₄, these neutrals are altered at the walls of the ion source via a Route D process prior to electron capture. In Figure 3, tetracyanoethylene and tetracyanoquinodimethane are altered by reactions with hydrocarbon free radicals generated in the CH₄ plasma. In both Figures 2 and 3, the use of CO₂ as the buffer gas provides EC spectra which are simplified and reflective of the molecular structure. Figure 4 provides the only case in which CO₂ did not provide simplified spectra which were free of negative ions originating from secondary processes. The trifluoroacetic anhydride (TFA) derivatives of the aminoanthracene (AA) and aminophenanthrene (AP) provide examples of compounds which undergo positive ion molecule reactions and neutralization (Route B) prior to EC. In Figure 4 we see that the major negative ions in the spectra of 3-AP-TFA isomer are the result of prior positive ion-molecule reactions, irregardless of which buffer gas is used. For those compounds which follow a Route B process, it may not be possible to eliminate the occurrence of unusual negative ions. To do so would require the elimination of positive ion-molecule reactions which may not be fundamentally possible.

There is evidence available which suggest that CO_2 may be an exceptional buffer gas with respect to electron energy thermalization^{3,4,5} and collisional stabilization⁶ of the initially formed negative ion. Additionally, our experience with CO_2 has shown that it consistently provides EC spectra which are free of the effects of secondary processes.

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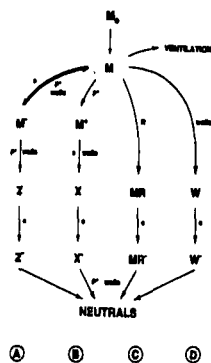


Figure 1

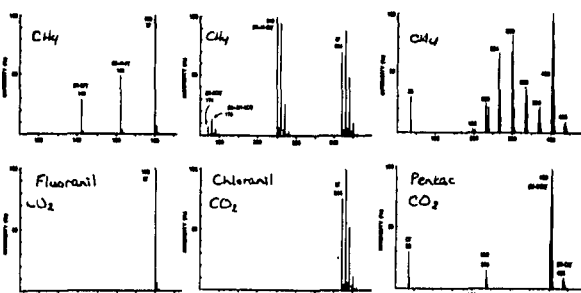


FIGURE 2

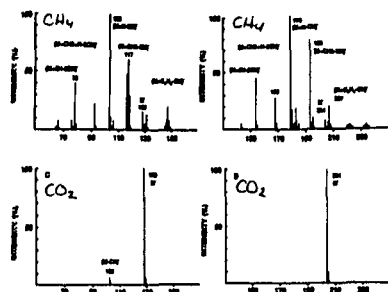


FIGURE 3

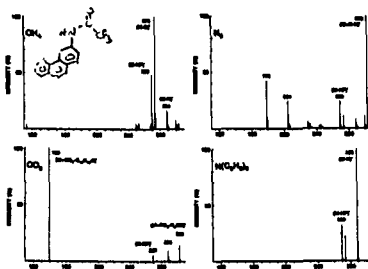


FIGURE 4

SENSITIVITY STUDY OF PERFLUOROCARBONS AND PERFLUOROCARBONS CONTAINING
NON-FLUOROCARBON GROUPS

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The detection of residual impurities such as perfluorocarbons (PFCs) containing one or two hydrogens, perfluorinated ketones, alcohols or ethers in PFCs by the negative ion chemical ionization mass spectrometry (NICIMS) has been a problem. The problem is associated with the relatively low electronegativity of these compounds with respect to PFCs which show strong response in the NICI condition. In an attempt to improve the signal strength of these impurities, the relative response of various model fluorocarbons with respect to the spiked internal standard, perfluoro-cis-decalin, in different NICI reagent gases was studied by an HP-5988 GC/MS system. As shown in Table 1, CH_4 is in general the best NICI reagent gas for the sensitive detection of perfluorinated alkanes, alkenes and aromatics. However CO_2 and NH_3 are good NICI reagent gases in detecting hydrogen- and oxygen-containing PFCs. Table 2 shows the relative sensitivity of the same model fluorocarbons with respect to the *E*-cis-decalin. The sensitivity ratio shown in this table has been normalized to the same number of molecules entering the ion source of the mass spectrometer by dividing the signal strength of each compound by its number of moles prepared for the solution. The sensitivity of the spiked *E*-cis-decalin was taken arbitrarily as one in this case. Some rough trends can be extracted from Table 2: 1) a highly cyclic PFC gives more sensitive response than a single cyclic PFC, 2) cyclic or aromatic PFCs seem also more sensitive than the open chain PFCs, 3) a branched PFC appears to be more sensitive than the straight chain PFC, and 4) PFCs containing heteroatoms are much less sensitive than PFCs in NICI.

Because of the effective wall reactions, PFCs undergo altered reactions (1) in NICI. As a result, a response factor variation as a function of sample amount is observed. As shown in Figure 1, the response ratios of the perfluorinated 1-methyl adamantane, 2,5-dimethyl [3,3,1] bicyclononane and 1,3-dimethyl-5-propyl cyclohexane to the internal standard, *E*-cis-decalin change with the injection volume from the same mixture. This phenomenon was also found to depend on the NICI reagent gas employed, which leads to a different linear dynamic range for each reagent gas as shown in Table 3. It was found that the employment of CF_4 yielded the largest linear range in the calibration curve. On the other hand, NH_3 gave the most sensitive response to the analyte's concentration.

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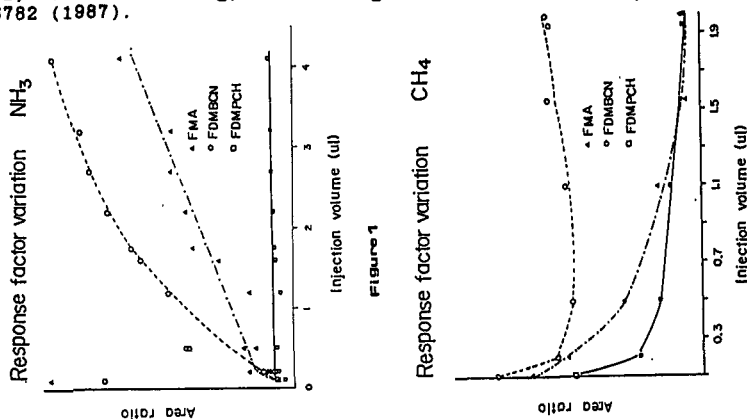


Table 1 Area ratio of fluorocarbons to perfluorodecalin
in different NICI reagent gases

	CF4	CH4	Ar	CO2	He	NH3	Air
F-hexane	0.24	0.535	0.355	0.461	0.131	0.278	0.475
F-benzene	0.065	1.1456	0.335	0.493	0.019	0.52	0.282
F-2-Bu-THF	0.111	0.322	0.204	0.82	0.022	0.517	0.4
F-2-Me-2-pentene	0.281	0.724	0.375	0.487	0.04	0.4	0.281
F-toluene	1.188	6.38	1.944	1.648	0.918	2.56	1.49
F-t-Butanol	0.006	0.015	0.006	0.05	0.002	0.134	0.074
F-t-decalin	1.014	0.975	1.230	1.342	0.811	1.146	1.162
F-adamantane	0.082	0.087	0.218	0.392	0.026	0.231	0.09
F-1-Me-adamantane	1.614	2.724	2.04	1.795	0.646	1.39	1.543
F-MeCyclohexane	0.371	1.314	0.52	1.214	0.317	0.98	1.02
F-1H-heptane	0.057	0.02	0.03	0.19	0.03	0.157	0.06
F-Bis(isopropyl) ketone	0.032	0.241	0.06	0.285	0.009	0.28	0.307

Table 2 Relative sensitivity of fluorocarbons with respect
to F-tet-decalin in different NICI reagent gases

	Formula	M.W	Sen. CF4	Sen. CH4	Sen. Ar	Sen. CO2	Sen. He	Sen. NH3	Sen. air
F-hexane	C6F14	300	0.136434	0.30413	0.20181	0.26206	0.07447	0.15803	0.27002
F-benzene	C6F6	186	0.029195	0.51455	0.15046	0.22143	0.00853	0.23356	0.11767
F-2-Bu-THF	C8F16O	416	0.106241	0.30819	0.19525	0.78484	0.02105	0.49483	0.38285
F-2-Me-2-pentene	C6F12	300	0.193421	0.49835	0.25812	0.33521	0.02753	0.27533	0.19342
F-toluene	C7F8	236	0.593640	3.18807	0.97141	0.82250	0.45872	1.27922	0.74454
F-t-Butanol	C4F9OH	236	0.002020	0.00505	0.00202	0.01683	0.00067	0.04511	0.02491
F-t-decalin	C10F18	462	1.080867	1.03950	1.31111	1.43049	0.86448	1.22157	1.23852
F-adamantane	C10F16	424	0.263651	0.27972	0.70092	1.26038	0.08038	0.74272	0.28937
F-1-Me-adamantane	C11F18	474	2.070130	3.49382	2.61652	2.30228	0.82856	1.78282	1.97906
F-MeCyclohexane	C7F14	350	0.232684	0.82411	0.32613	0.76140	0.19881	0.61463	0.63972
F-1H-heptane	C7F15H	370	0.012373	0.00867	0.00651	0.08238	0.00451	0.06807	0.02601
F-Bis(isopropyl) ketone	C7F14O	366	0.018458	0.13901	0.03460	0.16439	0.00519	0.16150	0.17708

Table 3 Calibration Curve by NICI/MS

reagent gas	analytes	linear dynamic range	slope	area ratio to FDEC**	RMV counts of FDEC
Air	FHA isomers	0-2.763 ng	1.533	0.035	5.5E8
	FMDCH isomers	0-2.275 ng	0.125	0.0013	
	FMDPC isomers	0-0.216 ng	6E-3	0.00016	
Argon*	FHA isomers	0-1.3 ng	3.8	0.0975	>8E8
	FMDCH isomers	0-1.5 ng	0.33	0.0111	
	FMDPC isomers	0-0.25 ng	0.018	0.0006	
CH4	FHA isomers	0-1.3 ng	2.21	0.0375	6.5E8
	FMDCH isomers	0-0.8 ng	0.11	0.002	
	FMDPC isomers	0-0.11 ng	4.6E-3	0.0001	
CH4*	FHA isomers	0-1.3 ng	3.68	0.1286	>8E8
	FMDCH isomers	0-1.14 ng	0.6	0.0153	
	FMDPC isomers	0-0.163 ng	0.095	0.0016	
CF4	FHA isomers	0-4.225 ng	1.719	0.042	4.5E8
	FMDCH isomers	0-2.65 ng	0.19	0.0009	
	FMDPC isomers	0-0.35 ng	0.013	0.0006	
CO2	FHA isomers	0-1.95 ng	2.25	0.07	>8E8
	FMDCH isomers	0-2.275 ng	0.175	0.0017	
	FMDPC isomers	0-0.162 ng	7.3E-3	0.00014	
NH3	FHA isomers	0-0.075 ng	4	0.1	2E8
	FMDCH isomers	0-2.055 ng	0.067	0.02	
	FMDPC isomers	0-0.23 ng	0.133	0.0026	
He	FHA isomers	0-0.6125 ng	0.94	0.015	7E8
	FMDCH isomers	0-0.474 ng	0.046	0.0004	
	FMDPC isomers	0-0.08 ng	6E-3	0.0001	

* double the initial sensitivity
** FDEC is the internal standard, the area ratios are taken for 0-25

Comparison of Gas Phase and Liquid Phase Ionization: Chemical Ionization at Atmospheric Pressure vs. Field Assisted Ion Evaporation

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The mechanism by which ions can be emitted from a liquid solvent into the gas phase has been termed Ion Evaporation (IE). Experimental evidence combined with a theoretical model suggested that as a charged droplet evaporates in air, a critical point is reached at which it is kinetically and energetically possible for ions at the surface of the liquid to "evaporate" into the air (1). Interest about this process has raised further questions about the details of the ionization mechanisms involved (2) and how it compares to other methods of liquid introduction. This presentation shows both positive and negative ion spectra with Ion Evaporation and APCI from a series of compounds ranging from low mass neutral compounds to high mass highly charged molecules which lend credence to the Ion Evaporation theory. The compounds shown are lovastatin (neutral in solution), a monosulfonated azo dye (singly charged), a disulfonated azo dye (doubly charged in solution), adenosine triphosphate (triply charged in solution), and β -lactoglobulin (21 charges). A comparison is made with Atmospheric Pressure Chemical Ionization (APCI) to highlight the fundamental differences in these two ionization techniques.

This work was done on a Sciex API III triple quadrupole mass spectrometer equipped with the standard *Atmospheric Pressure Ionization* source. The *Ion Spray* probe was used to generate Ion Evaporation spectra and the *Heated Nebulizer* probe with a corona discharge was used to generate APCI spectra.

Spectra were obtained by flow injection analysis in 50/50 methanol/H₂O 0.1 mM NH₄OAc, pH = 4.5. Flow rates were 40 μ L/min for the Ion Evaporation Spectra and 1 mL/min for the APCI spectra.

Conclusions drawn from the 20 spectra shown can be summarized as follows:

1. Field Assisted Ion Evaporation is a *pure desorption technique* having no gas phase or thermal component.

2. Compounds neutral in solution can produce ions by Ion Evaporation via association in solution with a charged molecule such as acetate or NH₄. Reagent ion monitoring experiments indicate that the neutral molecule and the charged adduct associate in solution and are then desorbed to the gas phase (3). The desorption process is so mild that these complexes do not fragment but remain intact during transfer to the gas phase. No fragmentation has yet been observed with this technique.

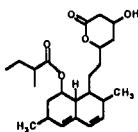
3. For a compound having a spectrum containing several multiply charged molecular ions, the charge state is quantized, i.e. a charged state is never skipped. This has important implications for the determination of the molecular weights of unknowns from multiply charged ions.

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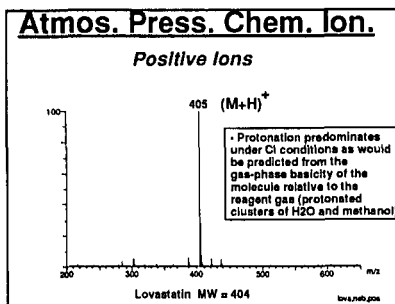
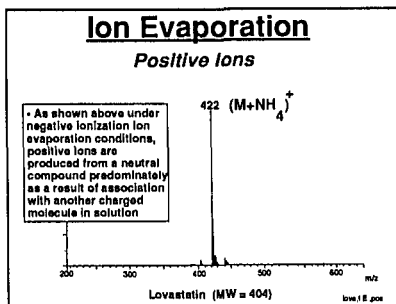
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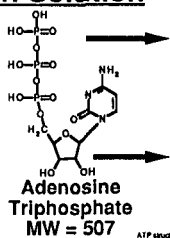
Neutral Compound



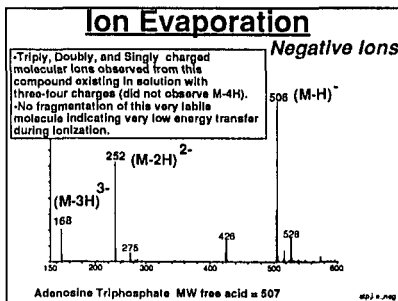
Lovastatin MW = 404



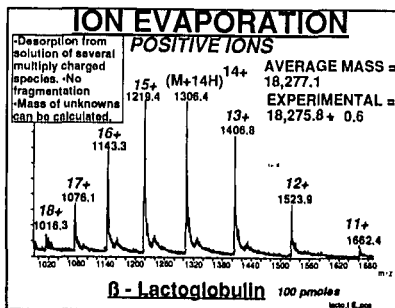
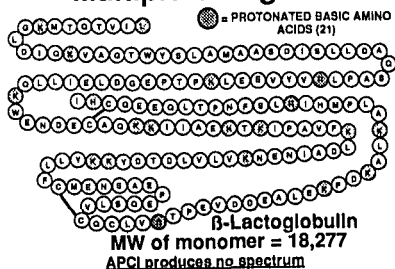
Four Charges in Solution



APCI produced no spectra



Multiple Charges



FIELD IONIZATION AT LOW VOLTAGE
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SRI International, Menlo Park, CA 94025

Historically, field ionization has required high voltages to achieve a field strength of 10^7 - 10^8 v/cm needed to induce electron tunneling from a molecule into the source. Ionization thresholds in excess of several kilovolts, and typical operating potentials of 7-15 kV are characteristic of the wire and foil-type sources used. The volcano-style field ionization source developed at SRI in the late 1970's produced field ionization above 800-900 volts, and usable mass spectra at voltages between 1.5 and 2.5 kV. FI at these voltages was possible because the physical dimension of the volcano opening was on the order of 15-25 μm , and the volcano-to-counter electrode spacing was typically 25 μm . Thus, high local field strengths could be developed with modest applied potentials.

More recently, we have developed a new microvolcano field ionizer that bears only a passing resemblance to the original volcano ionizer. Prototype devices have been microfabricated from a single silicon wafer, and consist of a 10×10 array of volcano structures, each surrounded on the top surface by a metallic counter electrode. The volcano rim diameter has been made as small as 0.75 μm , and as large as 2 μm . There are several major advantages of this design over the conventional volcano field ionizer, including: the integral counter electrode that ensures precise alignment; single unit construction requiring no assembly; and improved mechanical strength and ruggedness associated with the monolithic structure.

Because of its very much smaller dimensions, the microvolcano would be expected to exhibit a significantly lower threshold for ionization than the conventional volcano source. Figure 1 shows the measured voltage-current curves for a prototype microvolcano array. The curves were obtained by counting the total ion signal as a function of the applied voltage between the microvolcano rim and the counter electrode. The drive voltage in these experiments was half-wave rectified 60-Hz ac, and the ion extraction/acceleration potential was 1.5 kV. Note that no mass analysis was performed, however, other experiments (see below) confirmed that the ion signal was due primarily to parent molecular ions. The results obtained with 0.2 Torr of toluene vapor supplied to the back of the microvolcano array are shown by the solid symbols. The measured onset of field ionization is ≈ 48 volts ac. Similar results are obtained using a dc drive voltage. The apparent saturation of the current above 70 volts is an artifact of the limited dynamic range of the detection system. After operation for several hours, the ionization threshold voltage was found to decrease, with the lowest measured value being ≈ 30 volts. It is presumed that some type of activation process is responsible for the observed reduction in threshold voltage. The open symbols in Figure 1 show the measured signal level when the toluene vapor was pumped from the source. The ion signal is due to a combination of residual toluene within the inlet line, and background organic vapors within the vacuum test chamber.

Mass spectra were also obtained using microvolcano field ionizer arrays interfaced with a simple quadrupole mass filter. Spectra were obtained from a variety of simple hydrocarbon species, including toluene, benzene, n-propylbenzene, n-butylbenzene, acetone, and butane. In each case, the microvolcano source produced mass spectra that were very characteristic of field ionization, with $> 98\%$ of the observed signal attributable to M^+ ions. Spectra showed no appreciable change with applied voltage, other than the expected intensity variation.

By using a dual microchannel plate detector equipped with a phosphor screen, it was possible to image a portion of the ion signal produced by a microvolcano array. This magnified ion image clearly showed the discrete ionization sites associated with individual microvolcanos. A typical image consisted of 10-15 overlapping, circular spots aligned on an evenly spaced grid. Each spot showed a radially symmetric intensity pattern that was most intense in the center. Near threshold, individual image spots showed significant temporal intensity variations. At voltages well above threshold (2x), the patterns became very stable, eventually merging into a uniform intensity distribution on the microchannel plate detector.

The microvolcano field ionization source described here has a number of unique, and potentially useful features. Although the prototype device consisted of 100 microvolcanos, standard photolithographic techniques could be used to produce larger arrays of 100 x 100 microvolcanos, thus potentially providing a 100-fold increase in ion current. Moreover, multiple arrays could be fabricated in close proximity to each other to provide an even larger active area.

As a result of achieving field ionization at such low voltages, it is possible to increase the operating pressure of the microvolcano source without producing an electrical breakdown between the rim and counter electrode. The excellent mechanical strength of the silicon substrate will allow a pressure differential between the front and back sides of the device in excess of 200 Torr. Given the dimensions and potentials involved, it should be possible to observe field ionization at atmospheric pressure.

This work was supported by CommTech International.

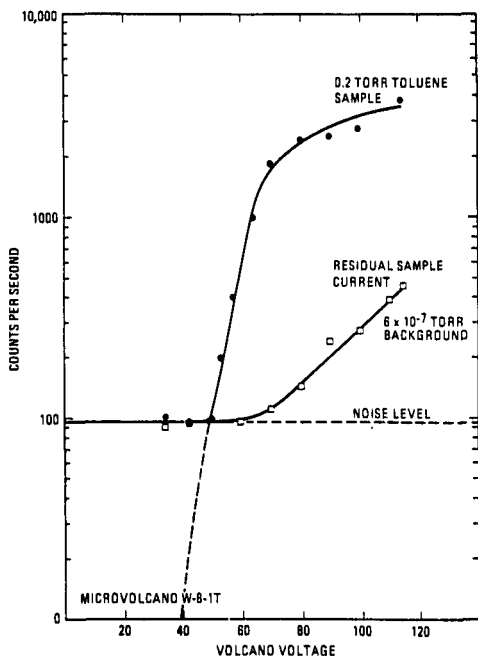


Figure 1. Measured voltage-current curves for a 10 x 10 microvolcano array. The volcano drive voltage was half-wave rectified 60-Hz ac, with the sample vapors indicated on the Figure. The effective onset of field ionization above the noise level is 48 volts ac. The apparent saturation above 70 volts is due to the limited dynamic range of the detector.

LD/FTMS. A Study of Variables Affecting Performance.

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Laser desorption mass spectrometry (LDMS) has been applied successfully to a number of important classes of compounds that are generally problematic for mass spectral analysis. This report describes a study whose purpose is to enhance the performance of LDMS and expand the set of compound classes that can be routinely analyzed by using laser desorption.

Performance criteria used in evaluating the performance of the LD/FTMS technique were resolving power (RP), high-mass detection, sensitivity, and detection limit. The production of abundant molecular or molecular-like ions is desirable to determine the molecular weight of the sample and to apply MS/MS experiments for structural information.

Because the compounds of greatest interest in the biosciences are isolated only in minute quantities, we have devised sample handling and manipulation procedures for reducing the detection limits in LD/FTMS. The microdeposition of the sample on the mass spectrometer probe target enables picomole detection limits to be achieved from biomolecules with a single laser shot and at high resolution.

The positive-ion LDMS mass spectrum of alpha-melanocyte stimulating hormone (MSH), which was obtained on 100 pmole of sample and with a single laser shot, is shown in Figure 1. This mass spectrum shows a resolving power of 7,200 (FWHM) and a S/N of greater than 10. Medium resolution positive- and negative-ion LDMS mass spectra were obtained with a single laser shot on 500 pmole of MSH (Figure 2). Peptide sequence information was obtained directly from the mass spectrum. The positive-ion spectrum (Figure 2A) shows the A and B series while the negative-ion spectrum (Figure 2B) shows the C and Z series of fragment ions. Other fragment ions resulted from internal and side-chain fragmentation. The absolute detection limit for LDMS when used with the microdeposition technique was estimated by studying RbCl. Sixteen attomoles of Rb was detected as $^{85}\text{Rb}^+$ at a S/N of 6/1, which corresponds to a limit of detection of five attomoles at a S/N of 2/1.

Metal-containing compounds are often problematic for mass spectral analysis because many are thermally unstable, air-sensitive or both. As a part of our study of this class of compounds, we have successfully analyzed by LD/FTMS a platinum complex. The positive-ion laser desorption mass spectrum (Figure 3) shows peaks corresponding to cationized molecules $[\text{M}+\text{Na}]^+$ (m/z 908) and $[\text{M}+\text{K}]^+$ (m/z 924), and fragment ions that correspond to the loss of a triphenylphosphine ligand. Fast atom bombardment (FAB) was unsuccessfully applied to the analysis of this compound. The LD/FTMS technique thus appears to hold promise for great progress in the field of mass spectral analysis of organometallics.

Figure 1.

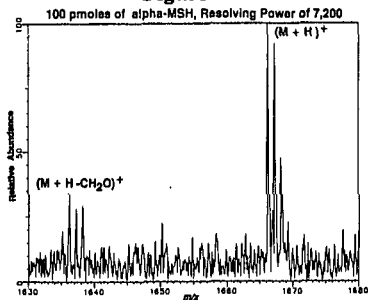


Figure 3.
PLATINUM ORGANOMETALLIC
 $\text{Pt}(\text{C}=\text{C}-\text{C}(\text{OH})(\text{CH}_3)_2)_2(\text{PPh}_3)_2 = \text{PtL}_2(\text{PPh}_3)_2 = \text{M}$

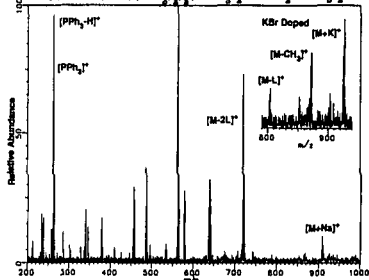
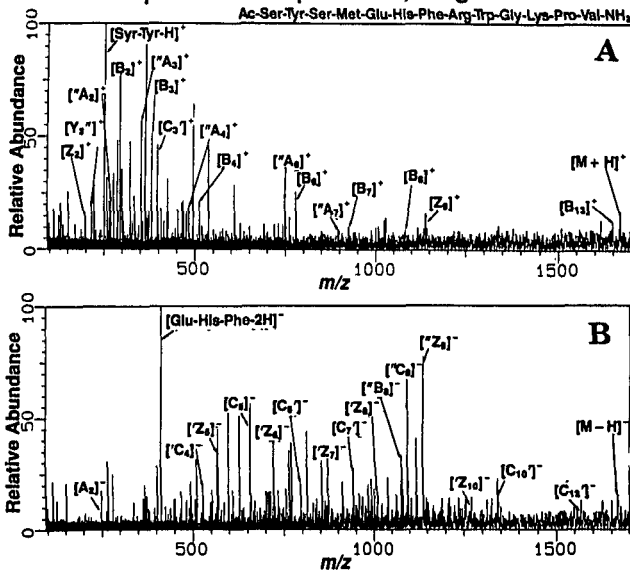


Figure 2.

500 pmoles of alpha-MSH, Fragment Ions

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂



MULTIPHOTON IONIZATION OF RHODAMINE 6G: STUDY OF FRAGMENTATION AS FUNCTION OF PHOTON ENERGY AND INTENSITY

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Multiphoton ionization followed by time-of-flight mass analysis, is a powerful method to obtain structural and molecular weight information of large neutral molecules. In many cases the fragmentation can be reduced to an extent, that only molecular ions are produced. Increasing the laser power increases the fragmentation, while different wavelengths select different fragmentation channels, i.e. give additional structural information.

Neutral molecules are vaporized by pulsed infrared CO₂-Laser radiation. The desorbed intact molecules penetrate a pulsed supersonic jet, which cools them and carries them into the multiphoton-ionization region. Thus this multistep desorption/ionization method allows in particular the examination of labile, nonvolatile substances. The measurements have been performed with a Bruker TOF 1. A more detailed description of the apparatus can be found in ref.1-3.

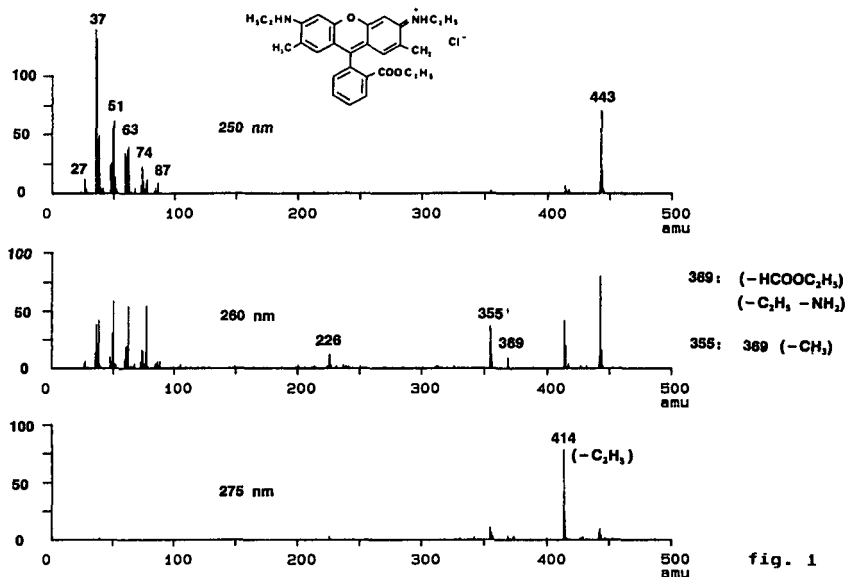


fig. 1

The results for Rhodamine 6G (fig. 1) show how different photon energies (wavelength) at constant intensities influence the multiphoton ionization mass spectra. Mass 443 corresponds to Rhodamine 6G without Cl and is desorbed as a neutral molecule. Different ionization laser wavelength drastically change the whole mass spectrum, especially in the mass range little below the molecular ion (i.e. Rhodamine 6G - Cl). Below 261 nm (= 4.75 eV) fragments of Benzene rings can be produced. Varying the laser power at constant laser wavelength (260 nm), leads to more small fragments and less signal on the molecular ion, but does not change the fragment ratio in the higher mass range (fig.2). The results show the necessity and the possibilities of desorbing in two separate steps, which can be optimized independantly.

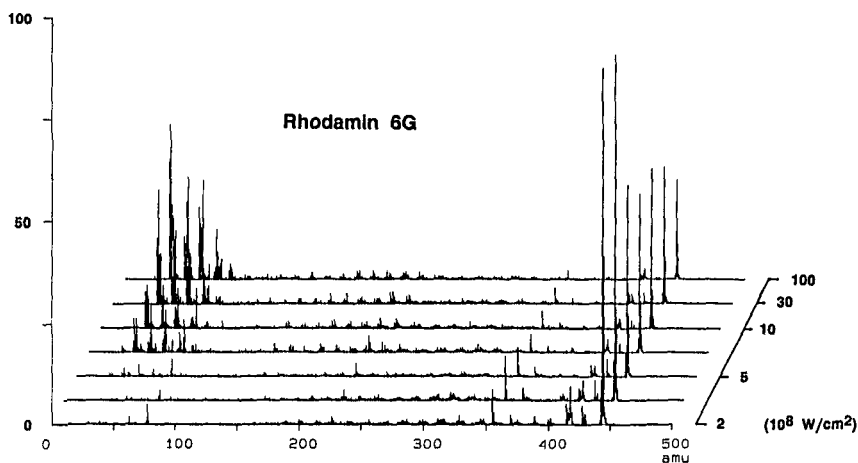


fig. 2

References:

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Aknowledgements:

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DESORPTION AND DISSOCIATION OF SMALL BIOMOLECULAR IONS BY UV-LASER IRRADIATION

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LASER DESORPTION:

Laser induced desorption of small organic molecules has been investigated in a linear time-of-flight spectrometer (Manitoba TOF I [1]). In contrast to previous work employing transient recorders for current measurement [2], we have used single ion detection. This enables measurements at lower laser irradiance (power density 10^3 to 10^6 W/cm²). An excimer laser (Lumonics Hyperex 460 HR) generated 10 ns pulses at 400 Hz and 308 nm. The laser light was focused by a single lens onto the target and spectra of compounds with different linear absorbance were recorded. (Examples are shown in Fig. 1, 2, 4, 5.) As expected, lower threshold irradiances were observed than in the work of Hillenkamp et. al. [2].

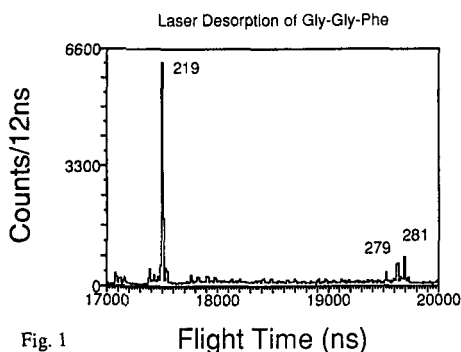


Fig. 1

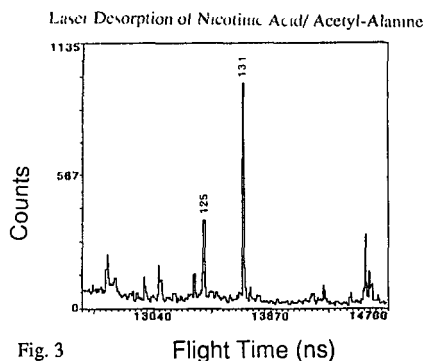


Fig. 3

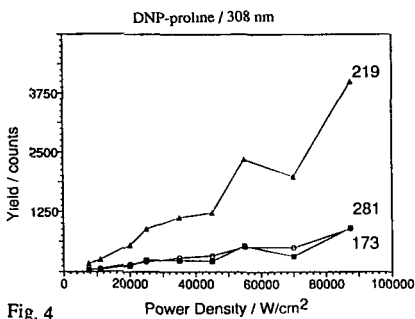


Fig. 4

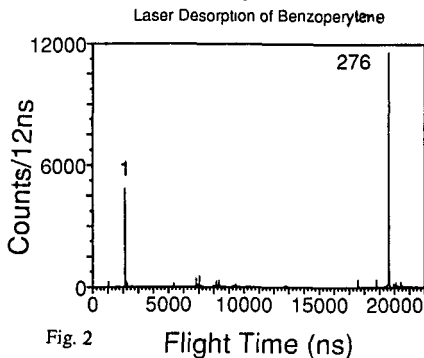


Fig. 2

Fig. 1 shows a laser desorption spectrum of GGE at a power density of 4×10^4 W/cm²; the first molecular ions were detected at a power density of 10^3 W/cm². In contrast to SIMS spectra and laser desorption spectra at high irradiance ($>10^7$ W/cm²) [2] neither sodium ions, silver ions from the substrate, nor cationized molecular ions were detected at these low power densities. Also the molecular ion yield is only weakly dependent on the power density as shown in Fig. 4, suggesting a single photon desorption mechanism.

Fig. 3 shows a laser induced matrix assisted desorption of Acetyl-Alanine. Here the frequency doubled output of an excimer laser pumped dye laser (Lumonics Hyperdye-300) was used. Pure Acetyl-Alanine does not show up in a laser desorption spectrum at 266 nm, but when combined with Nicotinic Acid in a mass ratio of 1/20, M^+ and $(M+2H)^+$ ions of Acetyl-Alanine appear. Further experiments on matrix assisted desorption of larger organic molecules are in progress.

Fig. 5 and Fig. 6 compare laser desorption vs. SIMS of the same sample of DNP-Proline.

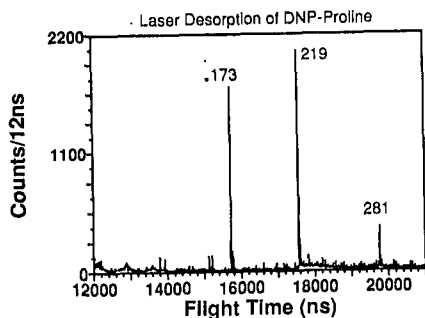


Fig.5

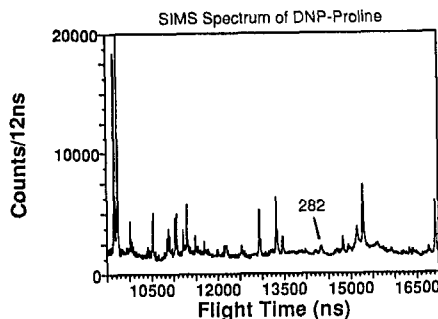


Fig. 6

LASER DISSOCIATION:

We are investigating photo-dissociation as a tool for obtaining sequence information on biomolecules. For that purpose the laser was focused onto the accelerated secondary ion beam of dinitrophenyl-derivatised peptides produced by a 20 keV Cs^+ ion beam in a reflecting time-of-flight spectrometer (Manitoba TOF II) [3]. Alternate-pulse data-acquisition of events with and without laser dissociation, and subtraction of the separately stored spectra, enables separation of different decay pathways.

Preliminary results show that photon bombardment can produce considerable additional fragmentation. Most of the normal metastable decay pathways are suppressed and the decomposition may follow only one of a few decay channels.

Fig. 7. shows the daughter ion mass spectra of a dinitrophenyl octapeptide, where only the ions around mass 86 u are enhanced by the laser interaction, as seen by comparison with the corresponding SIMS spectrum (Fig.8). In this case no new sequence information is produced. Further photodissociation experiments with different laser parameters are currently in progress.

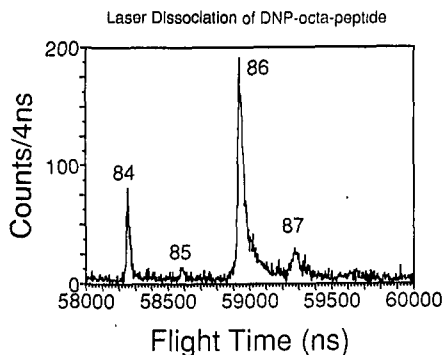


Fig. 7

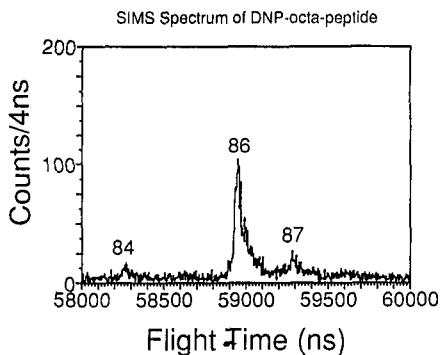


Fig. 8

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PULSED LASER INDUCED ELECTRON GENERATION
FOR ELECTRON IONIZATION MASS SPECTROMETRY.

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The advances of Multiphoton Ionization mass spectrometry compared to other methods has been described in literature [1]. The unique features of this method together with a reflectron-time-of-flight instrument include high mass range, ionization leading to radical cations, selective and very efficient ionization by resonant MUPI, the possibility to change the fragmentation from soft to hard by merely adjusting the ionizing photon density and simultaneous collection of all ions. Due to the intrinsic mechanism of the multiphoton activation of a molecule the ionization efficiency is largely dependent on the wavelength used.

A desirable feature for the instrument would be a simple EI-source with sufficient sensitivity to monitor the all neutral molecules produced in the desorption process [2]. Electron impact ionization complements the selective resonant MUPI, in the sense that all compounds are ionized, ionization efficiencies do not vary greatly from one compound to the next. For this reason a good ion source should give the possibility of producing MUPI- as well as EI-mass spectra nearly simultaneous.

Therefore we have investigated the possibilities of producing short electron pulses by shining UV-light from a frequency doubled dye laser to a metal surface. The ion source is shown schematically in figure 1. The modification of the MUPI ion source required for EI-ionization consists merely of a 0.25 mm Ta wire or a small stripe of Cu held in place between the repeller and the first extraction lens of the ion source at an electric potential intermediate to that of the two mentioned plates. The wire crosses both the molecular beam and the laser beam normally used for MUPI, and is perpendicular to both these beams. If photons in the wavelength region between 290 nm and 240 nm hit the wire or the stripe, a swarm of electrons instantaneously leave the surface according to the photo-electric effect. Using a 15 μ J pulse (10^{10} W/cm²) yield in the formation of $1 \cdot 10^7$ electrons from the Ta wire and $2 \cdot 10^8$ from the Cu stripe. These electrons are accelerated to the repeller and ionize incoming sample molecules in the supersonic jet. Molecules closer to the wire at the time of the electron pulse are ionized with less energetic electrons than those nearer to the repeller. However, the point of incidence not only defines the electron energy, but also the initial electric potential of the ion formed in the accelerating field. By adjusting the voltage on the ion reflector to selectively reflect ions of limited kinetic energy (and therefore initial electric potential), narrow peaks can be obtained at the detector. By realigning the laser beam (without focussing) to just miss the Ta wire,

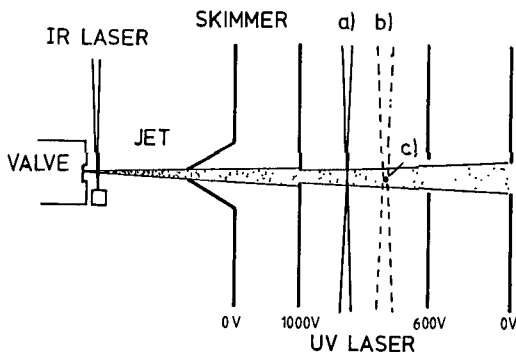


Fig. 1 Experimental setup of the combined multiphoton - electron ionization source. a) UV-laserbeam for MUPI, b) UV beam for EI, c) Ta-wire or Cu stripe.

all ions in the spectra disappeared completely, thereby eliminating the possibility of a contributing photoionization process.

Such experiments were already published by Honig et al. [3]. But in these cases electron beams in the microsecond timescale could only be obtained because the thermionic electron emission dominates when photon energies are involved that lie below the work function of the metal. In our case similar results can be obtained if photons with an energy below 292 nm strike the Ta wire (lower than the work function of Ta (4.25eV) or the Cu stripe (work function Cu (4.84eV)). In this cases the strong signals for the metal ions are observed.

Figure 2 and 3 displays some results from investigations with this EI-source. The mass spectrum in figure 2 shows the accumulation of 100 spectra of propene in He gas, introduced via the pulsed valve. The spectrum is nearly identical to literature EI spectra.

Figure 3 shows the accumulation of 100 EI spectra of caffeine, introduced into the pulsed molecular beam of He via IR-laser desorption from the probe tip indicated in figure 1. The spectrum shows good correlation with literature spectra and displays a molecular ion with enhanced intensity. This very desirable feature - also observed with other compounds - is probably due to the low temperature of the molecules in the supersonic beam rather than the slightly lower electron energies involved in the ionization process.

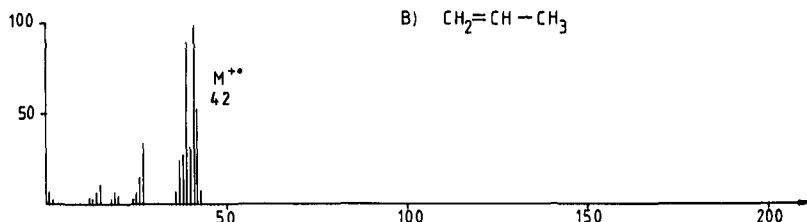


Fig. 2. Mass spectrum of Propene (Helium signal suppressed).

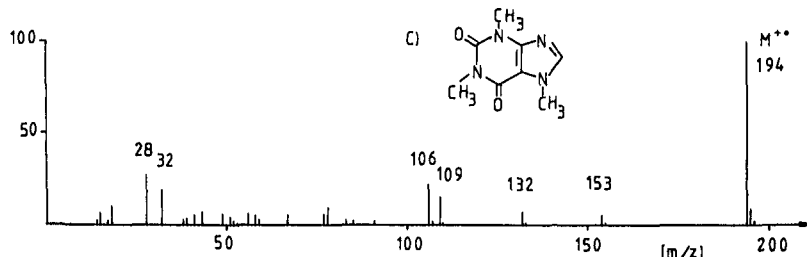


Fig. 3. Mass spectrum of caffeine obtained by laser evaporation - electron impact.

ACKNOWLEDGEMENTS.

Support from the Bundesministerium für Forschung und Technologie, the Deutschen Forschungsgemeinschaft and Bruker-Franzen Analytik (Bremen) is gratefully acknowledged.

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Multiphoton Ionization Mass Spectrometry :
Steroids and Nucleotides

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In this work we present the investigation of Steroids and Nucleotides using Infra-Red (IR) laser desorption of neutral molecules followed by resonantly enhanced Multiphoton Ionization (MUPI).

The soft and hard MUPI-MS of the following steroids are reported: 16-Epi-estriol, 17-Epi-estriol, 16,17-Epi-estriol, Estriol-16,17-diacetate and Estriol-3-methyl-ester.

For the first time we report MUPI-MS of nucleotides. The molecular ion was detected for DMT-thymidine triester (MW = 788,2) as well as for Bz-DMT-deoxyadenosine triester (MW = 901); Bz = Benzoyl, DMT = Dimethoxytrityl.

The mass spectrometer used is a BRUKER TOF1 and has been described in detail elsewhere [1]. The features of Multiphoton Ionization Mass Spectrometry include separation of desorption and ionization in time and space, selective and very efficient ionization by resonant multiphoton ionization, the possibility to change the fragmentation from soft to hard by merely adjusting the ionizing photon density and simultaneous collection of all ions [2].

Figure 1 shows the soft MUPI-MS of 16,17-Epi-estriol, the ionizing wavelength being 270.0 nm. The hard MUPI-MS of steroids show intensive breakdown pattern, due to the decomposition of the macrocycle. The EI- mass spectra of steroids reveal (M-18)⁺-peaks, which can be assigned as a dehydration reaction [3]. In contrast, no (M-18)⁺ was observed in the MUPI-MS of the investigated steroids.

The second, even more interesting group of organic compounds analyzed with MUPI-MS are the nucleotides. To improve the desorption process, the samples are encased in a polyethylene tablet mixed with finely divided gold powder. This minimizes the fragmentation during the desorption process. The ionization wavelength was 240.0 nm.

Figure 2 shows the partial hard MUPI-MS of Bz-DMT-deoxyadenosine triester (MW= 901,0). Beside the molecular ion an interesting peak at mass 598 Da occurs, which can be assigned as the counter ion of the DMT-ion, (M-303)⁺. In the MUPI-MS of DMT-thymidine triester (MW= 788,2) the molecular ion is clearly observable, whereas further mass peaks can be found in the lower mass region at m/z= 334, 320, 303 and 240. The dominant peak is mass 303 Da, corresponding to the DMT- ion, which is very stable.

The results of the hard MUPI-MS of the steroids demonstrate, that the absorption of three UV-photons leads to the generation of small fragment ions. In order to get larger fragment ions, the third photon has to be lower in energy. We will carry out two color experiments, to test the validity of this thesis.

The nucleotide MUPI-MS indicate, that the matrix assisted laser desorption is a good tool to desorb fragile involatile compounds.

ACKNOWLEDGEMENT.

The authors would like to thank the Bundesministerium für Forschung und Technologie, the Deutschen Forschungsgemeinschaft and Bruker-Franzen Analytik (Bremen) for supporting this work.

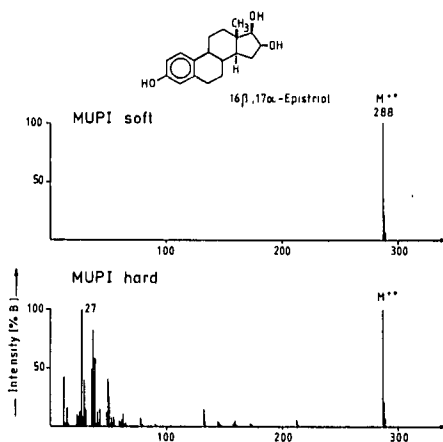


Fig. 1 Soft and hard multiphoton ionization mass spectra of 16β-17α Epiestrol.

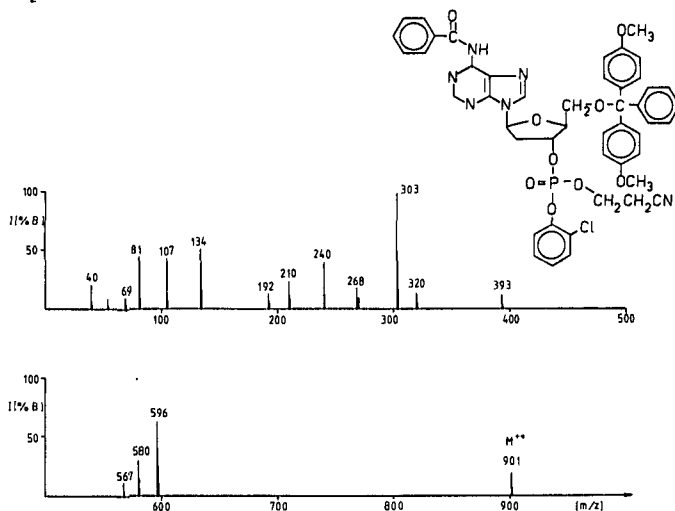


Fig.2 Soft and hard Multiphoton ionization mass spectrum of Bz-DMT-Deoxyadenosine-triester.

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RESONANCE ENHANCED MULTIPHOTON IONIZATION DETECTION OF THE THERMAL
DECOMPOSITION PRODUCTS INDUCED BY LASER DESORPTION OF OLIGOPEPTIDES

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In this work, laser desorption is used to volatilize labile peptides into the gas phase as neutral species. The molecules are then entrained into a supersonic jet expansion, transported into a time-of-flight mass spectrometer (TOFMS) and ionized using resonance enhanced multiphoton ionization (REMPI). It is shown that the products detected by REMPI in the TOFMS depend upon the conditions of the desorption process. If a thin layer of material ($\sim 1 \mu\text{m}$) or the material dissolved in a glycerol matrix is used in the desorption process then molecular ions of peptides can be detected by REMPI. When the laser power is raised then fragmentation characteristic of REMPI is observed. However, if a thick layer of material ($> 10 \mu\text{m}$) is used for desorption, a strong M-18 ion peak is observed in the TOFMS. As the laser power is increased, then fragmentation characteristic of the M-18 ion is observed rather than fragments characteristic of REMPI. It is demonstrated that the resulting thermal decomposition product from the laser desorption process in these thick samples is a 2,5-diketopiperazine which is formed by cyclization of the amino acid moieties in position 1 and 2 from the peptide with elimination of water. In addition, it is shown that 2,5-diketopiperazines can be formed in the laser induced thermal decomposition process for peptides and their derivatives whose N-terminal group is not blocked. This is demonstrated for species ranging from di- to pentapeptides as shown in Table I.

As an example, Figure 1(a and c) shows the R2PI/MPI induced mass spectra of the two isomeric dipeptides Tyr-Leu and Leu-Tyr desorbed from thin layer samples by pulsed infrared radiation ($10.6 \mu\text{m}$). These spectra were taken at an ionization laser power of $\sim 5 \times 10^6 \text{ W/cm}^2$ which induced some fragmentation which is useful for structural studies. The molecular ion (m/z 294) is observed as a strong peak in each case and the fragmentation observed is distinctly different for the two isomers, the laser power is decreased, the molecular ion only observed without fragmentation.

Figure 1(b and d) shows the R2PI/MPI induced mass spectra of the same two peptides desorbed from thick samples on the probe surface. The mass spectra obtained are almost identical for the isomeric dipeptides. The molecular ion is visible only as a very minor peak while the $[\text{M}-18]^+$ ion (m/z 276) forms the base peak in the mass spectrum. The ratio between the signal intensities of the M^+ and $[\text{M}-18]^+$ peaks can be varied by changing the position of the infrared beam on the surface. It is assumed that the decomposition is thermally induced as the thick sample absorbs the radiation.

The laser-induced R2PI/MPI mass spectrum of a synthetic sample of cyclo-(Leu-Tyr) is shown in Fig. 2. This spectrum is similar to that observed in Fig. 1(b and d) minus the molecular ion peak. The peak at m/z 86 in Fig. 1(d) is an MPI-induced fragment which originates from the intact molecule. Thus, we conclude that the species formed from thermal decomposition during the laser desorption process is indeed the cyclic dipeptide or 2,5-diketopiperazine.

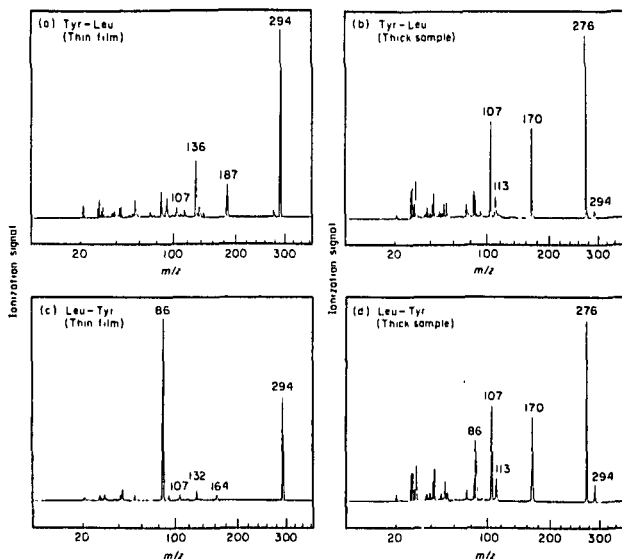


Figure 1. R2PI/MPI mass spectra of (a) Tyr-Leu and (c) Leu-Tyr obtained using a thin film of sample formed on the desorption surface. R2PI/MPI mass spectra of the thermally decomposed species induced during the desorption of (b) Tyr-Leu and (d) Leu-Tyr. Ionization was produced using $\lambda = 266$ nm at a power density of 5×10^6 W/cm². The desorption laser power density was $\sim 5 \times 10^6$ W/cm².

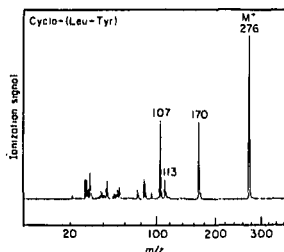


Figure 2. R2PI/MPI mass spectrum of cyclo-(Leu-Tyr)

Table I. The characteristic fragment ions of the 2,5-diketopiperazines obtained by R2PI/MPIA of the laser-induced thermal decomposition products of the linear peptides

Original linear peptides	Thermal decomposition product	Mol. wt.	Major fragments (m/z (rel. int. %) of cyclo-(di-peptide)
Gly-Tyr	cyclo-(Gly-Tyr)	220	220(100), 114(24), 107(77)
Tyr-Gly	cyclo-(Gly-Tyr)	220	220(100), 114(25), 107(73)
Tyr-Gly-Gly	cyclo-(Gly-Tyr)	220	220(100), 114(24), 107(76)
Tyr-Gly-Gly-Phe-Met	cyclo-(Gly-Tyr)	220	220(100), 114(24), 107(76)
Leu-Tyr	cyclo-(Leu-Tyr)	276	276(100), 170(48), 113(13), 107(47)
Tyr-Leu	cyclo-(Leu-Tyr)	276	276(100), 170(47), 113(15), 107(47)
Ile-Tyr	cyclo-(Ile-Tyr)	276	276(100), 170(45), 113(21), 107(58)
Trp-Leu	cyclo-(Leu-Trp)	299	299(86), 170(5), 130(100), 103(5), 77(15)
Leu-Trp-Met	cyclo-(Leu-Trp)	299	299(88), 170(8), 130(100), 103(5), 77(18)
Leu-Trp-Met-Arg	cyclo-(Leu-Trp)	299	299(80), 170(10), 130(100), 103(10), 77(20)
Leu-Trp-Met-Arg-Phe	cyclo-(Leu-Trp)	299	299(86), 170(10), 130(100), 103(9), 77(15)

^aThe ionization wavelength was 266 nm and the power density was 5×10^6 W/cm².

FRAGMENTATION OF CATIONIZED BIOMOLECULES IN LASER DESORPTION USING A CO₂ LASER

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Soft ionization techniques like Laser Desorption (LD), Fast Atom Bombardment (FAB) or Plasma Desorption (PD) have the valuable capability to create parent molecular ions of large organic molecules without significant fragmentation. Nevertheless, fragmentation is an important prerequisite for obtaining structural information about the analyte.

Compared to PD and FAB, LD can be more widely varied between soft and hard ionization. Laser fluence as well as wavelength are important factors controlling energy deposition and thus fragmentation. In addition, the process of cation attachment can strongly influence fragmentation in infrared laser desorption (IR-LD).

In this study the fragmentation behavior of organic molecules cationized by different alkali ions is described for IR-LD. K⁺-attachment leads to soft ionization for a wide variety of different compounds. However, Li⁺-attachment in general leads to an even higher amount of fragmentation and thus structural information. Increased fragmentation has been reported before in collision induced dissociation experiments of lithium attached molecules [1].

Studies on tryptophan support the assumption of gas phase attachment of neutral molecules by alkali ions in IR-LD, and show differences, as well as parallels to UV-LD.

IR-laser desorption mass spectra were obtained using a CVC-2000 time-of-flight mass spectrometer (Wiley-McLaren type), equipped with a pulsed CO₂ laser (10.6 μ m, $t=40$ ns) [2]. The delay between the laser pulse and the drawout pulse is variable (10-80 μ s), allowing the investigation of time dependant processes.

The mechanism of ion and neutral desorption, alkali attachment and fragmentation has been studied on tryptophan. Directly desorbed ions are detected at short delays. Because they have higher initial kinetic energies than desorbed neutrals [4-6] they will have escaped the acceleration region at longer delay times and thus can be distinguished from ions formed in the gas phase from desorbed neutrals. The generation of the desorbed ions is assumed to take place in the condensed phase or even in a high particle density region closely above the surface, where the collision probability is comparable to CI-conditions.

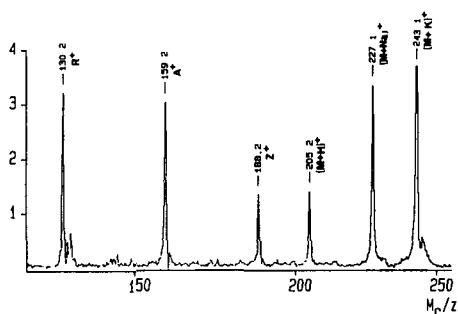


Figure 1
Tryptophan, short delay, no lithium added

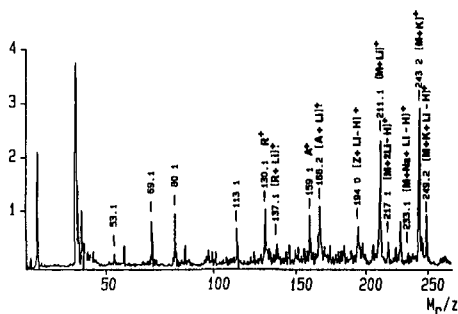


Figure 2
Tryptophan, long delay, lithium added

The IR-LD mass spectrum of tryptophan at short delay times shown in Figure 1 is very similar to the UV-LD mass spectrum [7], where generation of ions is assumed to take place in the condensed phase. Only the alkali attached parent molecules are not observed for tryptophan in UV-LD. They are observed both at short and long delays in IR-LD and are assumed to be generated by gas phase attachment of desorbed neutral molecules. At long delay times only the potassium attached molecule and the K^+ -ion are observed for tryptophan. The desorbed ions ($M+H^+$, F^+) have already moved out of the acceleration region.

Addition of Lithium (Figure 2) leads to increased fragmentation. Besides alkali free fragment ions ($M=130, 159$), lithium attached ($M=137, 166$) and lithium-hydrogen exchanged fragments ($M=194$) are observed.

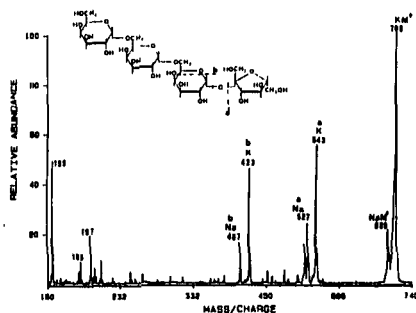


Figure 3
Stachyose, no lithium added

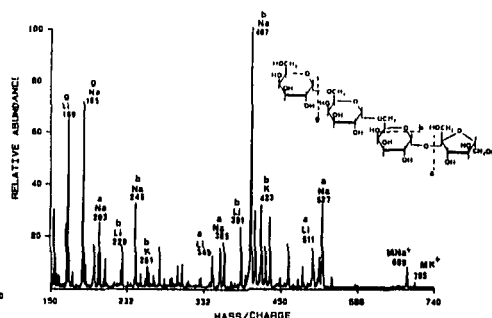


Figure 4
Stachyose, lithium added

Desorption of neutral fragments is not observed for tryptophan, indicated by the lack of potassium attached fragments. This is an important difference from UV-LD [6]. The data gives an explanation for the large amount of fragments, observed by UV positionization of UV-laser desorbed molecules [7], compared to UV-positionization of IR-laser desorbed molecules [8]. Another difference to UV-LD is that, in general, no alkali attached fragments are observed in UV-LD. This supports the idea, that alkali attachment is a gas phase process in IR-LD, whereas it is a condensed phase process in UV-LD [9].

Applications of admixing of lithium salts to substances of analytical interest show increases in structural information. Figure 3 shows the IR-LD mass spectrum of stachyose. IR-LD of stachyose after addition of half the molar amount of lithium bromide is shown in Figure 4. Fragmentations are characteristic two bond ring and glycosidic cleavages. Increase in fragmentation and thus structural information has been observed as well for peptides, like His-Ser-Gln-Gly-Thr-Phe and Leu-Trp-Met-Arg-Phe-Ala.

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ISOTOPE ANALYSIS IN INVOLATILE SAMPLE BY LASER DESORPTION ELECTRON IMPACT (LD/EI)

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Progress in desorption techniques has permitted the extension of mass spectrometry to analysis of involatile and thermally labile molecules such as glutathione conjugates. Most of these techniques produce even electron molecular ions, whose unimolecular fragmentation proceeds by closed shell processes and elimination of neutral molecules. Homologous cleavage of carbon-carbon bonds is not observed. The enzyme mechanism study reported here hinges on the analysis of deuterium labels on adjacent methylene groups in an involatile glutathione conjugate of cyclophosphamide. The bond between these methylene groups in unconjugated cyclophosphamide readily cleaved under electron impact. We have undertaken to combine laser desorption of neutral molecules and ionization by electron impact into an analytical technique that will accommodate the involatility of the sample and produce the carbon-carbon bond cleavage required for the isotope analysis.

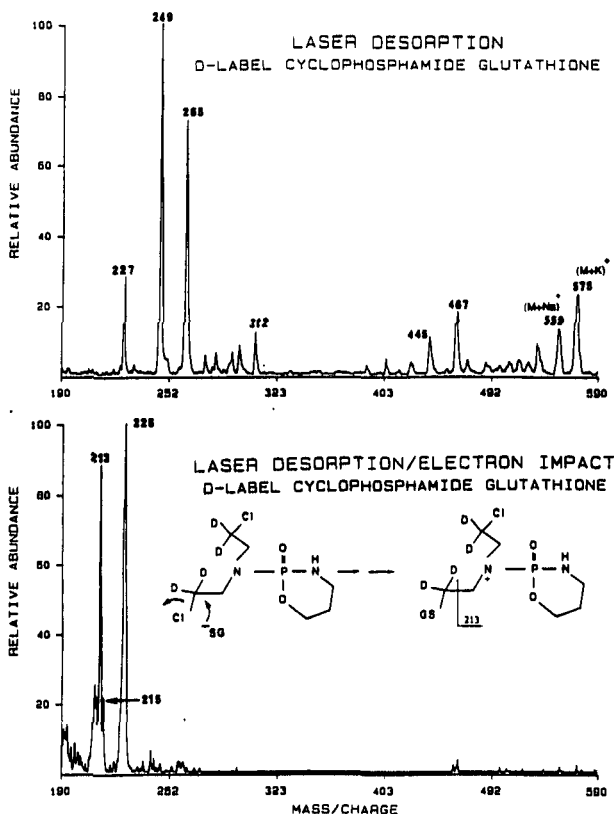
Toward the objective of understanding the role of glutathione in the cross resistance which some patients develop to alkylating antitumor agents. We have demonstrated that the conjugation with melphalan, chlorambucil and, in the present work, cyclophosphamide is catalyzed by glutathione transferases. In aqueous solution nitrogen mustards exist in equilibrium as amines and cyclic aziridinium ions. It was of interest to determine which (or both) of these forms is the substrate for glutathione transferase(s). To do this we took advantage of the fact that the alpha and beta methylene groups lose their positional identities in the symmetric aziridinium intermediate. Consequently, deuterium labels at one position in cyclophosphamide can end up either methylene group in the conjugated product. As a class, glutathione conjugates are involatile and thermally labile, and they have not been successfully analyzed by electron impact, even derivatised. The monoglutathione conjugate derived from cyclophosphamide was no exception. However, the combined LD/EI technique was successfully applied to this analysis to bring neutral sample molecules into the gas phase, where ion radicals were produced by electron impact. Cleavage of the carbon-carbon bond alpha to the mustard nitrogen indicated that no scrambling had taken place in either the enzymatic reaction or chemical reaction, i.e. the chloride ion of cyclophosphamide is directly displaced by glutathione during the conjugation reaction.

Experimental

LD and LD/EI mass spectra were obtained on a modified CVC Products (Rochester, NY) Model 2000 time-of-flight mass spectrometer. A Tchisto (Needham, MA) model 215G pulsed carbon dioxide laser provides 40 nS pulses with energies up to 700 mJ at a wavelength of 10.6 micrometers. The laser beam is focussed by a 19 inch focal length

ZnSe lens, passes through a ZnSe window in the vacuum housing and enters the pulsed electron impact source. LD mass spectra were obtained by turning the electron filament current off and the time lag focus to zero. Samples were applied to the probe tip in solution of KCl. The time delay between the laser pulse and the mass spectrometer trigger pulse was adjusted to produce well resolved M+K molecular ions. For the LD/EI mass spectra, the laser spectra, the laser power was reduced just below the onset of K⁺ ion emission and formation of M+K ions. This insured that only neutral species were desorbed. The electron filament current was then turned on, and the time lag focus was adjusted to produce the best energy focussing. Glutathione conjugates of cyclophosphamide were purified by RP-HPLC.

The figure below presents the LD and LD/EI mass spectra of deuterium-labeled monogluthathione conjugate of cyclophosphamide. The fragmentation shown locates the deuterium labels in the original carbon.



AN EFFECTIVE METHOD FOR ASSISTING LASER DESORPTION OF NON-VOLATILE COMPOUNDS

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INTRODUCTION

Laser Desorption Mass Spectrometry has found increasing applications for organic compounds. Matrices for assisting laser desorption of non-volatile compounds have been successfully used by several researchers. A simple method introduced here is using common solvents (water, methanol, toluene,...) as liquid matrices to assist laser desorption of non-volatile compounds.

EXPERIMENTAL

The instrument used is a LAMMA-1000 Laser Microprobe Mass Spectrometer.

1 μ l of sample solution is placed onto a thin Formvar film [Poly(vinylformaldehyde)]. The film is pulled together into a ball that keeps the sample solution sealed under vacuum in the ion source region. The ball is then introduced into the ion source chamber by the aid of a double sided sticky tape attached to a metal substrate.

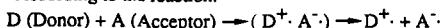
Another method is to use nitrocellulose membranes or soft, fine fibrous paper as sample substrate. It is easier to handle and gives more reproducible results.

RESULTS

Spectra of different kinds of compounds such as Vitamin B12, Chlorophyll a, Erythromycin, Stachyose, Bradykinin, Gramicidin S and Angiotensin have been obtained. The molecular ions or the pseudo molecular ions are greatly enhanced. Spectra shown in Fig. 1 and 2 demonstrate the ability of this method to keep common solvents under vacuum. The spectrum of toluene was taken after it was introduced into the ion source for 20 minutes. For comparison, a standard EI spectrum of Toluene is shown on the right top corner. the spectrum of glycerol with lithium carbonate is shown in Fig. 3, which cannot be obtained in the normal way by LAMMA-1000, and is similar to Cooks' (1) results. No cluster ions of glycerol appear in the spectrum. Spectra shown in Fig. 4, 5 and 6 are the results of different kinds of samples. The $(M+H)^+$ ions of Vitamin B12 shown in Fig. 4 demonstrate the "soft ionization" character of this method. It has not been observed by the use of nicotinic acid as matrix in the LAMMA-1000.

The method provides the possibility of the application of solution chemistry in LD-MS. An example of the charge-transfer derivatization is shown in Fig. 7 and 8.

According to the reaction:



Pauw (2) found that the FAB spectra match quite well with the solution chemistry by using N,N,N',N'-tetramethyl-1,4-benzenediamine (TMPD) as a donor and Benzoquinone (BQN) as an Acceptor. The spectrum of TMPD + BQN shows a prominent peak at M^+ (m/z 164), $\text{TMPD} + \text{NH}_4\text{Cl}$ at $(M+H)^+$ (m/z 165), and the neat TMPD at $(M-H)^+$ (m/z 163). Busch (3) found that the effect of using charge-transfer agents for derivatization will be more pronounced with the solid complex in the case of using glycerol as matrix.

Fig. 7 and 8 demonstrated that by using the method of keeping the sample solution in vacuum, the spectra of laser desorption match quite well the solution chemistry.

CONCLUSION

Using common solvents as liquid matrices has several advantages compare with other liquid matrices used in desorption techniques: Both polar and non-polar compounds are soluble; have very low viscosity; are chemically inert; no spectral background interference and have high dielectric constants.

The contributions of using common solvents as liquid matrices by this method for laser desorption are as follow:

- 1). Liquids are better adapted for promotion of ion formation.
- 2). The solution phase lowers the energy required to desorb ions by solvation and separation.
- 3). Desolvation in the gas-phase can carry away the excess internal energy of the molecular ions.
- 4). Explosive events happened during high power density laser radiation in the solution assisting desorption and clusters formation.

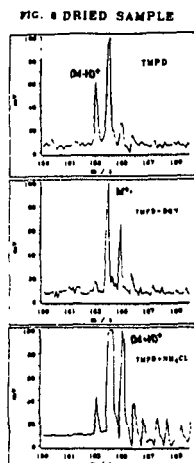
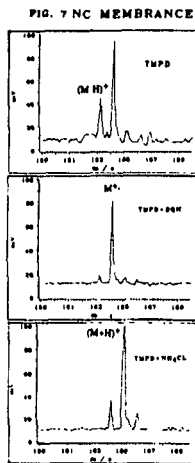
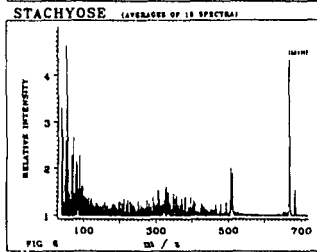
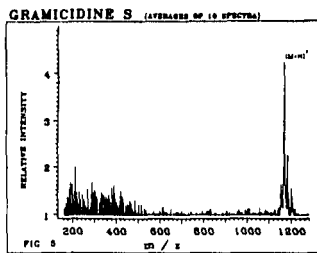
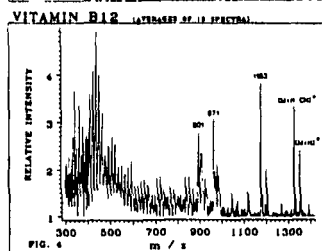
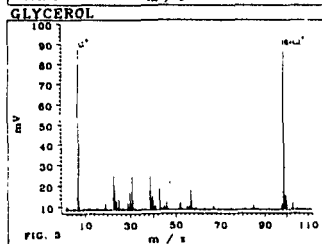
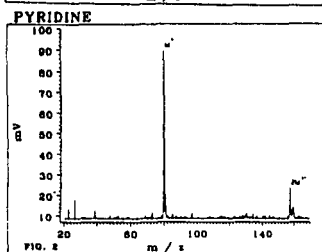
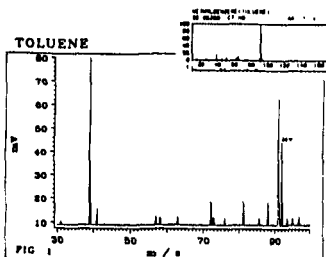
Moreover, it provides the possibility of application of solution chemistry in laser desorption mass spectrometry and the possibility of applying varieties of matrix used in other desorption techniques in LD-MS. Some of these applications are being investigated by the authors.

ACKNOWLEDGEMENT

The assistance of Dr. Kasi Somayajula and David Lineman is gratefully acknowledged.

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CHARACTERIZATION OF A GLOW DISCHARGE PLASMA AS A FUNCTION OF SAMPLING ORIFICE POTENTIAL

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INTRODUCTION

Glow discharges can provide stable ion sources for use with mass spectrometry. Much of the recent research regarding glow discharge mass spectrometry (GDMS) has focused on the development of glow discharge quadrupole mass spectrometry (GD-QMS) (1,2). In order to obtain optimum sensitivity in these devices, analyte ion signals must be maximized. It has been observed that placing a small positive bias on the sampling orifice plate (which is immersed in the plasma) results in a signal enhancement. For the purposes of optimizing instrument performance, the objective of this study is to characterize plasma parameters such as electron temperature, plasma potential, and charged particle densities as a function of the sampling orifice bias. This is carried out with the use of Langmuir probes. Details of the mathematical manipulations used here have been outlined elsewhere (3).

EXPERIMENTAL

Data were obtained with the use of an EXTREL EXT 1000 glow discharge mass spectrometer which is equipped with a sampling orifice plate to which a potential can be applied. The discharge was operated at 1 kV and 3 mA with an argon pressure of 700 mtorr. The cathode was 303 stainless steel. The Langmuir probe was a piece of tungsten wire 0.24 cm in length and 0.127 mm in diameter, which was positioned 4 mm from the cathode. The voltage on this probe was varied from -24 to +24 V during which time the collected current was measured. In this way, the I-V characteristic curves were generated from which all other plasma parameters were derived.

RESULTS AND DISCUSSION

A plot of plasma potential as a function of orifice bias is presented in Figure 1. This data illustrate that the plasma potential can be correlated directly to orifice bias for orifice voltages greater than 0V. When the bias on the sampling orifice is at or below 0V this relationship breaks down. In addition, the translational energy of the analyte ions with respect to the mass spectrometer is also directly related to the voltage placed on the sampling orifice.

The effect of sampling orifice bias on electron temperature is given in Figure 2. Here we observe that this parameter also correlates positively with the voltage

applied to the sampling orifice, but to a lesser extent than the plasma potential. The increase in electron temperature over the range of orifice potentials examined here is approximately 0.2 eV. In addition, our results indicate that there is no significant dependence of charged particle density on orifice bias.

CONCLUSION

The fact that plasma potential and ion energy both correlate with positive orifice bias indicates that ion energy is defined by the plasma potential, which is itself defined by the voltage placed on the sampling orifice. Furthermore, the weak dependence of electron temperature on orifice potential suggests that no significant increase in ionization is occurring in the plasma by either Penning ionization by Ar metastables or electron impact. This assertion would appear to be borne out by the relative insensitivity of the charged particle density to orifice bias. Therefore, we can conclude that mass spectrometric signal enhancements resulting from positive orifice voltages are due to improvements in ion transport to the mass spectrometer rather than being the result of increased ionization.

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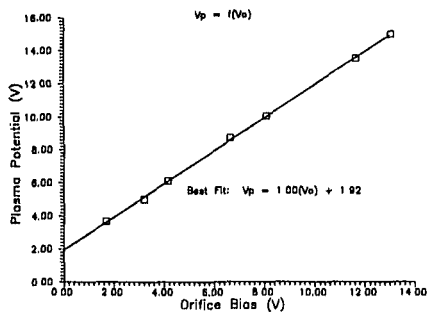


Figure 1.

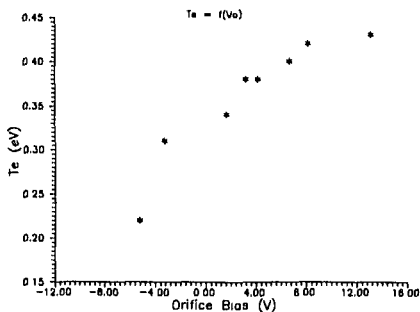


Figure 2.

ELECTROHYDRODYNAMIC EMISSION MASS SPECTRA OF PEPTIDES

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Electrohydrodynamic (EH) emission is an unusually mild ionization method which rarely if ever results in fragmentation of the sample molecule. Ions are extracted directly from solution by a high electric field. In our previous work, we have characterized the output from an EH source and found, in addition to sample and solvent ions, solvent cluster ions with mass to charge ratios extending beyond the range of our mass spectrometer (11,500). As a consequence of this "droplet emission, most of the sample is trapped in large solvent clusters and is not observed. Thus, the required sample amounts for EH spectra of peptides are unacceptably high for most applications. This work describes a modification of our EH source intended to circumvent some of these problems.

The output from the EH nozzle (Figure 1) is focussed with a series of lens elements into a gas filled collision cell. Ions are extracted from the other end of the collision cell and focussed onto the first slit of our JEOL HX100HF double focussing mass spectrometer. Length of the collision cell was 2.5 cm. Argon was used as the collision gas in most instances although some experiments were done with isobutane. Sample solution is transported to the EH nozzle by a 30 cm X 25 um ID fused silica capillary from sample tubes at atmospheric pressure. All samples were dissolved in 1.5 M ammonium acetate in water : methanol (60:40) at a concentration of 1 ug/ul. Spectra were acquired using a JEOL DA5000 data system.

The effectiveness of the collision cell for desolvating sample ions residing in charged solvent droplets was first evaluated by measuring the total ion transmission through the cell with a Faraday cup. Initially, the transmitted current sharply decreases with small changes in pressure. The current then reaches an inflection followed by a region of gradual decrease with increasing pressure. The shape of the curve suggests that the beam incident on the collision cell consists of two distinct components. The sharp decrease was attributed to the scattering of small ions. The gradual decrease following the inflection was attributed to charge loss from more massive droplets which undergo partial or complete desolvation. The shape of the curve has been successfully modeled by empirically fitting values for the scattering cross sections for the molecular ions and the large solvent droplets.

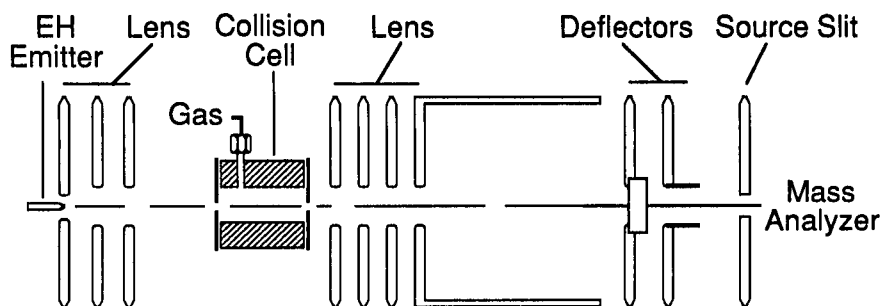
Spectral data appear consistent with the scattering model. When spectra were taken of a pentapeptide with no collision gas (Figure 4), a spectrum was obtained similar to those obtained with previous source designs. The ion and droplet components are clearly evident. As gas is introduced into the cell, the ion component

of the spectrum quickly disappears and the apparent distribution of the droplets is shifted to higher m/z values. Further increases in the pressure nearly eliminate the droplet component from the spectrum. The ion spectrum reappears, but only if the potential of the collision cell is carefully matched to the electric sector voltage.

With the new source, some of the objectives have been achieved. It is now possible to obtain molecular ions for peptides less than 1000 MW on a routine basis using much less sample than was previously necessary. In some instances, sample concentrations as low as 0.1 $\mu\text{g}/\mu\text{l}$ are sufficient. With increasing molecular weight, molecular ion signals decrease dramatically, and little success has been achieved beyond mass 1000. For the larger structures, adducts of sample with components of the solvent indicate full desolvation has not been achieved. Interestingly enough, although the instrument slits have been set for a resolution of 500, ions at higher mass are fully resolved, an indication that ions extracted from the collision cell are well focussed and have a uniform energy.

One apparent problem is the inability of the collision cell to stop all of the droplets. This problem may be solved by operating the EH nozzle at a lower potential. It remains to be seen whether full desolvation of large sample ions can be achieved under conditions compatible with our mass spectrometer.

Figure 1.



Positron Ionization Mass Spectrometry

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Physicists have extensively studied the interactions of positrons with matter since the initial prediction of positrons by Dirac and their discovery by Anderson in the early 1930's. These studies have taken several forms: measurements of scattering cross-sections, lifetime spectroscopy, and angular or energy analysis of the annihilation gamma radiation. The ways in which positrons interact with molecular gases have been elucidated and will be discussed elsewhere (1) in this meeting. Briefly, positrons can ionize molecules by impact at high energy, by positronium formation at energies up to 6.8 eV below the ionization energy of the molecule, and by non-resonant (pick-off) or resonant (attachment) annihilation at energies below 1 eV.

The goal of our research is to study all of the above processes in order to understand the energetics and physics involved by interacting positrons of a selected energy with target molecules and measuring the mass spectra of the ions with a time-of-flight mass spectrometer.

Our source of positrons is a new facility attached to the Oak Ridge Electron Linear Accelerator (ORELA) which is described in (2) and shown schematically in Figure 1.

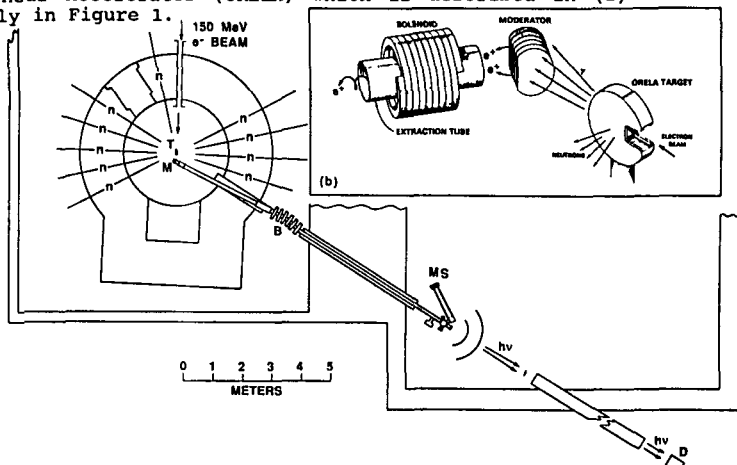


Figure 1. Overhead view of the ORELA positron source. T is the electron target, M is the positron moderator, B are bending solenoids, MS is the time-of-flight mass spectrometer, and D is a NaI(Tl) gamma-ray detector.

The ORELA is an induction-type accelerator which produces nanosecond pulses of 150 MeV electrons at an average power of 10 to 50 kW. The positrons are produced by the forward-scattered gamma bremsstrahlung striking a set of tungsten plates mounted directly behind the ORELA tantalum target. The tungsten moderator and pulsed extraction tube produce pulses of 3000 eV

positrons having pulse widths of 10 to 20 nsec at a repetition rate of up to 800 Hz. The number of positrons produced is 3×10^7 per second at 10 kW of ORELA power. These high-energy positrons can be used directly for positron impact ionization (yielding the spectra below) or they can be moderated with a thin tungsten film to produce low-energy (<3 eV) positrons with an efficiency approaching 40%. The pulses of positrons are directed into the ion source of a time-of-flight mass spectrometer where the ions produced are accelerated, mass-analyzed, and detected by a channelplate detector. The analyte species is introduced as a gas at pressures of 1×10^{-6} to 1×10^{-5} Torr. Figure 2, below compares the spectra obtained for isobutane and benzene under conditions of (a) 70 eV electron impact, (b) 2700 eV electron impact, and (c) 3000 eV positron impact. The spectra are quite similar, confirming the expectation that positrons and electrons act similarly at these energies. Current work is focussed on extending the energy range of the positrons down to below 1 eV where positron attachment can occur. Initial spectra from these studies are tantalizingly different.

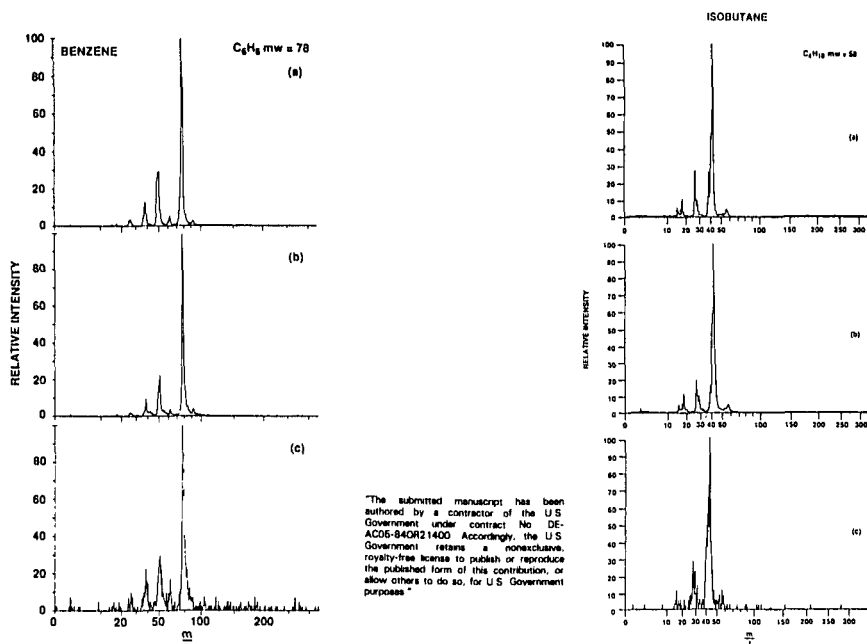


Figure 2. Mass spectra of isobutane and benzene under conditions of (a) 70 eV electron impact, (b) 2700 eV electron impact, and (c) 3000 eV positron impact.

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**ELECTROSPRAY IONIZATION-MASS SPECTROMETRY
AND TANDEM MASS SPECTROMETRY**

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Electrospray ionization coupled with mass spectrometry (ESI-MS) has its roots 20 years ago with the pioneering experiments by Malcolm Dole(1) and has been recently rejuvenated by the work from the laboratory of John Fenn, who first published elegant results of electrospray ionization mass spectra of large molecules with molecular weights up to 40 kilodaltons and demonstrated the unusual propensity for multiple charging produced by this soft ionization technique(2). The ability to produce multiply charged molecular ions for biomolecules allows mass spectrometers to analyze compounds with molecular weights (MW) much in excess of the instrument's mass (m) limit, but within the mass-to-charge (m/z) range of most conventional mass spectrometers. In fact, proteins with molecular weights in excess of 130,000 daltons have been successfully analyzed by ESI-MS with a quadrupole mass spectrometer of limited m/z range (1700) in our laboratory.

The mechanism for desorption of multiply charged molecular ions from highly charged liquid droplets is presently unclear. However, an ion evaporation model in which Coulombic explosion of rapidly evaporating liquid droplets of high charge density results in smaller sized charged droplets and continues this sequence until solute field-assisted desorption occurs is generally accepted. For peptide and proteins, we had previously observed(3) a crude correlation between the maximum positive (multiply protonated) charge state observed in our electrospray ionization mass spectra with the total number of basic amino acid residues (i.e., arginine, lysine, and histidine) in the primary sequence and to some extent, the N-terminal amino group. For example, glucagon (MW 3483) possesses 2 arginines, 1 lysine, 1 histidine, and the N-terminal amino group; its ESI mass spectra shows the $(M + 5H)^{5+}$ as the highest multiply charged species. However, the correlation for hen egg white lysozyme, and protein with 18 basic amino acids, is poor; the $(M + 14H)^{14+}$ is the highest charge state molecular ion observed. Little information is known regarding the effect of tertiary structure (either solution phase or gas phase) on the multiple charging phenomenon of electrospray ionization.

We have compared the ESI mass spectra of large proteins with and without reduction of its disulfide bridges. By cleavage of cysteine-cysteine bonds and thereby affecting the tertiary structure of the protein, a dramatic increase in the number of positive charges is observed. An ESI mass spectrum of egg white lysozyme shows the $(M + 20H)^{20+}$ as the highest charged species with the addition of 1,4-dithiothreitol (DTT). Disulfide-cleavage reactions may allow the protein to relax into more extended conformations, allowing "buried" basic amino sites to be more fully protonated. Over 175 positive charges are resolved in the mass spectrum for the albumin dimer molecule with the addition of DTT, an increase of over 50 charges relative to the unreduced form.

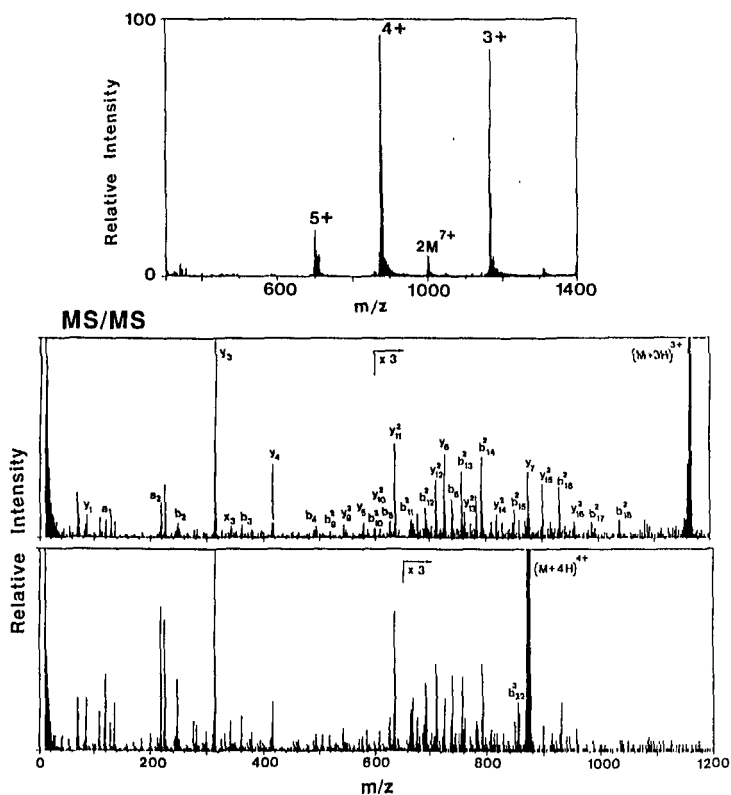
Highly charged ions desorbed from the droplets are sampled through a nozzle-skimmer orifice to be detected by the quadrupole mass spectrometer. The typical applied nozzle voltage for positive ion analysis is +200V with the skimmer at ground potential. Solvent clustering with the analyte is substantially reduced by collisions within this region. Increasing collision energies also yields significant fragmentation of large peptide and proteins. Singly and multiply charged sequence ions can be detected. Collisionally activated dissociation of selected multiply charged molecular ions for large peptides with MW > 3000 Da can easily be performed with a triple quadrupole MS to obtain structural information, with singly and multiply charged b and y type sequence ions generally dominating the spectra.

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*Pacific Northwest Laboratory is operated by Battelle Memorial Institute.

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B-Chain Insulin, Oxidized (MW 3496)



ISOTOPE RATIO MEASUREMENTS BY RESONANCE IONIZATION
TIME-OF-FLIGHT MASS SPECTROMETRY WITH A COPPER VAPOUR LASER
PUMPED DYE LASER SYSTEM

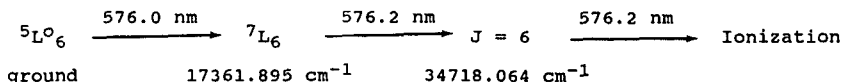
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In previous work we have shown that time-of-flight mass spectrometry (TOFMS) with multichannel gated pulse counting detection is well suited for quantification of the fast pulse ion signals generated by pulsed tunable dye lasers tuned to atomic resonant transitions. The precision and dynamic range of isotope ratio measurement was limited by the count rate of the ions which in turn was limited by the pulse repetition rate of the pump laser. We report here the application of a high repetition rate (5 to 7 kHz) copper vapour laser (CVL) and associated dye lasers to the measurement of 234/238 and 235/238 ratios in natural uranium.

An Oxford Lasers 40 W CVL was used to pump two kit dye lasers that had been adapted for high flowrate. Littman type oscillator cells were used with optional prism beam expanders; the dye laser bandwidths were 0.1 cm⁻¹ with the expanders, and 1.2 cm⁻¹ without. The pulse energy varied between 10 and 100 μJ. The TOFMS featured a two-stage acceleration region with static electrode potentials, a 5 m drift tube, and a microchannel plate detector. A mass resolution of 1400 could be obtained with this TOFMS, but a resolution of 600 was typically used for uranium isotopes. The gated pulse counter was configured for six signals: 234, 235, 238 and three background signals. Background counts were subtracted from isotope counts after each counting period.

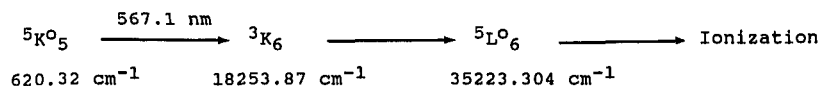
Well defined two colour resonance signals were observed for the 570 to 630 nm spectral region. For isotope ratio measurements, the prism beam expanders were removed and the scheme outlined below initially was used for resonance ionization.



Ion counts were accumulated at a rate 0.25 ²³⁸U ions/pulse until 1x10⁶ ²³⁸U counts were obtained (≈ 12 min) and the resultant isotope ratios are listed in Table 1, in which each ratio is an average of six consecutive measurements. The overall average 234/238 ratio is in exact agreement with the known value, and the precision is close to that expected (6% rsd) from Poisson statistics.

The results for 235/238 ratio showed that the observed uncertainty, 4%, was 8 times larger than that expected, 0.5%. This was attributed to steep dependencies of the isotope ratio on wavelength observed during the experiments (Figure 1), and to slight instabilities in the wavelength caused by mechanical vibrations and thermal drift. Steep dependence of the ratio on wavelength was observed over a narrow range, e.g. 0.02 nm, despite a laser bandwidth of 0.04 nm. Various excitation schemes were used, including one colour schemes and those with very small isotope shifts in the first step, but for none was the wavelength sensitivity low enough for precise 235/238 measurement. This problem was partly attributed to the unavoidable large isotope shifts, e.g. 0.4 cm⁻¹, in the second step. Possibly, the wavelength sensitivity could have been suppressed by use of sufficient dye laser pulse energy to oversaturate the transitions. Saturation of the second transition, in this case to a virtual state, was attempted by use of 50% of the CVL energy for ionization, but the wavelength sensitivity remained too high.

The 235/238 ratio was also dependent on orientation of the linearly polarized dye laser beams with respect to each other, for two-colour resonance excitation schemes. For example, a 12% enhancement of the 235/238 ratio was reproducibly observed for parallel vs perpendicular orientation, for the following scheme:



This result was consistent with the suppression of the ^{238}U photoexcitation rate predicted from polarization induced anisotropic distributions of the magnetic sublevels of the atomic states involved in the transitions.

Table 1. Observed Uranium Ratios

Set No.	Atomic Ratio to 238 $\times 10^6$	
	234	235
1	49	7111
2	60	7376
3	60	
4	51	7693
5	62	7869
6	55	7826
7	49	7300
$\bar{R}$	55	7.5×10^3
s	5	0.3×10^3
%rsd	9	4
known value	55	7.253×10^3

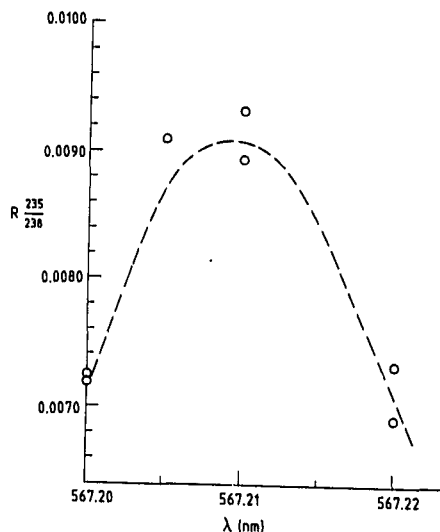


Figure 1. Wavelength sensitivity of 567.1 nm transition

DIODE LASER APPLICATION TO
RESONANCE IONIZATION MASS SPECTROMETRY

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We have demonstrated the use of semiconductor diode lasers to stimulate the first step in a resonant ionization process for lanthanum. These lasers are attractive for several reasons: They are inexpensive (\$75 for the laser and about \$5000 for the stabilized power supplies and temperature controller); they can be scanned by manipulating the temperature (coarse) and current (fine); they are rugged and reliable. The principal questions to be answered in the experiment were whether or not such lasers had sufficient power to produce appreciable numbers of ions and, if so, whether they would be stable enough for laboratory use. The answer to both these questions is yes.

Diode lasers are available with several central wavelengths about which they can be scanned over a limited range. One of these wavelengths is 750 nm, and we tuned the laser in question to 753.9 nm to stimulate the electronic transition in lanthanum from the ground state to the metastable state at 13260 cm^{-1} . Ionization required resonant absorption of a second photon to a higher state and subsequent absorption of a third photon to the continuum; these two photons were provided by a copper-vapor laser pumped dye laser.

Good signals were obtained, as shown in the figure. The bottom trace was taken with the laser detuned from 753.9 nm; the upper trace was taken with the laser tuned to that wavelength. The large peak centered at about 584.9 nm was due to the action of the diode laser. We thus demonstrated the suitability of diode lasers to applications such as these; we are presently assembling two more diode lasers so we can use them for all resonant steps leading to ionization.

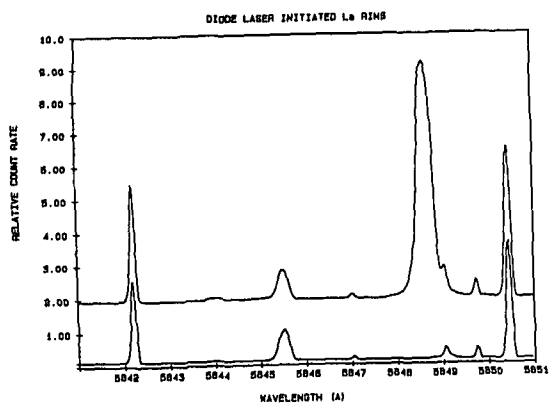
Using two of these lasers makes it possible to estimate the relative populations of various electronic energy levels in the atomic sample. This is of interest because resonance ionization can access only one level per laser; any atoms not in that state are lost to the experiment, reducing the efficiency of sample utilization. We estimated the ratio of the population of the ground state of lanthanum to that of the level at 1053 cm^{-1} . We obtained values (68 at 1350 deg. C and 38 at 1500 deg. C) that are higher than those predicted when one assumes a Boltzmann distribution. We ascribe this anomaly to optical pumping.

The optical spectra obtained with our apparatus had much higher resolution than we had anticipated. It was good enough to allow resolution of the hyperfine splitting of ^{139}La and to measure the constants associated with it. We believe this to be due to the selection of a velocity subset of the ions by the mass spectrometer's ion source, thus reducing the effects of Doppler broadening on the spectra.

It is possible to envision a mass spectrometer consisting entirely of a battery of lasers, a vacuum system, and a detector, with no conventional mass separating device. The low cost and high reliability of diode lasers make this a feasible concept. In the near term, it seems certain that application of these lasers to analytical problems will expand rapidly.

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MEASUREMENT OF MAGNESIUM ISOTOPIIC RATIOS IN THE STUDY OF BI-DIRECTIONAL
CELLULAR MAGNESIUM FLUXES

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Magnesium ions play a significant role in regulation of key cellular physiological and biochemical processes. Most research on magnesium fluxes has been done using radioactive ²⁸Mg, a short-lived isotope available irregularly from one supplier in the country, and can yield only unidirectional fluxes. Our work used the stable isotope ²⁶Mg (99.5% enriched, Oak Ridge National Laboratory, Oak Ridge, TN.) as a tracer for the measurement of bi-directional fluxes in the giant muscle fibers of the acorn barnacle. Bi-directional fluxes are obtained with a single isotope using dilution of the tracer by the natural isotopic distribution of Mg. This particular cellular model was chosen because of the ease of obtaining samples and because previous data on Mg fluxes exist in it.

Muscle fibers taken from a single animal were attached to a teflon slide and immersed into a specially made lucite chamber containing a medium free of Na and Ca (to prevent spontaneous contraction) consisting of TRIS, 10mM K and 1 of 3 ²⁶Mg enriched concentrations: 5mM, 25mM, 60mM (pH=7.6). Aliquots of medium were removed at time points 0, 15, 30, and 60 minutes and were analyzed directly using thermal ionization mass spectrometry and the silica gel technique (1). Mg masses 24, 25, and 26 were sequentially scanned for 8 seconds each (1 scan of data). A mean and relative standard deviation was calculated at the end of 10 scans (1 block of data). A mean of at least 2 blocks was collected for each sample.

Natural abundance accuracy and precision are reported in Table 1. Ratios were corrected for fractionation using the method described by Miller, et.al.(2,3). This method relates observed fractional intensities of individual masses with the deviation from expected fractional intensities. A mean slope and intercept were calculated from natural abundance magnesium measurements:

$$y = -0.069x + 1.666$$

where x = mass

y = the deviation from expected fractional intensity

i.e., $y = \frac{\text{observed fractional intensity} - \text{expected fractional intensity}}{\text{expected fractional intensity}}$

Table 2 reports the results of experiments at the 3 concentrations of highly enriched Mg. Absolute amounts of ²⁶Mg and ²⁴Mg are calculated from the isotope ratios at each time point. The initial ²⁶Mg level in the medium decreases due to both flow into the cell and efflux of natural abundance Mg from the cell. The relative standard deviation (RSD) for the determinations are somewhat higher than is expected for TIMS on our Finnigan THQ quadrupole mass spectrometer. We presume this to be due to the silica gel and not the unprocessed sample since natural abundance magnesium standards showed similar RSD. Results of the muscle fiber flux determinations are reported elsewhere (4).

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

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Table 1: Natural abundance isotopic ratios of magnesium.

ratio	observed ratio	expected ratio	corrected ratio	%RSD* (n=7)
26/24	0.12062	0.139385	0.13746	7.5
25/24	0.11741	0.126598	0.12559	3.6

* RSD is relative standard deviation.

Table 2: Time dependent isotopic ratio change of highly enriched ²⁶Mg extracellular medium. (Ratios have been corrected for fractionation.)

medium [Mg]	TIME (min)	26/24 (%RSD)	25/24 (%RSD)
5mM	0	73.649 (3.4)	0.1317 (3.3)
	15	40.795 (-)	0.2046 (-)
	30	48.854 (9.4)	0.1632 (3.6)
	60	47.342 (9.0)	0.1632 (6.0)
25mM	0	192.343 (4.5)	0.2177 (9.3)
	15	170.897 (4.0)	0.2390 (7.7)
	30	153.413 (1.3)	0.1846 (4.2)
	60	179.768 (4.1)	0.2001 (3.2)
60mM	0	278.745 (8.1)	0.2493 (4.9)
	15	217.347 (2.3)	0.2535 (3.2)
	30	207.076 (6.9)	0.2457 (9.8)
	60	228.870 (5.3)	0.2609 (3.7)

THE IMPORTANCE OF ABUNDANCE SENSITIVITY IN THE MEASUREMENT OF LARGE ISOTOPE RATIOS

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The maximum isotope ratio that can be measured by any mass spectrometer is ultimately limited by its abundance sensitivity. As the ions travel through the Spectrometer, their kinetic energy and direction can be changed as a result of collisions with residual gas molecules. This alters the position at which they arrive at the focal plane with respect to unscattered ions and is observed as an increase in the background level extending over several mass units from the peak centre and proportional to its intensity. Small peaks in the vicinity of a larger one can only be measured if they exceed this background level.

Abundance sensitivity depends upon the pressures inside the instrument. The pressure in the analyser section is critical because this forms the largest part of each ion's trajectory. The source pressure also influences abundance sensitivity when collisions in this region become important and gas diffuses into the analyser vacuum.

The abundance sensitivity of the ISOLAB instrument is minimised by use of ultra-high vacuum (UHV) techniques and is improved by the presence of electrostatic analysers (ESA) which filter out the scattered ions.

In the reverse geometry instrument, many unwanted ions are removed by the magnet and only those ions that collide after the ESA remain unfiltered. In the forward geometry instrument all of the ions are transmitted by the ESA and filtering of scattered ions is only carried out before the intermediate slit. The number of collisions after this will be fewer than in the single sector magnet instrument because the pressure will be held lower by the differential pumping regimes around both the source and the intermediate housing slits. Therefore the abundance sensitivity of the two sector ISOLAB 54 is expected to be inbetween the performance of the single sector and reverse geometry instruments at a level of 5×10^{-9} at (unit mass separation from U238).

The three sector ISOLAB 54 can be considered to be equivalent to an electrostatic analyser followed by a reverse geometry spectrometer with an effective source pressure of 1×10^{-9} mbar. The expectation that the abundance sensitivity of this enhanced configuration will be below 5×10^{-8} has been proven to be true.

For the ISOLAB 120, sophisticated UHV design enables source vacuum levels of 10^{-8} mbar to be maintained during measurements and an operating analyser pressure of below 10^{-9} mbar. The effect of the larger 96 cm radius electrostatic analyser and the larger 60 cm radius magnet has resulted in further enhancements because its dispersion length will increase the distance between peaks and reduce the background contributions from the tails of neighboring masses. It has been found that the abundance sensitivity of the two stage ISOLAB 120 is better than 1×10^{-7} and that of the three stage configuration is better than 5×10^{-9} , again demonstrating the advantages of the use of a large analyser. At low masses abundance sensitivity is further enhanced (see figure 1) due to the larger physical separation of adjacent masses.

ABUNDANCE SENSITIVITY
< 0.01ppb AT MASS 22

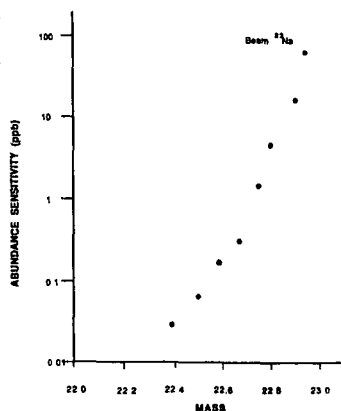


Figure 1 Abundance sensitivity of the ISOLAB 120 versus mass for a sodium beam.

THE EFFECT OF ULTRA-HIGH VACUUM ON
THERMAL IONIZATION PROCESSES

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We have observed an unusual delay in Pu and U ion production during a normal heating pattern when our triple-filament thermal-ionization source operates at a pressure less than 3×10^{-8} Torr. At lower pressures ($< 10^{-8}$ Torr) Pu⁺ ion current increases rapidly after the delay growing to 5 to 10 times the normal intensity as indicated in Fig. 1. Figures 1 and 2 show the general features of these phenomena for Pu⁺ and U⁺ ion production, respectively, for different ion source pressures. In Fig. 3, we show that normal Pu⁺ production can be produced by the introduction of a stable flow of O₂ from a dry air leak. Once the leak is turned off and the source pressure drops to about 10^{-8} Torr, the rapid increase in Pu⁺ ion current spontaneously occurs while the heating currents to the sample filament or the ionizing filament remain constant. This enhanced ionization of Pu is nearly independent (short term) of the sample filament current implying that the enhanced ionization phenomena for Pu occurs at the ionizing filament. The enhanced Pu ion current also decreases spontaneously as indicated in Fig. 3 at about 135 min. When the sample filament is turned off, the Pu⁺ signal drops rapidly but a residual 5 mV signal is seen from Pu deposited on the filament during the operation at 10^{-8} Torr source pressure. The equivalent residual signal for U is 0.7 mV. Residual Pu (2 ng) has been measured by alpha particle counting of an ionizing filament exhibiting the enhanced ionization. Additionally, at the lower pressures ($< 10^{-8}$ Torr) the Re ionization filament at 2080 °C exhibits a steady growth in Re⁺ ion output to a factor of two in one hour in the constant current mode of operation.

The data for this effect was obtained on a NBS-type 12"-90° sector mass spectrometer equipped with a 330 L/sec turbomolecular pump with a LN₂ trap for the source and a glass LN₂ cold finger directly above the ion source. The analyzer is ion pumped. When changing samples, the source is vented with dry N₂ (trapped with LN₂) to prevent accumulation of water vapor in the source. Additionally, the filament pins are vacuum baked as a final step in their cleanup process. This pumping system and the efforts to restrict water vapor from the source region reduced the source ion base pressure from 2×10^{-7} to 3×10^{-8} Torr after 8 months of the practice and the pressure during sample analysis was reduced to about 1×10^{-8} Torr. Pumpdown time was dramatically reduced reaching 5×10^{-8} Torr in 20 min of pumping after a sample change. As we approached these low pressures, we began to experience the delay effect and the finally the enhanced ionization phenomena. The improvement in vacuum basically reduce water vapor and other sources of oxygen; this fact was identified as the common cause of the loss of control of the ionization process. To restore normal operation, we first introduced an air leak by carefully loosening a bolt on the source flange to raise the pressure to about 10^{-7} Torr. This restored control but repeated use of this technique again accumulated water vapor and slowed pumpdown. The solution to this was the introduction of dry air through a variable leak valve to produce 8×10^{-8} Torr (1.6×10^{-8} Torr O₂ partial pressure) in the ion source. This produces normal operation.

* Work Performed under US-DOE Contract No. DE-AC04-88-DP43495.

There are two mechanisms involved in these phenomena. The first relates to the importance of oxygen in the thermal ionization process. Although Re probably does not form a stable oxide at the operating temperature, oxygen incident and partially adsorbed on the Re surface (in the form of H_2O , CO , O_2) together with the metal atoms incident appears necessary as a reagent for normal thermal ionization to occur. Many of the ionization enhancers reported in the literature (boric acid, silica gel) bring oxygen to the metal at the hot filament; in our case, the oxygen is presented as a reagent gas from the source region atmosphere. As the source pressure decreases, the delay time period lengthens but the product of the estimated oxygen partial pressure times the delay time gives a constant dose of oxygen equal to 5×10^{-7} Torr-min. The second mechanism of enhanced ionization in the absence of much oxygen could be the deposition of Pu on the bare Re ionizing filament surface to an extent that Pu collects in grain boundaries or as islands of Pu which surface ionize much like Re itself. The rapid growth could relate to the increased percent agglomeration as more Pu collects. The normal operating pressure of most commercial thermal ionization mass spectrometers is in the range of 5 to 20×10^{-8} Torr for which there is adequate oxygen for producing a stable ion beam.

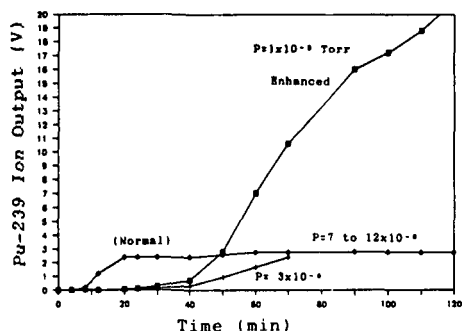


Fig. 1. Pu ion output versus time for three source pressures (Torr).
(♦) $P=7$ to 12×10^{-8} Torr
(+) $P=3 \times 10^{-8}$; (□) $P=1 \times 10^{-8}$

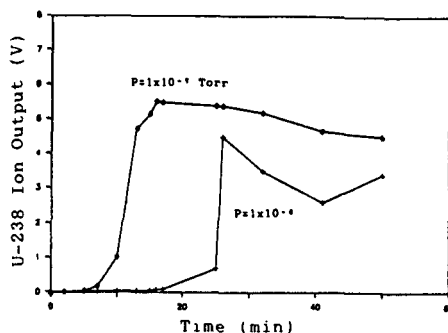


Fig. 2. U ion output versus time for two source pressures (Torr).
(♦) $P=1 \times 10^{-7}$ Torr; (+) $P=1 \times 10^{-8}$

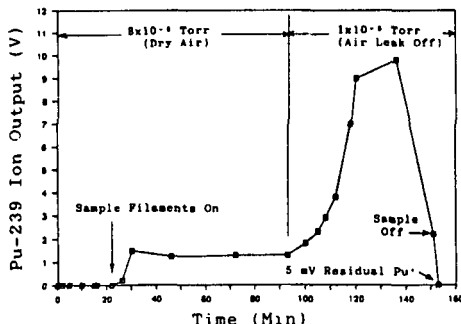


Fig. 3. Normal Thermal Ionization of Pu with dry air (8×10^{-8} Torr) introduced and the Enhanced Pu Ionization when the air leak is turned off (1×10^{-8} Torr).

EVALUATION OF MEMORY EFFECT IN THE DETERMINATION OF ALTERED ISOTOPE
RATIOS OF METALS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Introduction

Gas chromatography/mass spectrometry (GC/MS) using a general purpose mass spectrometer is a viable alternative available to the biomedical community for the determination of trace metals in biological samples. GC/MS offers the advantages of shorter analysis time and less specialized instrumentation compared to other mass spectrometric techniques (TIMS, ICP/MS) which are exclusively dedicated to metals determination. GC/MS methods involve the formation of volatile and thermally stable metal chelates and have been studied by a number of investigators. A critical flaw preventing the widespread applicability of GC/MS methods has been the observation of carry-over, or memory, in the sequential analysis of samples with different isotope ratios. We have investigated the memory effect using lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as a chelating agent for determining non-natural isotope ratios of Cr, Ni, Cu, Zn and Pb.

Experimental

Preparation of synthetic mixtures: A series of synthetic mixtures were prepared for each of the metals using standard solutions of the natural elements and solutions of the enriched isotopes (spikes) ^{50}Cr , ^{62}Ni , ^{65}Cu , ^{68}Zn and ^{204}Pb . Weighed amounts of these solutions were mixed to give four to five mixtures for each element with increasing amounts of the spike.

Preparation of metal chelates: The chelating agent Li(FDEDTC) was synthesized using bis(trifluoroethyl)amine, n-butyllithium and carbon disulphide. The metal chelates were prepared at a pH of 3 using an acetic acid-sodium acetate buffer. A 0.02 M solution of Li(FDEDTC) in distilled, deionised water was used to form the metal chelates which were then extracted into methylene chloride. For Cr, an oxidation step using $\text{KMnO}_4\text{-H}_2\text{SO}_4$ was included prior to chelate formation.

Gas Chromatography/Mass Spectrometry: A 10m DB-1 fused silica capillary column was used in a Finnigan-MAT 8230 GC/MS system. The isotope ratios were determined by recording the highest intensity ion containing the metal using selected ion monitoring in the voltage peak switching mode. The cluster of ion peaks used corresponded to the molecular ion for Ni, Cu and Zn and a fragment ion formed by the loss of one ligand for Cr and Pb. The mixtures were analysed in the sequence of increasing amount of the spike with three to four injections made for each sample.

Results and Discussion

The isotope ratios determined from the different synthetic mixtures of each element were used to calculate the concentration of the metal in the spike solution by the principle of reverse isotope dilution. The constancy in the isotope ratios obtained by replicate injections from each of the synthetic mixtures as well as the close agreement of the concentration values calculated from the synthetic mixtures was taken as evidence for the absence of any significant memory effect. The results obtained showed no measurable memory effect for Cr and a very slight memory effect for Ni. These results are illustrated in Figures 1 and 2 which show the measured ratios for the sequential analysis of natural and enriched isotope solutions of Cr and Ni, respectively. In these figures, the isotope ratios measured in the chelate correspond to the spike isotope (^{50}Cr , ^{62}Ni) and the highest abundant natural isotope (^{52}Cr , ^{58}Ni). Similar experiments for Cu, Zn and Pb showed significant memory effect. During the sequential analyses of different synthetic mixtures of Zn and Pb, there was a small memory effect as noted from the isotope ratio data obtained from the first injection. In the case of Cu, it was necessary

to reject the data of 2 to 4 injections before approaching a constant value for each synthetic mixture. The extent of memory effect will depend upon the differences in the isotope ratios of samples to be analysed sequentially. However, good experimental design for isotope dilution technique puts the isotope ratios in a relatively narrow range and GC/MS can still be used for these elements. As an example, the concentrations of Cu and Pb in the NIST urine reference material, SRM-2670, are shown in Table 1. As can be seen, good agreement was observed between the certified and measured concentration values. Zinc was not determined due to the non-availability of certified reference material.

Conclusion

GC/MS using Li(FD EDTC) as a chelating agent can be employed for the determination of isotope ratios and concentrations of Cr and Ni without any appreciable memory problem. For Zn and Pb, the method can be used by neglecting the data obtained from initial one or two injections, when analysing samples with different isotope ratios. Among the different elements studied, Cu showed a large memory effect. As demonstrated in the present work, it is possible to determine Cu concentrations by controlling the range of isotope ratios of different samples to be analysed by GC/MS. This can easily be achieved by performing optimum spiking during the isotope dilution step.

TABLE 1

Analyte	Certified Value ($\mu\text{g/mL}$)	Determined ($\mu\text{g/mL}$)
Cr (n=5)	$0.013 \pm ?$	0.013 ± 0.002
(n=11)	0.085 ± 0.006	0.089 ± 0.006
Ni (n=6)	$0.070 \pm ?$	0.084 ± 0.002
(n=6)	$0.300 \pm ?$	0.340 ± 0.020
Cu (n=4)	0.370 ± 0.030	0.360 ± 0.020
Pb (n=4)	0.109 ± 0.004	0.105 ± 0.008

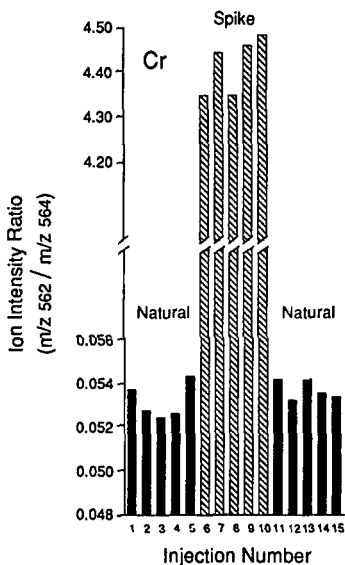


Fig. 1

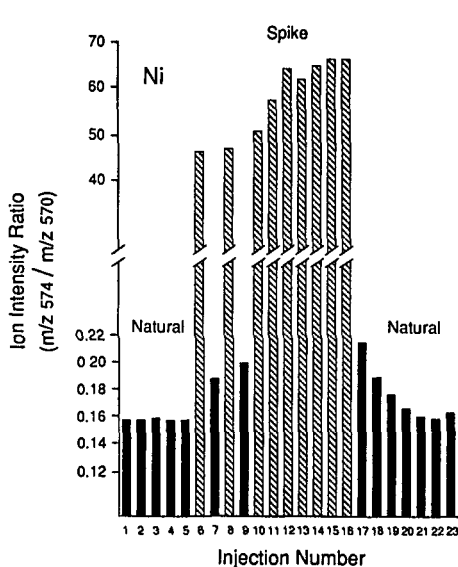


Fig. 2

MEASUREMENT OF TRACES OF CHD_3 IN CD_4

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1130 Pine Ave West, Montreal, Que., Canada, H3A 1A3

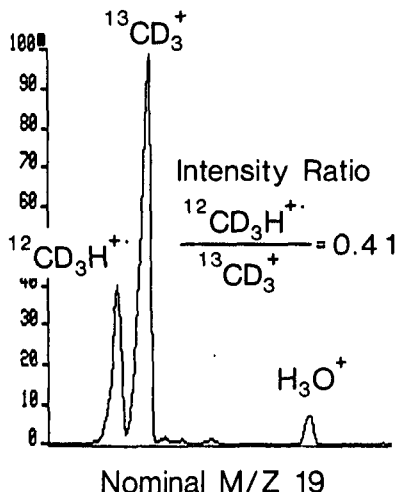
and

H. Koch, MSD Isotopes, Pointe Claire, Que., Canada, H9R 4P7

The determination of traces of CHD_3 in CD_4 by conventional mass spectrometry requires the measurement of very weak and very strong signals in the same analysis.

A simple approach to this problem is to measure the relative intensities at nominal mass 19 for the CD_4 sample with resolving power 5000. The $^{13}\text{CD}_3^+$ fragment ion in the spectrum of CD_4 has an abundance $A \times B$ that of the molecular ion of CD_4 , where A is the abundance of ^{13}C relative to ^{12}C (0.0111: natural abundance), and B is the usual relative intensity of the $^{12}\text{CD}_3^+$ fragment relative to the molecular ion $^{12}\text{CD}_4^{+}$ (0.862: measured in a separate analysis). The molecular ion $^{12}\text{CHD}_3^+$ (m/z 19.05013) and the $^{13}\text{CD}_3^+$ fragment (m/z 19.04566) are easily separated at 5000 resolution and will have intensities that are comparable and easily measured if the $^{12}\text{CHD}_3$ impurity is comparable with the ^{13}C content of the carbon in the methane. The contribution to $^{13}\text{CD}_3^+$ by H. loss from $^{13}\text{CHD}_3$ may be ignored in these calculations if the impurity is small.

The break-seal container is opened by manually breaking the fragile seal in a dry nitrogen atmosphere to exclude water as much as possible from the sample. The methane may be at sub-ambient pressure, and dry nitrogen will back-fill the tube as the seal is broken. A gas-tight syringe is used to remove approximately 50 μl of the methane-nitrogen mixture and is introduced to the EI ion source of a 2AB-HS via the septum inlet (50°C). The mass spectrometer is scanned over the nominal range m/z 18-20 and the intensity ratio for the molecular ion $^{12}\text{CHD}_3^+$ (m/z 19.05013) to the $^{13}\text{CD}_3^+$ fragment (m/z 19.04566) is measured. The figure shows an example of such a measurement in which the ratio has been measured to be 0.41. The CD_3H impurity relative to the CD_4 content then is equal to:



$$0.41 \times 0.0111 \times 0.862 = 0.00393 \text{ or } 0.39\% \text{ approximately.}$$

It is important to know with certainty the ^{13}C content of the carbon used to make the deuteriomethane. The method is simple and requires only a few minutes per determination.

EI INDUCED FRAGMENTATION OF 2-ARYLOXY-3-CYCLOHEXYL-6-(1,1-DIMETHYLETHYL)-3,4-DIHYDRO-2H-1,3,2-BENZOXAZA PHOSPHORINE-2-OXIDES

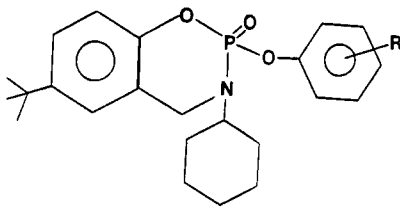
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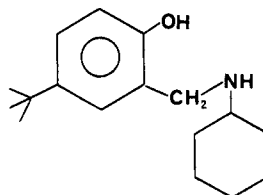
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A series of 2-aryloxy-3-cyclohexyl-6-(1,1-dimethylethyl)-3,4-dihydro-2H-1,3,2-benzoxaza phosphorine 2-oxides(A) have been studied by EI mass spectrometry in an attempt to understand their fragmentation behavior. These are potential anticancer drugs¹ and were synthesized by reacting 2-cyclohexylaminoethyl-4-t-butylphenol(B) and arylphosphorodichloride in benzene at room temperature for 2-4 hours.²



(A)



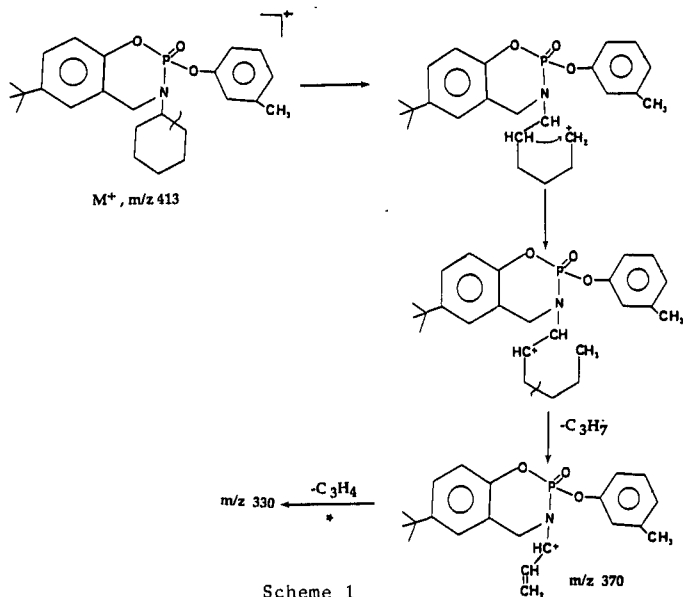
(B)

1A, R=H; 2A, R=2-CH₃; 3A, R=3-CH₃; 4A, R=4-CH₃; 5A, R=3,4-(CH₃)₂;
6A, R=2,3-(CH₃)₂; 7A, R=2,5-(CH₃)₂; 8A, R=2-Cl; 9A= R=4-Cl

The accurate mass measurements of some selected ions from compounds 3A and B were performed on a VG 70-70E-HF mass spectrometer interfaced to a 11-250M+ data system at a resolution of 10,000 by peak matching to a closest PFK reference ion. The CAD spectra were recorded by B/E linked scan in the 1st FFR employing helium as the collision gas.

The molecular ions generated from this series of compounds are very stable often giving rise to the base peak in their spectra. Elimination of cyclohexyl, propyl and isobutylene moieties from the molecular ions represent major fragmentation modes. The aryloxy and arylphosphone fragments are also lost from the M⁺ as evidenced by the CAD spectral data.

Based on the B/E linked scan spectra recorded for compound 3A on ions m/z 413(M⁺) and m/z 370(M-C₃H₇)⁺, it appears that the propyl radical is lost from the cyclohexyl ring and not from the t-butyl group. A mechanism involving an initial ring opening followed by a hydrogen transfer and subsequent C-C bond fission is proposed as in Scheme 1. The CAD spectrum of ion m/z 370 from compound 3A exhibited an ion at m/z 340 corresponding to a loss of C₃H₄ confirming its ionic structure (Scheme 1).



In an attempt to make a comparative study the mass spectrum of the starting material, 2-cyclohexyl-aminoethyl-4-*t*-butyl phenol(B) was also studied. The base peak in the spectrum of this compound is due to the M^+ ion at m/z 261 which again loses a propyl radical to give an intense fragment ion at m/z 218. This represents a major fragmentation pathway. Expulsion of methyl, C_4H_9 , pentyl and cyclohexyl fragments from the M^+ afford ions at m/z 246, 205, 190 and 178, respectively. All these fragmentations and the resulting ionic structures were confirmed by CAD spectral data and accurate mass measurements. A similar mechanism shown in Scheme 1 appears to be operative in the elimination of propyl radical in compound(B). Furthermore, the ring opening of cyclohexyl moiety prior to fragmentation also supports the loss of C_3H_{11} fragment from the molecular ion of compound B.

In conclusion, all the fragmentation processes observed support the assigned molecular structures for these compounds.

Acknowledgement: One of the authors (GSR) is thankful to Shrader Analytical & Consulting Laboratories, Inc. for their financial support.

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MASS SPECTRAL FRAGMENTATION OF 2,10-DICHLORO-6-ARYLOXY-DIBENZO[d,g]
[1,3,6,2]DIOXATHIAPHOSPHOCIN-6-OXIDES

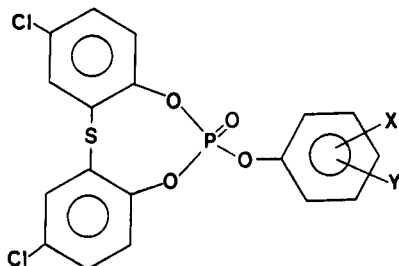
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During our investigations on the synthesis of organophosphorus esters bearing anticholinesterase activity, we have prepared a series of 2,10-dichloro-6-aryloxy-dibenzo[d,g][1,3,6,2]dioxathiaphosphocin-6-oxides (1-6).¹ The electron ionization mass spectra of these compounds have been examined since they contain a novel eight membered dibenzo dioxathiaphosphocin ring system.



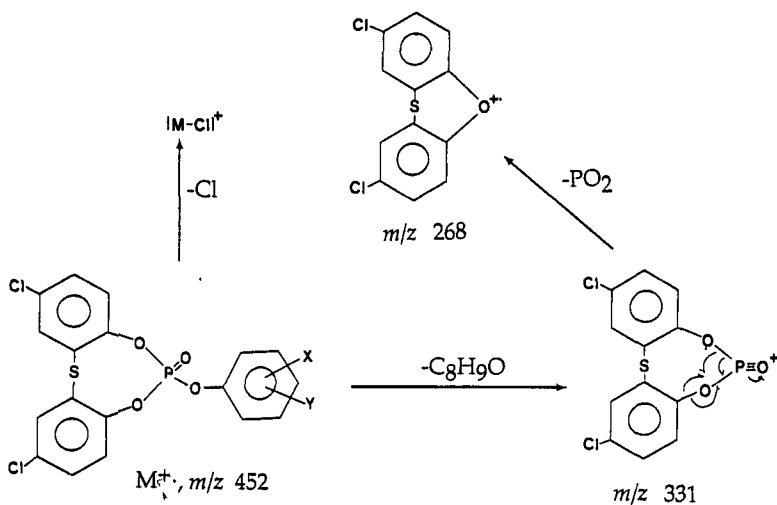
	X	Y
1.	H	H
2.	H	2-CH ₃
3.	2-CH ₃	3-CH ₃
4.	2-CH ₃	6-CH ₃
5.	H	2-Cl
6.	3-CH ₃	4-Cl

The low resolution mass spectra were recorded on a JMS-300 mass spectrometer using direct inlet probe at 70 eV. The high resolution mass measurements of some selected ions from Compound 3 were performed on a VG 70-70E-HF mass spectrometer at a resolution of 10,000 by peak matching to a closest PFK reference ion. The CAD spectra were taken in the 1st FFR using helium as the collision gas.

The mass spectra of this series of compounds exhibited stable M⁺ ions. The molecular ion further expels an aryloxy ion to give a (M-OR)⁺ ion which forms the base peak in their spectra. The (M-OR)⁺ ion decomposes into various ions by other competing reaction pathways.

Based on the accurate mass measurements and metastable transitions recorded for Compound 3, the major fragmentation reactions are interpreted as shown in Scheme 1.

The molecular ion gives rise to an ion at m/z 330 with the elimination of C₈H₇O (aryloxy) moiety. The (M-C₈H₇O)⁺ ion further loses PO₂ radical to form an ion at m/z 268. It can also expel PO₃ to yield a weak



Scheme 1

ion at $m/z\ 252$. Expulsion of C_8H_3ClS moiety from the $(M-C_8H_9O)^+$ results in an ion at $m/z\ 189$. Similarly, a loss of C_8H_3ClOS from this ion forms an ion at $m/z\ 173$. All these fragmentation reactions are supported by CAD spectral data. The proposed ionic structures are in good agreement with the accurate mass measurements.

Acknowledgement: One of the authors (GSR) is thankful to Shrader Analytical & Consulting Laboratories, Inc. for their financial support.

References:

1. K. Anuradha, Ph.D. thesis to be submitted to Sri Venkateshwara University, India, 1989.

MASS SPECTRAL FRAGMENTATION OF SOME 1,4-DITHIANE-1,1-DIOXIDE AND
1,1,4,4-TETROXIDE AND RELATED HETEROCYCLIC COMPOUNDS

D. Bhaskar Reddy^a, S. Reddy^a, B. Seenaiah^a,
M. V. R. Reddy^a and G. Sudhakar Reddy^b

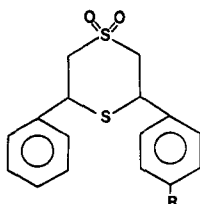
^aDepartment of Chemistry, Sri Venkateshwara
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^bShrader Analytical & Consulting Laboratories, Inc.
3814 Vinewood, Detroit, Michigan 48208

In the course of our investigations on novel heterocyclic systems we have synthesized a series of 1,4-dithiane dioxide, 1,4-dithiane tetroxide and 1,4-thiomorpholine dioxide derivatives in good yields.¹ The mass spectral studies on these systems are undertaken to understand their fragmentation behavior in the gas phase.

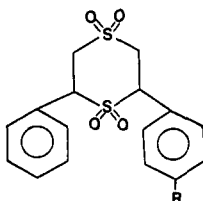
The 70 eV low resolution mass spectra were recorded on a JMS-300 mass spectrometer using a direct inlet system. The high resolution spectra of some selected compounds were recorded on a Kratos MS-50. The accuracy of masses for several ions measured was within ± 3 ppm.

The following representative compounds from each series were examined under EI conditions. The M⁺ ion in all of these compounds is less



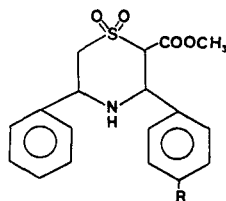
1, R=H

2, R=Cl



3, R=H

4, R=NO₂



5, R=H

6, R=CH₃

7, R=NO₂

significant and undergoes further decomposition depending upon the structure of the molecule. For example, an expulsion of SO₂ molecule from the M⁺ of dithiane tetroxides(3,4) is seen as one of the competing processes while it is absent in dioxides(1,2). Similarly, the elimination of SO₂H radical from the molecular ion is predominant in dioxides while it is less significant or absent in tetroxides. It is interesting to note that both SO₂ and SO₂H moieties are lost from the M⁺ of thiomorpholine dioxides(5-7). Furthermore, the loss of methoxy radical from the M⁺ of 5-7 represents another competing reaction. The base peak in these compounds is due to the styrene radical cation. A similar trend is seen in the mass

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spectra of dithiane tetroxide where the styrene ion appeared as the base peak. On the contrary, the formation of the styrene ion at m/z 104 is less significant in the case of dithiane dioxides. The base peak in these compounds is due to a substituted thiophenyl ion which results from the M^+ by SO_2H loss.

The proposed ionic structures and fragmentation reactions are supported by high resolution data.

Acknowledgement: One of the authors (GSR) is thankful to Shrader Analytical & Consulting Laboratories, Inc. for their financial support.

Authors thank Kathy Lathrop for her technical assistance.

References:

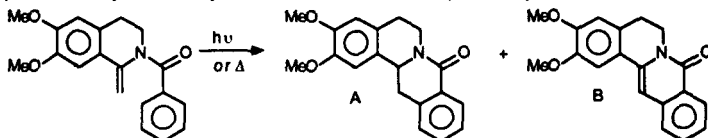
1. S. Reddy, Ph.D. thesis to be submitted to Sri Venkateshwara University, Tirupati, India, 1989.

PHENYL AND PYRIDYL GROUP MIGRATION ON ELECTRON IONIZATION OF ENAMIDES DERIVED FROM 1,3-BENZOTHAZINES AND ISOQUINOLINES:

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Enamides derived from 3,4-dihydro-1-methylisoquinolines and 3,4-dihydro-1-methyl- β -carboline have been used extensively in the synthesis of alkaloids and related heterocyclic systems. The utility of these enamides rests upon their ability to cyclize either photochemically or thermally as shown below for the isoquinoline system.



Enamides of the type shown in figure 1 were examined by EIMS to see if a similar process might occur in the mass spectrometer ion source and also to examine the mass spectral behavior of this group of compounds. Full scan electron impact mass spectra (EIMS), mass analysed ion kinetic energy scans (MIKES) and collisional activation spectra (MIKES, He collision gas, parent ion transmission = 50%) were recorded on a VG ZAB-E mass spectrometer (VG Analytical, Wythenshawe, UK) using a direct insertion probe. Typical experimental conditions were: source temperature, 200°C; electron energy 70eV; resolution, 1000 for low resolution and MIKES, and 5000 for elemental composition determinations.

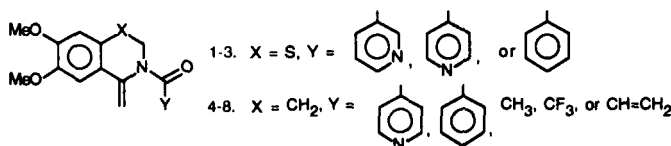


Figure 1

Compounds 1-5, and 8 exhibited intense ions corresponding to loss of CO from the M⁺ and [M-H]⁺ ions. The EIMS of compounds 3 and 6 are shown in figure 2. The loss of CO implies that aryl (and vinyl) group migration must occur. The [M-CO]⁺ ion then fragments further by loss of H, CH₃, OCH₃, Y, CH₂Y, and CH₂NY. Table 1 summarises the MIKES data and elemental composition determinations for compound 3. In contrast, 6 and 7 showed no loss of CO but simple cleavage reactions were observed.

Examination of the literature of related systems was not helpful. Simple amides^{1,2a} do not lose CO from the M⁺ ion. The cyclic enamides, α - and γ -pyridones^{2b}, and the fused α -pyridones³, 2(1H)-quinolinone and 1(2H)-isoquinolinone, all exhibit ions corresponding to loss of CO from the M⁺ ion. However, compounds in which the 1(2H)-isoquinolinone system or its naphthyridine analogue are incorporated into more complex ring systems show no tendency to lose CO in their EIMS as is the case for compounds A^{4a} and B^{4b} shown above.

The EIMS of compounds 1-3 (X = S) are similar. Loss of the neutrals shown in table 1 are common to all three compounds. The molecular ion loses CO with migration of the phenyl group to

nitrogen yielding an ion at m/z 299 which, by loss of phenyl in the case of compound 3, affords the m/z 222 ion. The latter is also formed directly from the molecular ion by loss of the aryl group. The ion at m/z 222 loses CH_2N to yield m/z 194 which is also formed from m/z 299 by loss of $\text{C}_7\text{H}_7\text{N}$. The ion at m/z 179 is formed from m/z 194 by loss of CH_3 . The process leading to m/z 208 is more complex. It may indicate that the $[\text{M}-\text{CO}]^+$ ion has a different structure than that discussed previously. This second ion may form by migration of phenyl to carbon rather than nitrogen. The expulsion of C_7H_7 would yield the ion at m/z 208. In compounds 4 and 5 the same processes are observed, however, the intensities of the ions are low (<5%).

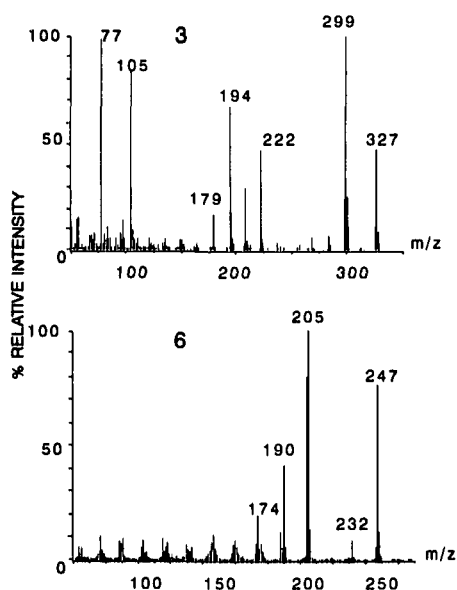


Figure 2

Cyclization of these compounds was not observed under EIMS conditions, CO elimination followed by aryl group migration to either the nitrogen or the exocyclic methylene was observed.

(1) F.W. McLafferty. Interpretation of Mass Spectra, 3rd ed., University Science Books, Mill Valley, CA, 1980, p. 223.

(2)(a) H. Budzikiewicz, C. Djerassi and D. H. Williams. Mass Spectrometry of Organic Compounds, Holden-Day, San Francisco, CA, 1967, Chapter 9. (b). *ibid.*, Chapter 20, p. 571.

(3) A. Kubo et al., Chem. Pharm. Bull., 16, 1533 (1968).

(4) (a) Jahangir, D.B. MacLean, and H.L. Holland, Can. J. Chem., 65, 727 (1987). (b) Jahangir, D.B. MacLean, and H.L. Holland, Can. J. Chem., 64, 1031 (1986).

ION (M/Z)	DAUGHTERS
M^+ (327)	326 (-H) 299 (-CO) 222 (-C ₆ H ₅ CO) 105 (-C ₁₁ H ₁₂ NSO ₃)* 77 (-C ₁₂ H ₁₂ NSO ₃)*
$(\text{M}-\text{H})^+$ (326)	325 (-H) 298 (-CO)
$(\text{M}-\text{CO})^+$ (299)	298 (-H) 284 (-CH ₃) 268 (-OCH ₃) 222 (-C ₆ H ₅) 208 (-C ₇ H ₇) 194 (-C ₇ H ₇ N)

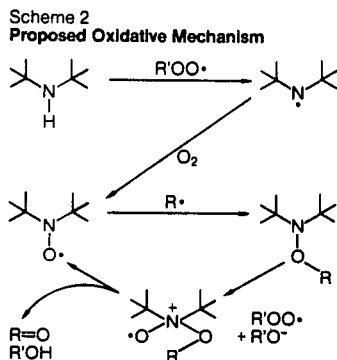
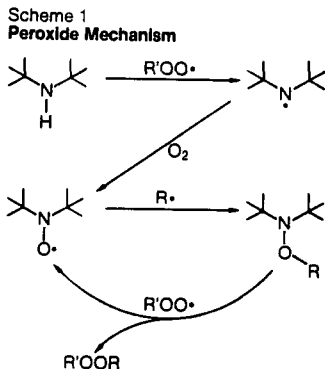
* THESE IONS OBSERVED ONLY UNDER COLLISIONAL ACTIVATION CONDITIONS

Table 1

Investigation of the Hindered Amine Light Stabilizer Mechanism using Isotopic Labeling

NG K., Piatek B., Klemchuck P.,* Gande M.,* Corporate Analytical Research and Additives* Research Departments. CIBA-GEIGY Corporation, Saw Mill River Road, Ardsley, N.Y. 10502

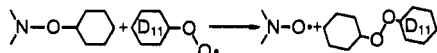
2,2,6,6-tetramethyl piperidiny compounds are very effective light stabilizers for polymers. Literature reports that nitroxyl and polymer bound hydroxyl amine ethers are the intermediates. N-alkoxy-tetramethylpiperidines react with peroxy radicals to generate nitroxyls and dialkyl peroxides as shown in Scheme 1. This mechanism is generally accepted as the "HALS CYCLE" mechanism. An alternate OXIDATIVE mechanism is being proposed. Reaction of the N-O-R ether with an alkyl peroxy radical results in the cleavage of the R group to form a ketone and the nitroxyl as shown in Scheme 2. In this study the R group of the light stabilizer is cyclohexane. By the use of a variety of isotopic labeling experiments and gas chromatography/ mass spectrometry analysis as shown in Figures A, B, and C, the generally accepted "HALS CYCLE" mechanism has been disproven and the alternate OXIDATIVE mechanism is supported



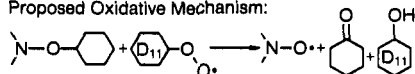
CIBA-GEIGY

Figure A
Comparison of Literature "PEROXIDE" Mechanism with Proposed Oxidative Nitroxyl Regeneration Mechanism

Deuterium Labeling Experiment 1
Peroxide Mechanism:



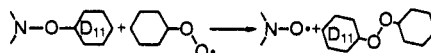
Proposed Oxidative Mechanism:



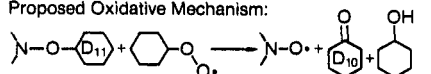
Mechanism	Predicted Ratio:
Peroxide	0.0
Oxidative	0.8
Ratio Found:	0.7

Figure B
Comparison of Literature "PEROXIDE" Mechanism with Proposed Oxidative Nitroxyl Regeneration Mechanism

Deuterium Labeling Experiment 2
Peroxide Mechanism:



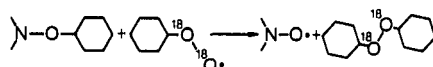
Proposed Oxidative Mechanism:



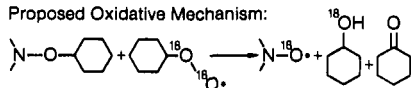
Mechanism	Predicted Ratio:
Peroxide	∞
Oxidative	1.7
Ratio Found:	1.6

Figure C
Comparison of Literature "PEROXIDE" Mechanism with Proposed Oxidative Nitroxyl Regeneration Mechanism

Oxygen - 18 Experiment
Peroxide Mechanism:



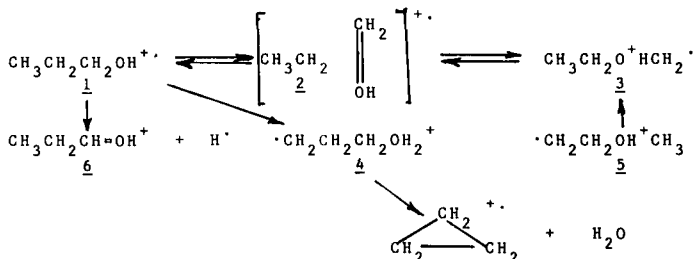
Proposed Oxidative Mechanism:



Mechanism			
Peroxide	$\frac{16}{18}\text{O}$	$\frac{18}{18}\text{O}$	$\frac{18}{16}\text{O} + \frac{18}{18}\text{O}$
Oxidative	$\frac{16}{18}\text{O}$	$\frac{18}{18}\text{O}$	$\frac{18}{16}\text{O} + \frac{18}{18}\text{O}$
Found:	$\frac{16}{18}\text{O}$	$\frac{18}{18}\text{O}$	$\frac{18}{16}\text{O} + \frac{18}{18}\text{O}$
			3:1

1,2-Shifts in $C_3H_8O^+$ Ions
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Marine Biomedical Institute, University of Texas Medical Branch
Galveston, Texas 77550

Cleavage beta to a radical site to give an olefinic product is a favorable fragmentation of ions in the gas phase. Near threshold, such cleavages can form ion-neutral complexes followed by readdition at the other end of the double bond. We here extend this concept by proposing the reaction $\underline{3} \rightleftharpoons \underline{2}$ (Scheme I). The distonic species $\underline{5}$ has previously been studied.



Metastable spectra and associated translational energy releases starting from $\underline{1}$ and $\underline{5}$ are given in Table 1.

Table 1. Metastable Spectra and Translational Energy Releases ($T_{0.5}$) from $C_3H_8O^+$ Isomers.

		$-H^+$		$-H_2O$
$CH_3CH_2CH_2OH^+$ ($\underline{1}$)	10	(46.2 meV)	100	(19.5 meV)
$\cdot CH_2CH_2OH^+CH_3$ ($\underline{5}$)	100	(116 meV)	98	(21.0 meV)

The loss of water requires isomerization to a species containing only one C-O bond, placing the oxygen in a position to be lost in water. The two losses of water have similar energy releases, consistent with decomposition from the same reacting configuration.

Studies of labeled ions (Table 2) demonstrated that hydrogen is lost predominantly from the methyl group of $\underline{5}$. About 2/3 of the water lost contained the hydrogen initially on the oxygen of $\underline{5}$, as did nearly all of the water lost from $\underline{1}$. Only a small portion of the methyl hydrogens of $\underline{5}$ were lost in water, and the hydrogens of the C_2H_4 moiety were lost in water about equally.

Table 2. Metastable Decompositions of $C_3(H,D)_8O^{+}$ Ions

m/z	$CD_3OH^+CH_2CH_2^+$	$CH_3OD^+CH_2CH_2^+$	$CH_3OH^+CD_2CH_2^+$	$CH_3OH^+CH_2CD_2^+$
31			2	1
32			1	
33	16			
42		2		
43		≤ 1	3	2
44	8		11	
45	45			
60		100	≤ 2	
61	100		100	100
62	≤ 2			

Comparison of CAD spectra of the products of H^+ loss from metastable 1 and 5 to reference CAD spectra of $C_3H_7O^+$ ions demonstrates that the product of both transitions is the same, and that the product is protonated propanal. This seems good evidence that both ions lose H from structure 1, justifying the proposals in Scheme I. However, the translational energy releases are quite different for the two H losses (Table 1), demonstrating that the two reactions differ in some way.

Table 3. CAD Spectra of $C_3H_7O^+$ Produced by $C_3H_8O^{+}$ $C_3H_7O^+ + H^+$

m/z	<u>1</u>	<u>5</u>	$CH_3CH_2CH=OH^+$	$CH_3^+O=CHCH_3$	$CH_2=O^+CH_2CH_3$
26	16	15	16	7	4
27	41	34	39	17	13
28	14	13	14	14	7
29	55	58	44	27	29
30	8	6	9	1	4
31	72	69	100	16	100
37	7	4	7	6	
38	8	8	11	7	
39	30	30	37	2	2
40	4	4	4		
41	30	30	49	2	48
42	12	11	12	12	1
43	23	25	26	34	3
44	8	10	10	7	2
53	3	2	3		
55	8	8	10		
57	52	52	69	2	7
58	100	100	82	100	5

We thank Professor M.L. Gross and the Midwest Center for Mass Spectrometry for use of a Kratos MS50TA mass spectrometer and the Robert A. Welch Foundation and the Petroleum Research Fund for financial support.

1. a. D.J. McAdoo and C.E. Hudson, Adv. Mass Spectrom. 823 (1985). b. D.J. McAdoo, C.E. Hudson, M. Skyiepal, E. Broido and L.L. Griffin, J. Am. Chem. Soc., 109, 7648 (1987).
2. F.W. Crow, M.L. Gross and M.M. Bursey, Org. Mass Spectrom. 16, 309 (1981).

THE OXIRANE ISOMERS $\cdot\text{CH}_2\text{OCH}_2\cdot$, $\cdot\text{CH}_2\text{CH}_2\text{O}\cdot$, AND HCOCH_3
AND THEIR IONIZED COUNTERPARTS

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C-C and C-O bond rupture in oxirane leads to $\cdot\text{CH}_2\text{OCH}_2\cdot$ and $\cdot\text{CH}_2\text{CH}_2\text{O}\cdot$, respectively. These biradicals and methoxycarbene, HCOCH_3 , have been proposed as intermediates in the thermal and photochemical reactions of acetaldehyde and oxirane, but they have never been experimentally detected. We prepared these neutrals in the gas phase via neutralization from the corresponding radical cations and studied their structures and reactivities by collisionally activated dissociation (CAD), reionization into cations and anions, and further CAD analysis (MS/MS/MS) of the neutralized-reionized parent ions.

The precursor radical cations $\cdot\text{CH}_2\text{OCH}_2^+$, $\cdot\text{CH}_2\text{CH}_2\text{O}^+$, HCOCH_3^+ , and oxirane $^+$ were prepared by electron ionization of 1,3-dioxolane, ethylene carbonate, methyl glyoxylate, and oxirane, respectively. Their structures were confirmed by CAD and neutralization-anionization (NR^-) experiments. $\cdot\text{CH}_2\text{OCH}_2^+$ and HCOCH_3^+ yield upon NR^- stable molecular anions which show dominant, structurally diagnostic $\text{CH}_2=\text{CHO}^-$ (m/z 43) and OCH_3^- (m/z 31) fragments, respectively (Figure 1). Collisional excitation of oxirane $^+$ in the field-free region preceding that of CAD does not change its CAD spectrum, indicating that ionized oxirane does not ring-open readily to $\cdot\text{CH}_2\text{OCH}_2^+$, as predicted by MO theory.

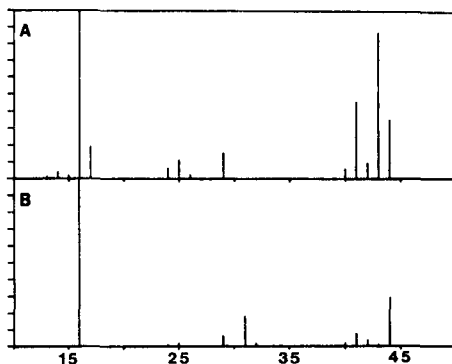


Figure 1. NR^- , benzene (30% Transmittance), spectra of
(A) $\cdot\text{CH}_2\text{CH}_2\text{O}^+$ and (B) HCOCH_3^+ .

All four $\text{C}_2\text{H}_4\text{O}^+$ precursor ions lead upon neutralization-cationization (NR^+) to recovered $\text{C}_2\text{H}_4\text{O}^+$ molecular cations showing that a substantial proportion of the neutrals are formed below their fragmentation thresholds. CAD of the mass-selected recovered molecular ions (MS/MS/MS) combined with NR^- data reveals that both the biradicals and methoxycarbene as well represent bound structures with finite barriers towards isomerization and fragmentation.

Upon CAD all $\text{C}_2\text{H}_4\text{O}$ neutrals studied yield mainly CH_3^+ + $\cdot\text{CHO}$ (e.g., Figure 2). Neutralization-CAD-cationization (NCR^+) of $\cdot\text{CH}_2\text{OCH}_2^+$ leads to recovered $\text{C}_2\text{H}_4\text{O}^+$ molecular ions shown by MS/MS/MS to be composed of 22% $\cdot\text{CH}_2\text{OCH}_2^+$, 76% oxirane $^+$, and 2% CH_3CHO^+ ; thus, undissociated $\cdot\text{CH}_2\text{OCH}_2\cdot$ has partly isomerized to oxirane and a trace of acetaldehyde. The 1,3-H rearrangement $\cdot\text{CH}_2\text{OCH}_2\cdot \rightarrow \text{HCOCH}_3$ is not observed. Similarly, NCR^+ of

$^+\text{CH}_2\text{CH}_2\text{O}^+$ produces reionized $\text{C}_2\text{H}_4\text{O}^+$ parent cations consisting of 56% $^+\text{CH}_2\text{CH}_2\text{O}^+$ and 44% CH_3CHO^+ , which indicates partial $^+\text{CH}_2\text{CH}_2\text{O}^+$ isomerization to CH_3CHO ; no detectable amount of oxirane or vinyl alcohol is found.

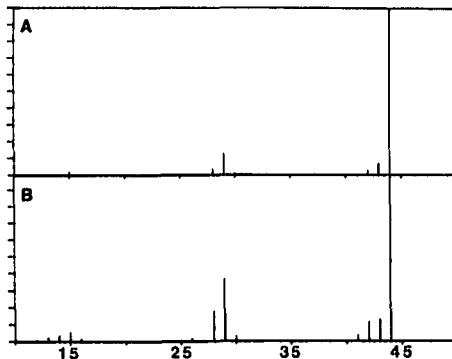


Figure 2. (A) $^+\text{NR}^+$, Xe(70% T)/ O_2 (70% T) and (B) $^+\text{NCR}^+$, Xe(70% T)/He(30% T)/ O_2 (70% T), spectra of oxirane $^+$.

$^+\text{NR}^+$ of oxirane $^+$ under multiple collision conditions yields recovered $\text{C}_2\text{H}_4\text{O}^+$ ions whose CAD spectrum is identical to that of the original oxirane $^+$ precursors and characteristically different from the CAD spectra of $^+\text{CH}_2\text{OCH}_2^+$ and CH_3CHO^+ . This is consistent with high isomerization barriers not only for the oxirane ion (vide supra), but also for the oxirane molecule. Our experimental results are summarized in the potential energy diagram of Figure 3.

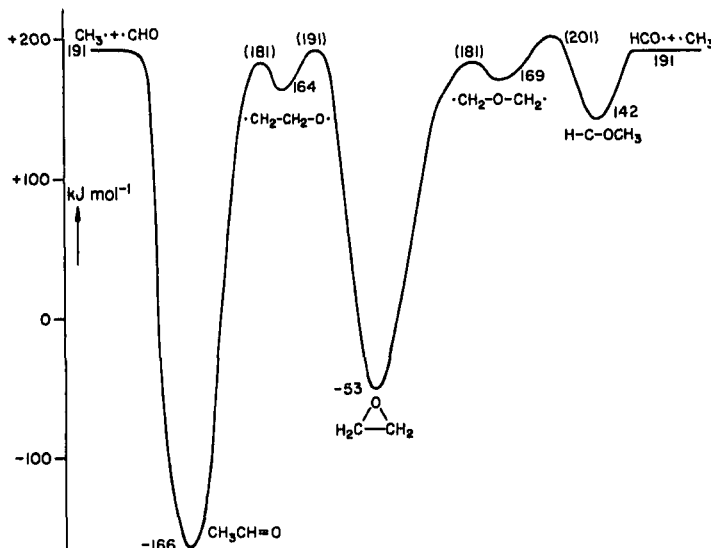


Figure 3. Potential energy diagram of the neutral $\text{C}_2\text{H}_4\text{O}$ isomers.

TRIMETHYLSILYL GROUP MIGRATIONS IN THE MASS SPECTRA OF
DERIVATIZED CHOLESTEROL OXIDATION PRODUCTS

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Northeastern University, Boston MA 02115

The high susceptibility of cholesterol to undergo oxidation in the presence of air and/or light results in the formation of a variety of oxides, many of which have been suspected of causing the cell death and lesion development associated with atherosclerosis. As a part of a study aimed at the characterization and quantification of cholesterol oxides, the fragmentation behavior of the trimethylsilyl (TMS) derivatives of these compounds was examined.

Of interest was the presence of an ion, $[M-71]^+$, which is thought to be analogous to the $[M-56]^+$ ion commonly seen in the spectra of TMS derivatives of 16- and 17-ketosteroids. The ion, $[M-56]^+$, which was initially thought to be due to D-ring cleavage, was later reported by Brooks¹ to be a result of TMS group migration followed by A-ring cleavage at the C_1-C_{10} and C_3-C_4 bonds. We report here related evidence of TMS migrations towards electron rich centers in the electron impact mass spectra of cholesterol oxides.

Deuteriated cholesterol oxide analogues, d_9 -TMS derivatives and TMS derivatized d_3 -(2,2,4)-oxides, as well as linked scanning in the B/E mode were employed in order to demonstrate the occurrence of and further understand the nature of these TMS migrations. The oxides examined were 5 β ,6 β -cholesterol epoxide, 5 α ,6 α -cholesterol epoxide, 6-ketocholestanol, 7-ketocholesterol, 25-hydroxycholesterol, and 3 β ,5 α ,6 β -cholestantriol. All of the spectra were obtained using a VG-70-250SE double focussing mass spectrometer.

A table of ions resulting from TMS migration followed by A-ring cleavage at the C_1-C_{10} and C_4-C_5 bonds is shown below. While several of the compounds, 5 β ,6 β -cholesterol epoxide, 6-ketocholestanol, 7-ketocholesterol, and 25-hydroxycholesterol, also exhibited the analogous migration/cleavage process leading to the $[M-56]^+$ ion, fragmentation leading to the $[M-71]^+$ ion is more prevalent. Included in this table is an ion, $[M-56-113]^+$, possibly arising from a concerted loss of the side chain and A-ring cleavage initiated by TMS migration.

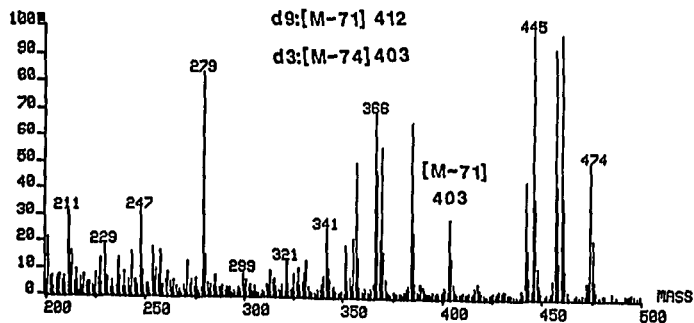
TABLE 1

OXIDE	TMS- d_0	d_9 -TMS	TMS-2,2,4- d_3	NEUTRAL LOSS
α -epoxide	403	412	403	-71,-71,-74
β -epoxide	403	412	403	-71,-71,-74
6-keto	403	412	*	-71,-71
	305	314	*	-56-113,-56-113
7-keto	403	412	401	-69,-69,-71
triol	403	412	403	-90-71,-90-71, -90-74
25-hydroxy	403	-	404	-71,-74

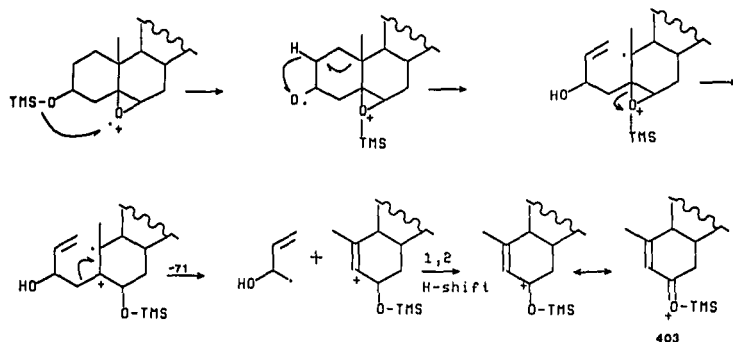
In the EI mass spectrum of the β -epoxide (Figure 1), the $[M-71]^+$ ion is indicated as well as the ions observed in the spectra of the d_9 -TMS-epoxide and the TMS derivatized d_3 -(2,2,4)-epoxide. Similar results confirming TMS migrations were obtained for the other oxides as well. The table and the spectrum show that there is some degree of variation, among the different compounds, in the number of hydrogens transferred to the B-ring during TMS migration and A-ring cleavage. The presence of an $[M-70]^+$ ion as well as an $[M-71]^+$ ion is, in fact, common. A proposed mechanism for the generation of the more frequently observed $[M-71]^+$ ion is shown in Scheme 1. In general, however, the generation of ions such as those listed in the table involves the transfer of a trimethylsilyl group to an accessible electron rich center followed by radical induced cleavage of the A-ring. Of interest is the occurrence of an $[M-71]^+$ ion in the spectra of 25-hydroxycholesterol, which is another possible example of long range TMS migration.

These data, as well as the results of linked scans are quite consistent with TMS migrations. Furthermore, they demonstrate that this process is general in the sense that it takes place in a wide variety of compounds having an electron rich center within proximity of the TMS group. The effect of structure on the mass and intensity of ions resulting from TMS migrations is, as yet, unknown.

FIGURE 1



SCHEME 1

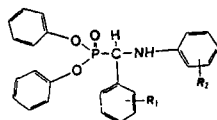


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SUBSTITUENT EFFECTS IN THE MASS SPECTRA OF SUBSTITUTED DIPHENYL 1-PHENYLAMINO-1-PHENYLMETHANEPHOSPHONATES M. Rahi,¹ K. Jayasimhulu,² S.J. Smith,¹ and H. Zimmer¹ ¹Department of Chemistry, ²Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati Ohio 45221, 245267-0056

Alpha-Heterosubstituted phosphonate carbanions are very useful in organic synthesis (1). Zimmer et al. (2) have found that chemical shifts of ³¹P, ¹³C, and ¹H NMR of diphenyl 1-phenylamino-1-phenylmethane phosphonates, I, and their anions are influenced in a Hammett fashion by the



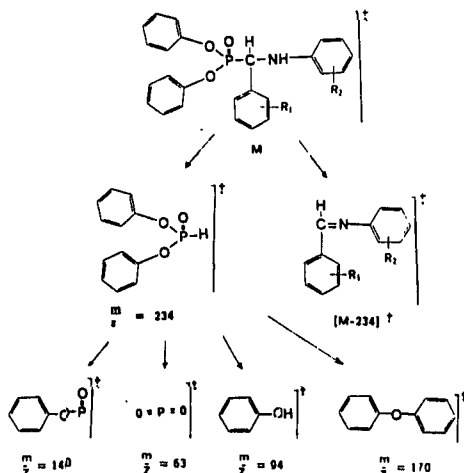
I

substituents R₁ and R₂. Based on these results, we became interested in investigating whether substituent effects also could be observed in the mass spectra of these compounds. Therefore, we investigated the influence of the substituents R₁ and R₂ on the abundance of certain ions in their mass spectra. A series of compounds substituted in the meta and para positions on the aniline moiety and/or the benzyl moiety were prepared, Table I, by the method outlined by Zimmer and Seemuth (1), and their mass spectra were recorded.

TABLE I

	R ₁	R ₂	Log Z	σ _{m,p}	σ ^o
1	H	H	1.32	0	0
2	H	m-Br	.86	.38	.39
3	H	p-Br	.90	.23	.29
4	H	m-NO ₂	1.12	.71	.70
5	H	m-OCH ₃	1.22	.12	.06
6	H	p-OCH ₃	1.28	-.27	-.15
7	m-Br	H	1.07	.39	.38
8	p-Br	H	.96	.23	.29
9	m-NO ₂	H	1.21	.71	.70
10	p-NO ₂	H	1.07	.78	.83
11	m-OCH ₃	H	1.25	.12	.06
12	p-OCH ₃	H	1.25	-.27	-.15
13	p-Br	p-Br	.96	.46	.52
14	m-Br	m-Br	.86	.78	.76
15	p-OCH ₃	p-OCH ₃	1.34	-.54	-.30
16	m-OCH ₃	m-OCH ₃	1.34	.24	.120
17	p-Br	p-NO ₂	.95	1.03	1.12
18	p-Br	p-OCH ₃	1.14	-.04	.10
19	m-Br	m-NO ₂	.68	1.10	1.08
20	m-Br	m-OCH ₃	.67	.51	.42
21	p-NO ₂	p-Br	.68	1.03	1.12
22	p-NO ₂	p-OCH ₃	1.21	.51	.66
23	m-NO ₂	m-Br	.62	1.10	1.08
24	m-NO ₂	m-OCH ₃	1.18	.83	.76
25	p-OCH ₃	p-Br	1.20	.04	.10
26	m-OCH ₃	m-NO ₂	1.11	.83	.76
27	m-OCH ₃	m-Br	.88	.51	.42
28	H	p-CN	1.23	.69	.66
29	p-CN	H	1.14	.69	.66

The molecules undergo fragmentation under electron impact corresponding to the scheme in which the major peaks of the spectra are illustrated.



The electron impact fragmentation data indicates significant changes in the fragmentation patterns in terms of relative intensities of various peaks with respect to different substituents in the phenyl groups.

In general, the molecular ions are low to medium in abundance. The fragment ion $m/z = [M-234]^+$, the most abundant in most cases, appears to have a good correlation with Hammett σ values. The intensity of this ion is enhanced by electron-releasing groups, while electron withdrawing groups tend to decrease its intensity. Plots of $\log Z$, where Z is the relative percent abundance of the ion, versus $\sigma_{m,p}$ and $\log Z$ versus σ^0 , results in linear fits in both cases. This linear relationship can be described by the equation.

$$F_m = 1.22 - 0.31 \Sigma \sigma_{m,p}$$

$$F_m = 1.23 - 0.35 \Sigma \sigma^0$$

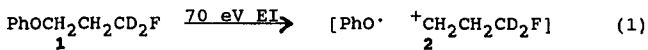
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REARRANGEMENTS OF FLUORINE-CONTAINING CATIONS

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The rearrangements of 3- and 4-carbon mono-fluorinated cations have been experimentally probed. The technique which has been employed involves generating the ions as a component in an ion molecule complex. The ion-molecule complex in this case is an intermediate in the unimolecular decomposition of an ionized fluoroalkyl phenyl ether. Ionized primary alkyl phenyl ethers have previously been shown to yield phenol molecular ion and an alkene through the intermediacy of an ion-molecule complex¹. Placement of a fluorine substituent on the alkyl group will yield, upon ionization and carbon-oxygen bond heterolysis, a complex in which the fluoroalkyl cation is predicted to be unstable with respect to rearrangement. Subsequent proton transfer to the more basic phenoxy moiety will occur to give PhOH^+ and a neutral fluoroalkene. The structure of the neutral fluoroalkene thus formed reveals information regarding the pathways by which the fluoroalkyl cation has rearranged in the ion-molecule complex.

In our experiments, we collect and analyze the neutral products from the 70eV electron impact on primary fluoroalkyl phenyl ethers at 1×10^{-4} Torr. For this we use the EBFlow apparatus². Electron impact on phenyl ether **1** may initially form the ion-molecule complex **2**. The ion in this complex is a primary carbocation, destabilized by the electron withdrawing effect of fluorine, and is expected to rapidly rearrange. *Ab initio* molecular orbital calculations on the $\text{C}_3\text{H}_6\text{F}^+$ system have indicated that a number of rearrangement pathways should be facile³.



The neutral fluoroalkenes formed from **1** are shown in Scheme 1. The mixture of neutrals which was collected was analyzed by 282 MHz ¹⁹F NMR. Integration of the NMR signals yielded the relative amounts of the products which enabled us to estimate the branching ratios shown. The results indicated that about 70% of the products arise from a single 1,2 hydride shift, and about 30% come from a double 1,2 shift. Small amounts (<2%) of products arising from more than two 1,2 hydride shifts are also observed.

In a second set of experiments, we have examined the question of whether there is competition between a 1,2 methyl migration and a 1,2 fluoride migration, as predicted by *ab initio* calculations. In these experiments phenyl ether **3** was used as the precursor to the ion of interest. A summary of the neutral product analysis for the EBFlow experiment on this compound are shown in Table 1. The experiment was also performed on the hexadeuterio-analogue of **3** in which both methyl groups are perdeuterated. These results show that products arising from both methyl and fluoride shifts are formed. Alternative pathways, such as elimination of HF followed by re-addition of HF which would form the same products as a fluoride shift, can be ruled out from the deuterium labeled experiment.

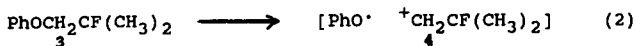
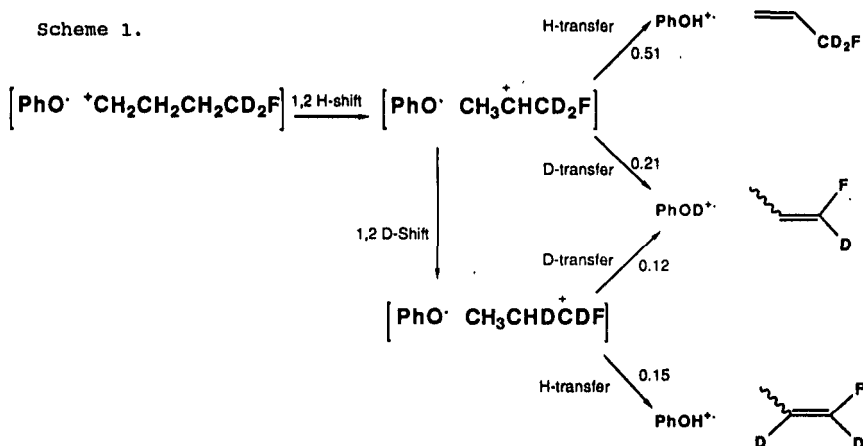


Table I Isomer distribution from 70 eV EBFlow radiolyses of **3** (d_0) and (d_6) based on integration of ^{19}F NMR signals (corrected for differences in T_1 relaxation times).

Methyl shift	Rel. amount		Fluoride shift	Rel. amount	
	X=H	X=D		X=H	X=D
	1.0	1.0		0.29	0.25
	0.32	0.50		0.27	0.37
	0.58	0.79			

Scheme 1.



Acknowledgment. This work was supported by NSF Grant CHE88-02086.

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BENZOPHENONE IMINE FORMATION FROM BENZOPHENONE OXIME

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A GC/EIMS study was made of both unsubstituted and substituted benzophenone oximes (YArC(=N-OH)Ar) by varying the GC injection port, transfer line and ms source temperatures to confirm the origin of the unusual $[\text{M-O}]^+$ ion found by several groups in EIMS studies of benzophenone oxime⁽¹⁻³⁾.

EXPERIMENTAL

Experiments were performed with the Hewlett Packard 5890 GC-5970 MSD system. An HP-1 (Methyl silicone) capillary column was used for the separation. Benzophenone oximes were prepared by adding excess hydroxylamine hydrochloride to the corresponding benzophenones (in ethanol) at room temperature. After the reactions were completed, the solutions were injected into the GC injection port at different injection port temperatures or at different transfer line and source temperatures. The resulting GC traces and the mass spectra were investigated.

RESULTS AND DISCUSSIONS

(1) The formation of benzophenone imines (YArC(=NH)Ar) from neutral benzophenone oximes was found in the GC injection port at about 200-300 °C. However, no benzophenones were found under these conditions, in contrast to earlier work on the formation of benzophenone imines from the oximes⁽⁴⁾. The formation of the imines is strongly temperature dependent within an injection port temperature range of 100 °C (Fig.1,2). Thus thermal decomposition and ionization of the neutral products may explain the previously observed variations in the relative peak intensities in the mass spectrum of the oxime.

(2) Experiments were also done by changing the transfer line/source temperatures at a constant injection port temperature. Mass spectra of the "oximes" under high and low transfer line/source temperatures than were compared, and any imine formation should be observed from the characteristic ions of the imine in the mass spectra of the "oximes".

Formation of the unsubstituted benzophenone imine from the oxime is not obvious due to both the short contact time and overlapping ions in the spectra. Formation of substituted benzophenone imines was clearly indicated by an increase in the intensity of the ion at m/z 180, an ion that is characteristic of all imines. Figure 3 shows the mass spectrum of para-chloro benzophenone imine and the mass spectra of the "oxime" at two different transfer line temperatures. The difference spectrum between the spectra of the para-chloro benzophenone oxime at high and low transfer line temperatures compares well with the spectrum of the imine.

CONCLUSIONS

From our studies, it is evident that neutral benzophenone oximes can decompose thermally to imines in the hot injection port or ion source prior to ionization. The formation of $[M-O]^+$ ion in the mass spectra of the oximes and the variations in the relative intensities of some fragment ions are primarily due to imine formation.

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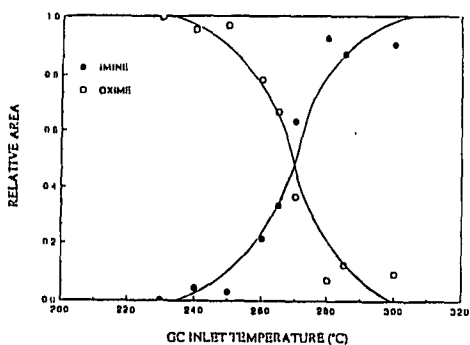


Figure 1. Effect of the injection port temperatures on the conversion of benzophenone oxime to the imine.

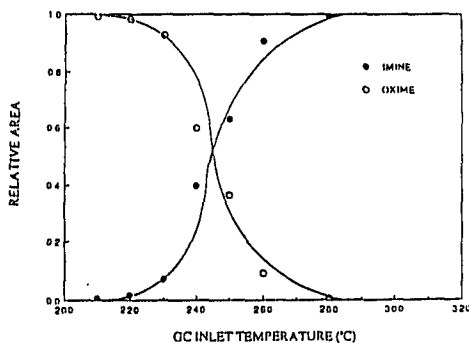


Figure 2. Effect of the injection port temperatures on the conversion of para-fluoro benzophenone oxime to the imine.

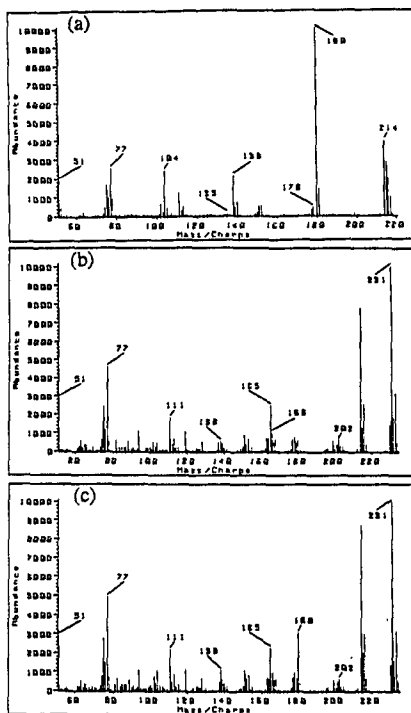


Fig. 3 Mass spectra of para-chloro benzophenone imine(a) and para-chloro benzophenone oxime obtained at 260 °C injection port temperature and 250 °C(b), 310 °C(c) transfer line temperatures.

DEUTERIUM ISOTOPE EFFECT IN SOLID PROBE MS ANALYSIS.

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Clairol Inc., Stamford, CT 06922

OBJECTIVE

To enhance the analytical capability of the GC/MS system for structure elucidation by solid probe MS.

INTRODUCTION

It is a well known fact that an active hydrogen (e.g., -OH, -NH₂, or -NRH) in a compound can be replaced with deuterium in the presence of deuterium oxide. Indeed this is a common technique for making assignments in NMR. The advantage of using this technique in MS analysis is less obvious since a pure D-labeled species is difficult to maintain through the entire experimental process. A deuterium in an active site will exchange with the hydrogen in the surrounding area. The mass spectrum of a D and H mixture may not provide useful information. We have found, however, that the H- and D-species may show differences in thermal desorption processes. This paper presents a utilization of this technique to study HC Blue No. 1 (1) and its analogue (2) which is N₄,N₄-(2-Hydroxyethyl)-2-nitro-(p-phenylene-diamine).

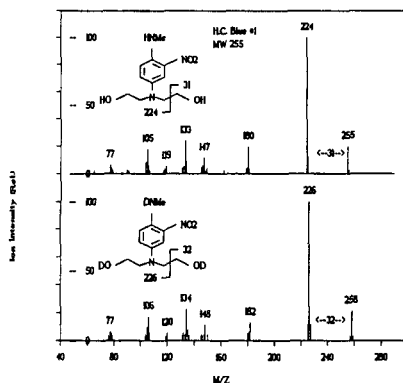
EXPERIMENTAL

In this study, all analyses were carried out using a Finnigan 4000 mass spectrometer equipped with a solid probe. The ballistic mode was selected for heating samples. D-labeled compounds were prepared by dissolving a small portion of the sample in a few drops of D₂O (Aldrich, Milwaukee, WS 53233). Compounds 1 and 2 were provided by Mr. J. Anderson (Clairol Inc.).

RESULTS AND DISCUSSION:

N-hydroxyethyl is a functional group commonly found in dyes or intermediates. The major characteristic ions found in the mass spectra of this class of compounds are the molecular ion and the M-31 ion. The M-31 ion is usually the base peak. The mass spectra shown in Figure 1 are HC Blue No. 1 and its deuterated species. These spectra clearly suggest that the M-31 ion is formed by a simple cleavage of the hydroxyethyl group. The spectra also indicate that there are three active hydrogens in this compound. This D-labeled sample is actually a mixture of various D- and H-species. These species, however, show slight variations during the

Figure 1.



sample heating process. Figure 2 shows the RIC of this deuterated sample and the single ion chromatograms of the various molecular ions.

Figure 3 is the mass spectra of compound 2 and its deuterated species. Again the spectrum of the D-labeled was obtained from a D- and H-mixture. The D-labeled species in the various locations of the RIC are shown in Figure 4. Based on these spectra we are able to derive the formation of the base peak and to assign the number of active hydrogens in this compound.

Figure 2.

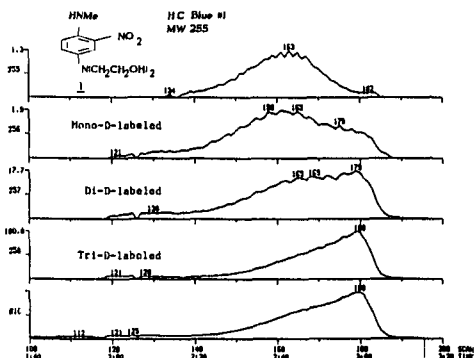
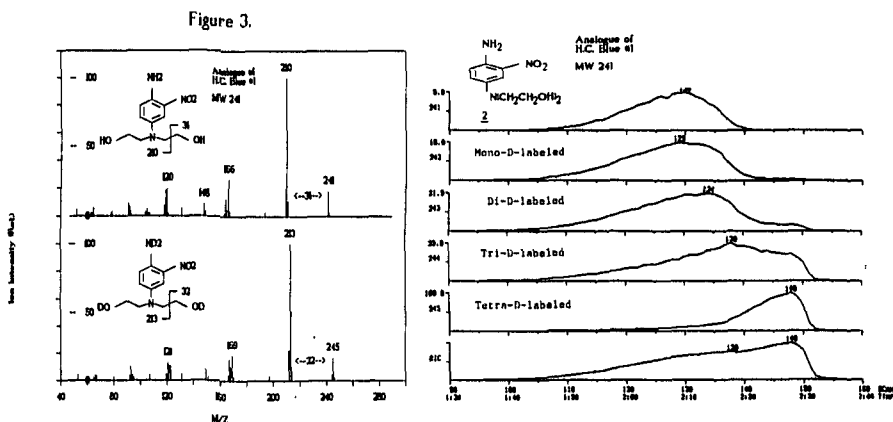


Figure 4.



CONCLUSION

The results have demonstrated that solid probe MS, which is generally considered to be a low resolving power method can provide significant separation for D,H-mixtures. As a result, the combined utilization of solid probe MS and D-labeling techniques can greatly enhance the analytical capability of a MS system for making structure assignments.

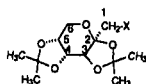
THE ELECTRON IONIZATION MASS SPECTRA OF NOVEL 2,3,4,5-BIS-O-(1-METHYLETHYLIDENE)-B-D-FRUCTOPYRANOSE DERIVATIVES AND RELATED SUGAR SULFAMATES. G.W. Caldwell, B.E. Maryanoff and J.A. Masucci; Dept. of Chemical Research, Janssen Research Foundation, Spring House, PA 19477-0776

The electron ionization (EI) mass spectral fragmentation of bis-Q-(1-methylethylidene) fructopyranose derivatives (Chart 1) and related sugar sulfamates (Chart 2) have been investigated by accurate mass measurements, isotopic labeling and metastable scans. The mass spectra of the 2,3:4,5-bis-Q-(1-methylethylidene) fructopyranose derivatives were not very dependent upon the nature of substitution - the mechanisms of the common fragmentation patterns are given in Scheme 1 and 2¹⁻⁹. Several pieces of evidence corroborate the proposed fragmentation pathway outlined in Scheme 1. Considering our example with X = OSO₂NH₂, both the elemental composition of the proposed fragments and the deuterium incorporation agreed with the fragmentation paths. The elemental composition of fragment A was found to correspond to C₁₁H₁₈O₈NS (found 324.075₉₁; -0.5 mmu), that of fragment B to C₆H₁₂O₇NS (found 266.033₄₀; +0.05 mmu), and that of fragment D to C₆H₈O₅NS (found 206.013₄₂; -1.1 mmu). The deuterium incorporation observed in the EI mass spectrum of 1-d₁₂ is consistent with Scheme 1. The deuterium incorporation for 1-d₁₂ at m/z 333 (324+9) and 269 (266+3) and the lack of deuterium incorporation at m/z 206 was observed. The loss of acetone (58 amu), from fragment A, and acetic acid (60 amu), from fragment B, were confirmed by neutral mass loss metastable link scans. There are eight prominent peaks in the lower mass range for compounds 1-10, common to all 2,3:4,5-bis-Q-(1-methylethylidene) fructopyranose derivatives: m/z 229, 171, 127, 113, 85, 69, 59, and 43 (Scheme 2). The loss of CH₂X (X = OSO₂NH₂), from compounds 1-10 produced a relatively abundant peak (3-78%) at [M-CH₂X]⁺ (m/z 229; fragment H) - the loss of acetone from fragment H gave [M-CH₂X-58]⁺ (m/z 171; fragment I). The ion abundance of fragment I ranges from 9-100% and thus is easily recognized in most EI spectra. A peak at [M-CH₂X-58-28]⁺ (m/z 143; fragment J) was observed in all EI mass spectra (except 2) at a low ion abundance (1-3%). These fragmentation pathways are outlined in Scheme 2. The elemental composition of fragment H was found to correspond to C₁₁H₁₇O₅ (found 229.107₄₅; +0.1 mmu), that of fragment I to C₉H₁₁O₄ (found 171.064₉₆; +0.8 mmu), and that of fragment J to C₇H₁₁O₃ (found 143.069₄₂; +1.4 mmu). The 1-d₁₂ and 6-d₁₂ analogues exhibited m/z 241 (229+12), 117 (171+6) and m/z 149 (143+6). The loss of acetone from fragment H was confirmed by neutral mass loss metastable link scans. The fragmentation pathway leading to fragment J could not be confirmed by metastable link scans. The EI spectral data for bis-Q-(1-methylethylidene) derivatives of several isomeric sugar sulfamates (Chart 2; 11-13) were investigated. While many of the fragments were similar to 1-10, there were structurally significant ions which could be used to distinguish these structures. Compound 11 contained a unique fragment at m/z 100 (C₅H₉O₂; 100.052₂₇; +0.2 mmu). The most pronounced peak in 12 was the m/z 143 ion (C₇H₁₁O₃, 143.071₅₆; -0.7 mmu), Compound 13 contained two unique ions at m/z 248 (C₈H₁₀O₆NS, 248.022₁₇; +0.7 mmu) and 238 (C₇H₁₂O₆NS, 238.037₉₂; +0.6 mmu).

Experimental

Electron ionization (EI) mass spectra were obtained using either a VG 70-250E mass spectrometer or an HP 5970 gas chromatograph/mass selective detector (GC/MSD) mass spectrometer.

Chart 1



X	X
1 OSO ₂ NH ₂	6 OH
2 OSO ₂ NHCH ₃	7 Cl
3 OSO ₂ N(CH ₃) ₂	8 NH ₂
4 OSO ₂ N ₃	9 OC(O)NH ₂
5 OSO ₂ NH-	10 OC(O)NH-

Scheme 1 (X = OSO₂NH₂)

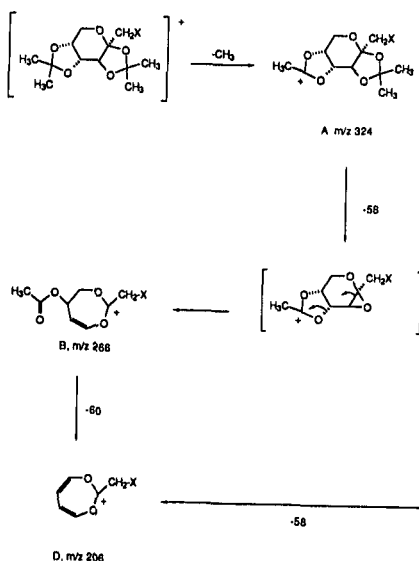
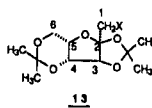
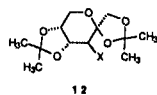
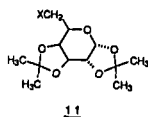
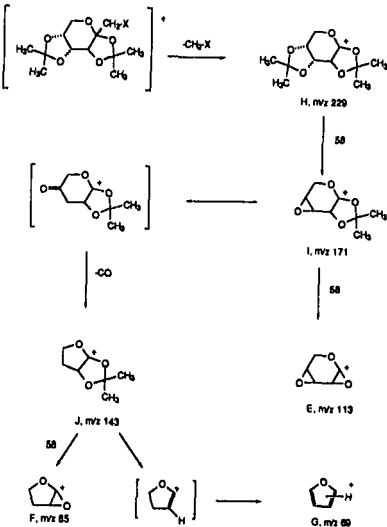


Chart 2

X = OSO₂NH₂



Scheme 2



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THE INVESTIGATION OF [M-35]⁺ IONS IN 2-ALKYL-4,6-DINITROPHENOLS
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An unusual fragment ion, the loss of 35 mass units, was observed in several 2-alkyl-4,6-dinitrophenols. This ion fragmentation process was characterized to establish if the [M-35]⁺ fragment could be useful for the identification of these compounds in environmental samples, as well as to study the *ortho* effect (1-3) chemistry believed to be involved in this reaction. Selected compounds were studied by electron impact with a VG-70-250SEQ tandem hybrid mass spectrometer (EBQQ geometry); low resolution unimolecular and CAD MS/MS and high resolution (25,000 10% valley) spectra were recorded. In addition, one compound was studied by photoelectron-photoion coincidence (PEPICO) time-of-flight mass spectrometry (4,5) to determine some of the energetics of the reaction.

An analysis of the low resolution data revealed that the [M-35]⁺ ion could be correlated with the formation of [M-18]⁺ ions. Also, the alkyl group has to be larger than methyl for the [M-35]⁺ fragment to be observed. High resolution measurements of these fragments yielded that the [M-18]⁺ ion corresponds to H₂O loss, and the [M-35]⁺ ion represents further loss of OH. The MS/MS results, shown in Figure 1 for 2-(1-methylpropyl)-4,6-dinitrophenol, indicate the [M-18]⁺ ion is formed directly from the molecular ion, and CAD enhances [M-35]⁺ formation. A breakdown diagram for 2-ethyl-4,6-dinitrophenol obtained by PEPICO is shown in Figure 2.

A mechanism for the formation of [M-35]⁺ ions is shown in Figure 3. The initial water loss can occur via hydrogen rearrangement going through a cyclic six-membered transition state, and the actual water loss may be driven by anchimeric assistance (6). The subsequent OH radical loss is an example of the *ortho* effect; an alkyl hydrogen atom rearranges to a nitro oxygen. Since the [M-35]⁺ ion is enhanced under CAD conditions and is dominant in the MS/MS spectrum of the [M-18]⁺ ion, these two neutral losses are stepwise. The PEPICO results indicate that [M-18]⁺ and [M-35]⁺ ion formations occur at parent ion internal energies of 1.55 and 2 eV respectively. Therefore, the potential barrier for the second step in the [M-35]⁺ formation is considerably smaller than the barrier for [M-18]⁺ formation and this would result in the [M-18]⁺ to [M-35]⁺ reaction to be fast relative to the [M]⁺ to [M-18]⁺ reaction. This suggests that the *ortho* effect is a strong driving force for secondary fragmentation of the [M-18]⁺ ion.

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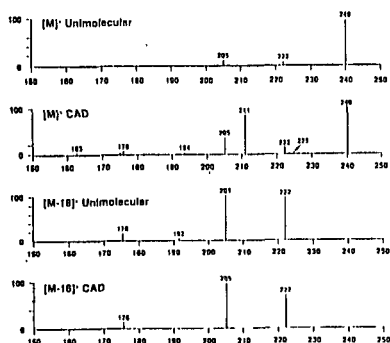


Figure 1: Unimolecular and CAD MS/MS spectra of $[M]^+$ and $[M-18]^+$ ions in 2-(1-methylpropyl)-4,6-dinitrophenol.

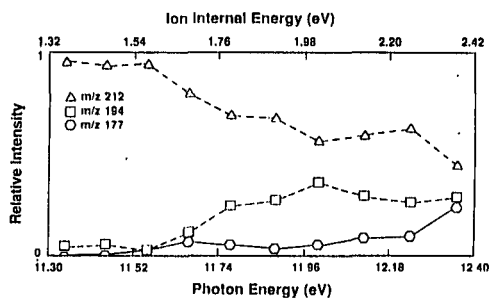


Figure 2: PEPICO breakdown diagram for $[M]^+$, $[M-18]^+$, and $[M-35]^+$ for 2-ethyl-4,6-dinitrophenol.

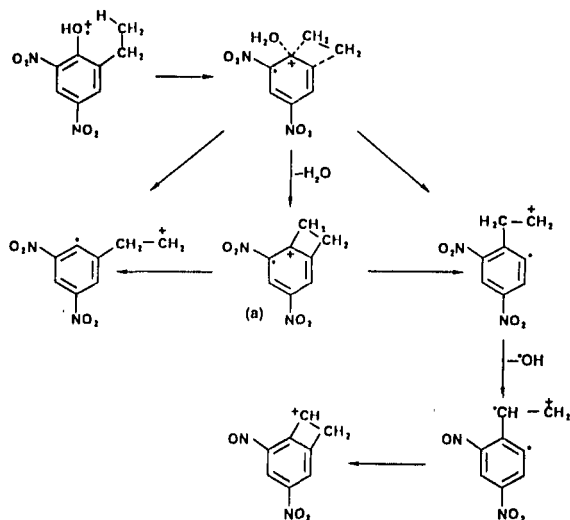


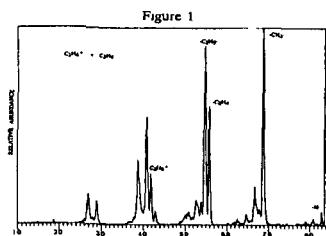
Figure 3: The proposed mechanism, shown with R =Ethyl, for the formation of $[M-35]$ fragment ions in 2-alkyl-4,6-dinitrophenols.

THE GAS-PHASE REACTION OF THE CYCLOPROPANE RADICAL CATION AND CYCLOPROPANE: FORMATION OF THE HEXAMETHYLENE DISTONIC ION

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The principle aim of this study is to investigate whether a gas-phase adduct is formed in a reaction between the cyclopropane radical cation and cyclopropane, and if so, characterize the adduct. This reaction is of particular interest because several studies have shown that the cyclopropane radical cation may react as a distonic radical cation (i.e., charge-radical site separate radical cation).^{1,2}

In order to obtain structural and kinetic information concerning the reaction adduct we employed two complimentary tools.^{3,4} First, tandem mass spectrometry combined with collisional activation (CAD) was used to determine the structure of an adduct formed in a chemical ionization (CI) source. Secondly, FTMS was used to determine possible changes in an adduct structure over time.



When cyclopropane is ionized in a chemical ionization source an adduct, $C_6H_{12}^{+•}$, is formed that fragments upon CAD to give the following structurally significant ions (see Figure 1); viz, m/z 83 $[M - H]^{+•}$, m/z 69 $[M - CH_3]^{+•}$, m/z 56 $[M - C_2H_4]^{+•}$, m/z 55 $[M - C_2H_5]^{+•}$, and m/z 42 $[M - C_3H_6]^{+•}$.

Gas-phase bimolecular adducts may exist as loosely-bound complexes. Complexes of this nature are not expected to undergo rearrangements, and upon collisional activation they should return to starting materials. The adduct ions formed in the reaction between $C_3H_6^{+•}$ and C_3H_6 undergo, upon CAD, multiple dissociation processes. Clearly, a major proportion of the adduct ions formed in this reaction possess a significant degree of covalency. Moreover, the metastable dissociations of the adduct closely resemble the CAD dissociations and also do not include any significant reversion to starting materials.

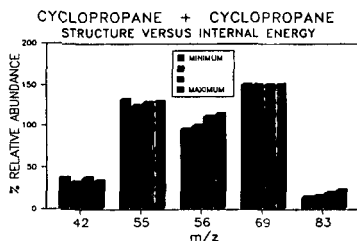


Figure 2

To investigate further the nature of the bonding in these adduct ions, several CAD spectra were obtained as a function of internal energy. This was accomplished by raising the pressure in the ion source of a third body, CS_2 , from zero to 40 times that of cyclopropane thereby changing the degree of collisional stabilization. Thus, the internal energy of the adduct may be decremented in a controlled manner. A histogram (Figure 2) showing the relative abundances of several key ions in the CAD spectra as a function of internal energy shows little change in going from the most to the least stabilized adduct. Therefore, the adduct must possess a stable structure.

The kinetics of adduct formation were investigated by varying the delay time between ionization and detection using an FTMS spectrometer. The relative abundance of the ion of m/z 42 ($C_3H_6^{+ \cdot}$) decreased while the relative abundances of ions of m/z 55, 56 and 69 all increased steadily from 300 msec to 3.25 sec (Figure 3). Clearly, the reaction has first order rate kinetics with respect to the cyclopropane radical cation.

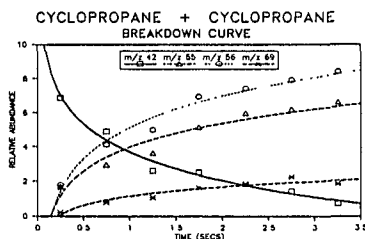
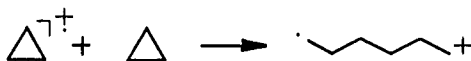


Figure 3

On the basis of earlier gas-phase and solution chemistry, we propose that a linear adduct is formed between $C_3H_6^{+ \cdot}$ and C_3H_6 (Scheme 1).



Scheme 1

A strength of the CAD MS/MS technique is that an adduct structure may be determined by a comparison of the CAD mass spectra of appropriate model compounds with the CAD spectrum of the adduct. Of four model compounds studied only the 1-hexene radical cation closely resembles that of the adduct. This is perhaps not surprising as a facile 1,5-hydrogen shift of the initially formed 1-hexene radical cation would yield a distonic structure identical to that of the adduct. Conversely, a 1,5-hydrogen shift of the adduct would give rise to the 1-hexene radical cation.

The CAD spectra of two cyclic models, cyclohexane and methyl cyclopentane, are quite different from the adduct. Specifically, cyclohexane dissociates to give more abundant ions of m/z 83 [$M - H$] $^{+ \cdot}$ and 56 [$M - C_2H_4$] $^{+ \cdot}$, and the ion of m/z 69 [$M - CH_3$] $^{+ \cdot}$ is much diminished. The CAD spectrum of methylcyclopentane is dominated by the ion of m/z 69, whereas the losses of C_2H_4 and C_2H_5 are attenuated.

The losses of C_2H_4 and C_2H_5 from the 3-hexene radical cation are more abundant whilst the loss of CH_3 is less significant than that of the adduct.

In conclusion, the cyclopropane radical cation reacts with cyclopropane to give rise to a linear adduct whose structure most closely resembles that of the 1-hexene radical cation. Moreover, the CAD spectra of several possible precursors of $C_6H_{12}^{+ \cdot}$ are distinguishable while being similar as an isomeric group. The adduct has covalent bonding and is formed by a reaction that proceeds with first order kinetics.

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MASS SPECTROSCOPIC STUDY OF SOME PERFLUOROCARBONS AND PERFLUOROCARBONS
CONTAINING NON-FLUOROATOMS OR GROUPS

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It has been reported that the fragmentation pattern of perfluorocarbons (PFCs) under the negative ion chemical ionization (NICI) condition was little influenced by the change of the reagent gases, the pressure of the reagent gas, and the ion source temperature, etc. The most abundant molecular radical anion of PFCs was usually obtained in a typical NICI condition. The fragmentation pattern of PFCs containing heteroatoms or groups in a typical NICI condition however was found to be very different from of PFCs. Fragmentation patterns of some PFCs containing heteroatoms or groups, ionized by different ionization modes, were studied by an HP 5988 GC/MS system. A compilation of ions and their relative abundance by the ionization of perfluoroadamantane and its derivatives containing non-fluorocarbon groups under different ionization conditions is shown in Table 1. For the comparison purpose, some of the simpler fluorocarbons such as 1H-perfluoroheptane, perfluoro-1-t-butanol, perfluoro-2-butyl THF and perfluorinated isobutyl-1-bromobutyl ether were also studied.

As shown in the table, under typical NICI conditions, PFCs containing chlorine(s), methyl ester and hydrogen have a tendency to lose Cl, CO₂CH₃, and HF respectively. Although the molecular ion is still the major ion in NICI for perfluoroadamantanes containing one or two chlorines, the ion corresponding to an HF loss becomes the major ion (100) with respect to the molecular ion (91) for 2-H-Perfluoro-1,3-dimethyl adamantane. An ion corresponding to the loss of CO₂CH₃ is the major ion observed for methyl perfluoroadamantane in which very little or no molecular ion was detected. No molecular ion was observed in the NICI condition for 1H-F-heptane and F-t-butanol. Instead, ions corresponding to a loss of HF and H, respectively are the most abundant. Therefore it seems that non-cyclic or structurally unstable fluorocarbons containing non-electronegative atoms or groups can easily eliminate these heteroatoms or groups in NICI.

In the methane chemical ionization (CH₄/CI) condition, the abstraction of an F from PFCs by the reagent gaseous ions results in the formation of (M-F)⁺ which accounts for the most abundant ion for most of the fluorocarbons studied in this work. (M-Cl)⁺ and (M-Br)⁺ were the major ions observed for 1,3-dichloro-F-adamantane and F-isobutyl-1-bromobutyl ether, respectively. (M+H)⁺ was an abundant ion observed for F-t-butanol and methyl-F-adamantane. MS/MS spectral studies of several ions of these compounds by a VG-ZAB BEQQ hybrid mass spectrometer suggests that the protonation of F-t-butanol and F-2-butyl THF in the CH₄/CI condition was occurred on the fluorine atom not on the oxygen atom.

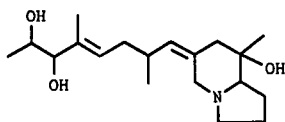
A common stable ion observed in EI for F-adamantane and its derivatives listed in Table 1 is C₈F₉X⁺ where X is F or Cl or CF₃. A higher percentage of M⁺ and (M-OCH₃)⁺ was found for methyl-F-adamantane. It appear that the CO₂CH₃ group is more competitive with fluorines on the -adamantane cage for the electron ionization than are other heteroatoms or groups. Therefore the CO₂CH₃ group appears to be a good "heat sink" or an energy moderator for F-adamantane through its stable resonance structures of F-Ad-C≡O⁺.

Table 1

		C1			C2, C3			C4, C5			C6, C7		
		100	111	102	100	111	102	100	111	102	100	111	102
2		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	2 9 4 4 61 231 100 203		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	3 5 4 4 100 421 100 421		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	3 5 4 4 100 421 100 421		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 440 44 440 65 440	
3		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	3 7 4 4 10 21 100 221		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	7 8 4 4 100 421 100 421		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	7 8 4 4 100 421 100 421		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 456 37 456 87 456	
4		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	7 12 100 203		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	97 425 13 386 100 203		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	97 425 13 386 100 203		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 421 36 426	
5		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	6 4 10 367 46 201 14 187 18 166 33 131 43 117 18 95 100 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	57 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	57 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 406 58 405 49 386	
6		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	6 4 1 367 47 201 32 187 47 131 35 113 21 83 100 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	48 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	48 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 406 50 386	
7		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	6 4 1 367 47 201 32 187 47 131 35 113 21 83 100 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	48 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	48 406 100 367 100 367		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 386 58 386	
8		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	33 446 28 445 47 433 100 131		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 405 2 5 445 100 405		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 405 2 5 445 100 405		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	1 446 4 433 100 405 6 386	
9		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	0 15 5 365 30 251 13 181 22 131 100 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 505 13 427 100 505		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 505 13 427 100 505		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 521 28 453	
10		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	5 506 1 447 30 251 38 181 19 131 100 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 437 32 449 100 437		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 437 32 449 100 437		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	91 506 100 486	
11		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	5 476 13 455 17 251 100 203 27 161 47 131 92 69		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 455 42 437 100 455		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 455 42 437 100 455		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 476 7 456 23 485	
12		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	2 231 1 219 17 151 47 131 27 119 46 161 100 49 75 51		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	4 8 369 77 231 100 217 100 217		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	4 8 369 77 231 100 217 100 217		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	22 6 100 8 3 369 100 216 100 216	48 403 100 369 12 354
13		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	5 517 51 197 41 137 47 137 100 87		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	5 7 251 61 8 217 100 217 100 217		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	5 7 251 61 8 217 100 217 100 217		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	47 471 16 462 38 255 100 235 39 239 42 166	
14		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	3 7 397 100 169 17 131 46 100		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	51 417 100 397 100 397		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	51 417 100 397 100 397		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	100 416 2 5 400 4 9 338	
15		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	0 3 11 21 8 15 17 100		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	22 463 11 363 45 403 100 363 9 297 23 286		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	22 463 11 363 45 403 100 363 9 297 23 286		m ⁺ (M-1) ⁺ (M-2) ⁺ m ⁺ C ₆ H ₄ Cl ⁺ m ⁺ C ₆ H ₅ Cl ⁺	98 515 100 395	

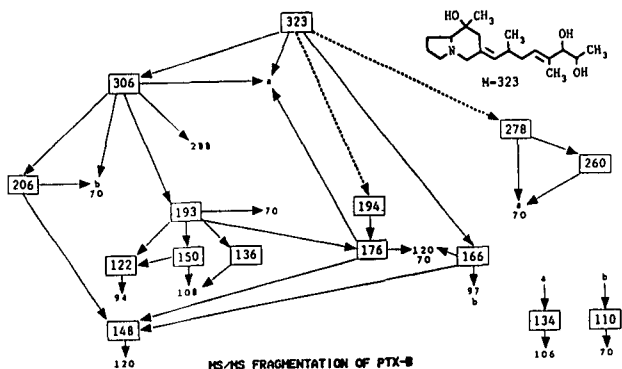
TPA61: A Study of the Fragmentation Pathway of a Series of Frog Alkaloids of the Pumiliotoxin Class.
Pu Quan-long, L.K. Pannell, H.M. Fales and R.T. Mason, NIDDK and NHLBI, National Institutes of Health, Bethesda, MD 20892.

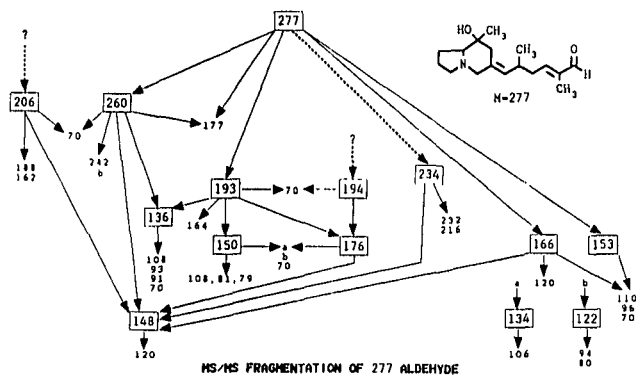
AIM: Skin secretions from amphibians contain a wide range of biologically active substances, including lipophilic alkaloids. One of the largest group is the compounds referred to as the Pumiliotoxins. The structure of Pumiliotoxin B (PTX-B) which has a molecular weight of 323 is shown below:



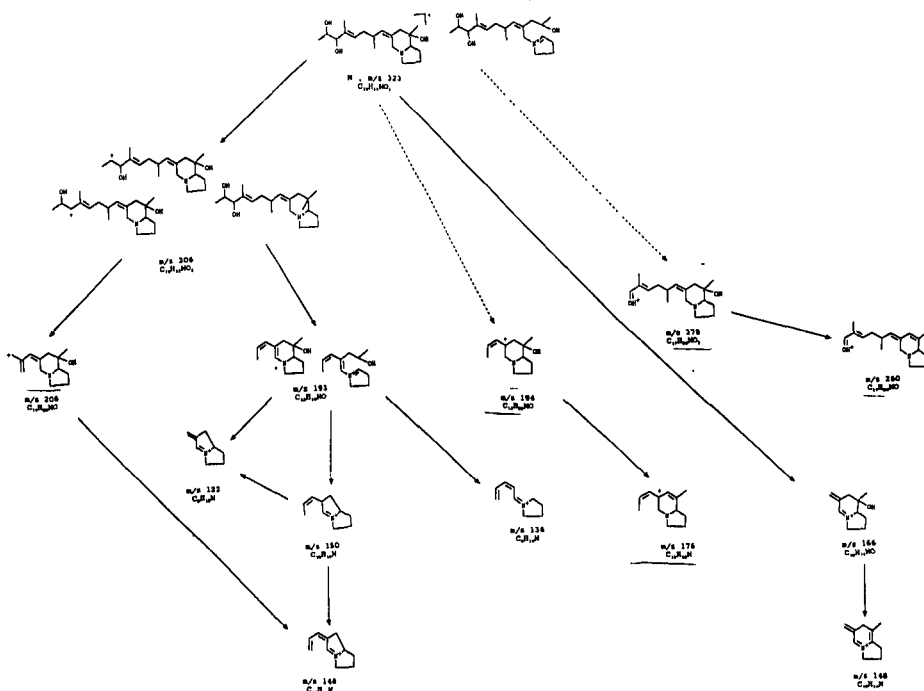
As in the case of PTX-B, the structure of the major alkaloids have been solved by chromatographic purification followed by a combination of mass spectrometry, NMR and crystal structure methods. In these cases a large amount of extract is required. However, many new potent extracts are being isolated in which the activity is thought to be from the minor components or there is too little of the extract for isolation. Therefore a thorough understanding of the fragmentation pathways of the alkaloids will be useful in identifying these unknowns. While a comparison of the spectra of several related alkaloids provides a lot of information, the source of the fragmentation ions has previously been based on speculation.

METHOD: A Finnigan Ion-Trap Mass Spectrometer equipped with a "DC box" and a "Tickle" frequency generator allows the isolation and fragmentation of a single ion from a compound under GC/MS conditions. Following the MS/MS dc/tickle procedure, the daughter ion spectrum for all the ions in the EI spectrum of a compound may be obtained. After several compounds have been run, similarities in their fragmentation pathways can be seen like the group of ions at 150, 176, 193, 194 in the maps below:





However the saturated analog of PTX-A at mass 311 shows a different pattern as much of the fragmentation in the above compounds was directed by the unsaturation. Using these fragmentation diagrams, an assignment of the structure to the EI ions is possible.



CONCLUSION: With a good understanding of the shape of the fragment ions and of the fragmentation pathway, structures can often be assigned to unknown compounds solely on the basis of the mass spectra generated as they elute from the gas chromatograph, without need of larger amounts of pure compound.

CLEAVAGE MECHANISMS OF CHROMIUM- β -DIKETONATES
BY ELECTRON IONIZATION

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Electron ionization mass spectra (70 eV EI/MS) of tris chromium- β -diketonates of undecanedione (Hud), 2,2,6,6-tetramethyl-3,5-heptanedione (Hthd), and 2,2,7-trimethyl-3,5-octanedione (Htod) show characteristic fragmentation pathways. A loss of one ligand from each of the three isomeric chromium complexes yields the most abundant ion at m/z 418 [$\text{Cr}(\text{L})_2^+$].

As suggested by MacDonald and Shannon [1] an open chain structure of a ligand in the beta-diketonate is possible. A loss of 42 daltons from $\text{Cr}(\text{ud})_3$ and $\text{Cr}(\text{tod})_3$ (m/z 601 based on Cr52 isotope) and their respective $\text{Cr}(\text{L})_2^+$ can be explained by a McLafferty rearrangement due to sufficiently long ω -substituents (n-butyl and s-butyl) [2].

$\text{Cr}(\text{thd})_3$ (only t-butyl as ω -substituent) shows losses of 15 dalton and up to 3 subsequent losses of 42 daltons. The corresponding ions have very low intensities. EI-MS-MS spectra of the parent ion yield ions of these fragments with much higher abundancies (fig. 1).

The loss of methyl can be rationalized by the formation of a tertiary radical. The subsequent mass losses of 42 dalton could be explained by a radical mechanism with a three membered ring as intermediate before loss of propene occurs (fig. 2). Another possible explanation has been suggested elsewhere [3] and assumes a cyclopropane as intermediate.

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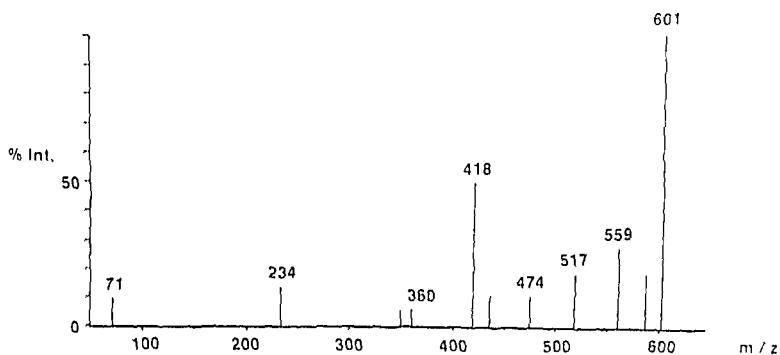


Figure 1 EI-MS-MS of $\text{Cr}(\text{thd})_3$; daughter ions of m/z 601

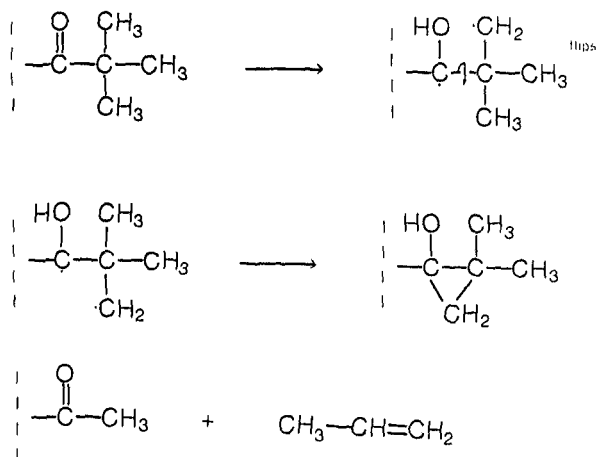


Figure 2 Suggested fragmentation for loss of 42 dalton from one thd ligand on $\text{Cr}(\text{thd})_3$.

FUNDAMENTAL STUDIES OF CHEMICAL IONIZATION
OF PORPHYRINS AND METALLOPORPHYRINS

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Under both ammonia and hydrogen chemical ionization conditions, porphyrins and metalloporphyrins yield pyrrolic ions useful in structure elucidation. These pyrrolic ions result from reduction and cleavage of the meso-bridge bonds. There has been some debate as to whether this reduction occurs in the gas phase, or if it is surface-induced. Data presented here support the theory that the pyrrolic ions arise from surface-induced decomposition. These experiments also indicate that the surface of the ion volume may have a significant impact upon both positive and negative chemical ionization mass spectra of non-volatile compounds. Ions which appear as fragment ions in the CI mass spectra of such compounds may actually be surface-induced decomposition product ions.

A porphyrin standard (2,3,7,8,12,13,17,18, -octaethyl-21H,23H-porphine) known as OEP, and its cobalt(II) derivative were studied under positive and negative H_2CI and NH_3CI conditions. All of the experiments were performed on a Finnigan MAT TSQ 45 triple quadrupole mass spectrometer, with removable CI ion volumes. The source was pressurized with 0.9 torr H_2 for the H_2CI experiments, and 0.4 torr NH_3 for the NH_3CI experiments. The porphyrin and metalloporphyrin samples were dissolved in methylene chloride and deposited on the rhenium filament of a direct exposure probe (DEP). The DEP was then inserted into the ion source and heated from ambient to 1350C at 600C/min. Collision energies and argon pressures were 25 eV and 1.0 mtorr for positive ions and 30 eV and 1.5 mtorr for negative ions.

When porphyrins or metalloporphyrins are introduced via a DEP into a high pressure ammonia or hydrogen chemical ionization source, two peaks typically arise. The first peak occurs as the compound vaporizes off the probe filament (about 250-350C). Some of the sample condenses on the ion volume, which can only be heated to 190C. As the DEP is heated to a maximum temperature of 1350C, the second peak appears, beginning around 900C. This second peak results from condensed porphyrins on the ion volume surfaces reducing and decomposing to form pyrroles, which vaporize as the ion volume temperature increases, and are ionized.

The positive NH_3CI and H_2CI mass spectra of both OEP and Co(II)OEP are characterized by these two peaks. The first peak consists of molecular (in the H_2CI case only) and protonated molecular ions and some monopyrrolic ions resulting from reduction and cleavage of the meso-bridge bonds. The second peak in both cases consists of much higher abundances of the monopyrrolic ions, but no molecular or protonated molecular ions. This second peak also includes ions which do not appear in the first peak. Daughter ion mass spectra indicate that these are reduced monopyrrolic ions resulting from surface-induced reduction. These results do not rule out the possibility of some reduction and fragmentation occurring in the gas phase, but they indicate that surface-induced decomposition is an important mechanism by which the monopyrrolic ions arise. In fact, such surface-induced decomposition is useful in structure elucidation because it greatly enhances pyrrolic ion formation.

When the porphyrin or metalloporphyrin solution is deposited directly on the inner surface of the CI ion volume, and a clean DEP filament is inserted into the H_2 plasma and heated, only one peak is observed. This peak appears at high DEP temperatures, and yields mass spectra very similar to those from the high temperature peak in conventional DEP experiments. An example of this is shown for OEP in Fig. 1.

Both H_2CI/MS and NH_3CI/MS analyses of free-base (metal-free) porphyrins often yield corresponding iron metalloporphyrin ions, even when care is taken to avoid contact of the porphyrin sample with metal during sample preparation. In order to determine whether this metalloporphyrin results from impurities in the solution or from the stainless steel ion volume, H_2CI/MS data were obtained from identical samples. Positive ion mass spectra yielded very little if any iron porphyrin ions, but these metalloporphyrin ions can be seen clearly in the negative ion mass spectra. When the stainless steel ion volume was electroplated with copper, and the $-H_2CI/MS$ experiment repeated, a remarkable decrease in the abundance of the iron metalloporphyrin was observed. This

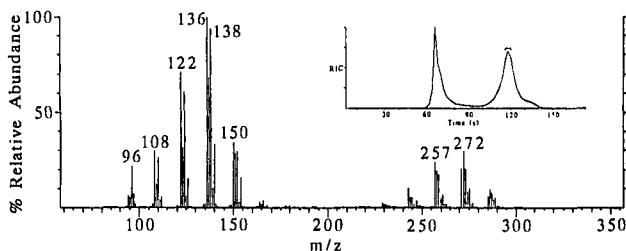
indicates that pick up of iron most probably occurs on the surface of the ion volume. It is noteworthy that m/z 588, the iron metalloporphyrin ion, is only detected at lower DEP temperatures (around 300C), indicating that products of porphyrin/surface interactions are observed at both low and high DEP temperatures. It is also interesting that copper plating does not seem to result in metalation of OEP by copper to the extent that stainless steel results in pick up of iron by OEP.

Upon $-H_2CI$, $Co(II)OEP$ yields two distinct peaks as the DEP is heated. The first appears at about 300C, and consists entirely of the negative molecular ion, m/z 591. This negative ion is very stable and difficult to fragment via collisionally activated dissociation (CAD). The second peak arising in $-H_2CI$ mass spectra of $Co(II)OEP$ appears when the DEP reaches 900C, and consists of hydrogenated cobalt OEP adducts resulting from surface-induced reduction. The $[Co(II)OEP+6H]^+$ ion (m/z 597) was selected for a daughter ion MS/MS experiment. It fragmented much more efficiently upon CAD than did the m/z 591 ion, yielding m/z 568 and m/z 449 daughter ions.

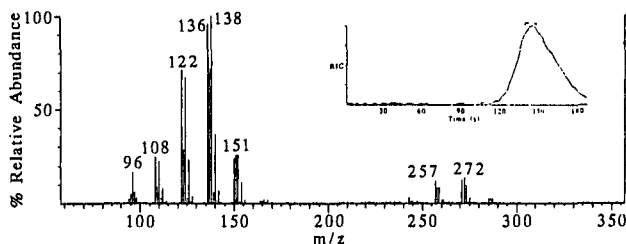
In conclusion, it was shown that under $+H_2CI$ and $+NH_3CI$ conditions, porphyrins and metalloporphyrins decompose to a significant extent on the ion volume surface, yielding pyrrolic ions. In addition, metal-free porphyrins tend to metalate upon interaction with stainless steel ion volumes; this can lead to misinterpretation of the $-H_2CI$ and $-NH_3CI$ mass spectra of such porphyrins. It was also demonstrated that negative molecular ions of hydrogenated porphyrins fragment more easily upon CAD than do M^+ ions of the corresponding non-reduced metalloporphyrins. While this work does not rule out the possibility of reduction and decomposition of porphyrins and metalloporphyrins in the gas phase, it demonstrates the importance of the surface-induced decomposition mechanism. This work leads to interesting implications concerning mass spectra of other types of non-volatile compounds which may undergo surface-induced decomposition reactions within the ion source. Perhaps the nature of the ion volume surface is a variable such as choice of the CI reagent gas, pressure, and source temperature, which can be utilized to optimize certain mass spectra.

$+H_2CI$ Mass Spectra of OEP

OEP Deposited on DEP Filament



OEP Deposited Directly on Ion Volume



Studies of the Ion/Molecule Reaction of Esterification
by Ion Trap Mass Spectrometry

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The quadrupole ion trap mass spectrometer (QITMS) has the ability to perform MS^n and to permit ion residence times as long as a few seconds inside the trap. These capabilities make the QITMS especially useful for the study of ion/molecule reactions such as the investigation of reaction mechanisms and the elucidation of ion structures via mass-selective storage and MS/MS daughter scans in a single analyzer^{1,2}.

The ion/molecule reactions of esterification and transesterification of alcohols (e.g. ethanol, s-butanol and ethanol- d_6) and benzoic acid, benzoyl chloride, methyl benzoate and ethyl benzoate, with water or deuterium oxide as reagent gas, and the elucidation of the daughter spectra of alkyl benzoate have been demonstrated by MS^4 using a Finnigan MAT IIMS operated with both RF and DC voltages applied to the ring electrode of the ion trap analyzer.

Protonated water ions m/z 19 (H_3O^+) are isolated and allowed to react with ethanol neutrals to form protonated ethanol via proton transfer. The protonated ethanol ion m/z 47 ($C_2H_5OH+H^+$) is then isolated and reacted with benzoic acid. The resulting benzoic acid adduct ion m/z 169 ($C_6H_5COOH+C_2H_5OH+H^+$) then undergoes an elimination reaction (loss of H_2O) to form protonated ethyl benzoate m/z 151 ($C_6H_5COOC_2H_5+H^+$). When the protonated ethanol ion, m/z 47, is reacted with benzoyl chloride, there is a lower yield of protonated ethyl benzoate. This effect is probably due to competition between the elimination reaction (loss of HCl), and the dissociation of the adduct ion to form $C_6H_5CO^+$ ion (m/z 105). The transesterification of methyl benzoate with ethanol was performed by two methods: isolation of protonated ethanol m/z 47, which is then reacted with the neutral methyl ester; and, isolation of protonated methyl benzoate ion m/z 137, with subsequent reaction with neutral ethanol. Both experiments resulted in the formation of protonated ethyl benzoate, m/z 151, but selective isolation of protonated methyl benzoate resulted in cleaner spectra.

The D_3O^+ ion m/z 22 was selected and reacted with ethanol- d_6 to form the deuterated ethanol- d_6 ion m/z 54, ($C_2D_6OD+D^+$). Deuterated ethyl- d_5 benzoate m/z 157 ($C_6H_5COOC_2D_5+D^+$) was formed in the ion trap through the reaction of isolated m/z 54 with benzoic acid. The esterification schemes for benzoic acid with ethanol- d_6 , the MS^4 scan function and mass spectra obtained following the different steps of scan function are shown in Figs. 1-3, respectively. The deuterated benzoic acid ion, m/z 125, was not observed in the esterification of benzoyl chloride with ethanol- d_6 . The hydrolyzed product of benzoyl chloride was therefore not a by-product in this reaction. The transesterification of methyl benzoate with ethanol- d_6 can be carried out either by reacting isolated deuterated ethanol- d_6 m/z 54 with neutral methyl benzoate, or by reacting isolated deuterated methyl benzoate m/z 138 with neutral ethanol- d_6 . Upon transesterification of ethyl benzoate with the deuterated ethanol- d_6 ion, the deuterated ethyl benzoate ion m/z 152 is not formed. This means the transesterification of the ethyl ester to form deuterated ethyl benzoate occurs directly from the reaction of ethyl benzoate with deuterated ethanol- d_6 . CAD of the parent ion m/z 157, ($C_6H_5COOC_2D_5+D^+$), resulted in loss of C_2D_5 (and not CO) to form the benzoic acid- d_4 ion m/z 125, ($C_6H_5COOD+D^+$). These results for deuterated compounds indicate that the loss of 28 as from CAD of protonated ethyl benzoate ion m/z 151 corresponds to C_2H_5 rather than CO . Increasing the C_2D_5OD pressure from 0.5 to 1.0 to 1.5×10^{-5} torr increases the intensity of the reactant ion m/z 54 and product ions m/z 157, as shown in Fig. 4. The ion current for m/z 157 increased more rapidly at higher ethanol- d_6 pressures.

The protonated s-butyl benzoate ion can be obtained by the reaction of s-butanol and either benzoic acid or benzoyl chloride, with water as reagent gas. The transesterification reactions were studied both with and without isolation of the protonated alkyl benzoate ions. The base peak m/z 151 ion can be found in the spectrum of transesterification of methyl benzoate with s-butanol, as shown in Fig. 5. The m/z 151 ion, the fragment ion of m/z 179 through a-cleavage by the elimination of C_2H_5 , is confirmed by CAD of m/z 179 ion.

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IONIC DISSOCIATION OF 7-R-O-METHYLNAGAROL
AND ITS DEGRADATION PRODUCTS

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7-R-O-methylnogarol is an antibiotic of the anthracycline family showing activity against gram-positive microorganisms and is an anticancer agent. The synthesized 7-R-O-methylnogarol contains process and degradation impurities. A variety of possible process and degradation impurities were identified by reverse phase ion-pair HPLC. Major degradation products under fluorescence and ultraviolet irradiation are nogarol, 7-deoxynogarol and nogarene. In this work dissociative ionization of these compounds synthesized were investigated using several mass spectrometric techniques (Electron ionization (EI) with low impact energies, isobutane chemical ionization (CI) mass spectrometry, collision induced dissociation (CID) mass spectrometry/mass spectrometry (MS/MS) (Fig. 1)).

Experimental conditions:

Instrument	Finnigan 4600 and TSQ-70
Mode	direct exposure: 20 C° to 600 C°
Manifold pressure	ca. 3×10^{-7} Torr
Mass resolution	ca. 1 mass unit
Scan range	m/z 35 - 1,000
Scan rate	1 scan / second
Electron multi.	- 1.2 kV
Conv. Dinode	- 5 kV for positive ion + 5 kV for negative ion
CI gas	Isobutane
source pressure	ca. 0.5 Torr
CID gas	Xe
pressure	ca. 0.5 Torr
collision energy	ca. 15 eV

7-R-O-methylnogarol The formations of m/z 523 and 509 ions result from losses of a water and methanol from the molecular ion (m/z 541). The m/z 491 ion is produced via simultaneous or successive eliminations of both water and methanol from the A-ring. This ion is common to the analogues, and was found to be the nogarene ion from MS/MS studies (Fig.1). This process is predominant in low energy EI mass spectra. The productions of m/z 454 and 116 ions are due to fragmentation at the dimethyl amino end. The m/z 454 ion is formed by elimination of $(CH_3)_2NCH=CHOH$ [87] from the molecular ion via a concerted mechanism while the formation of m/z 116 corresponding to $(CH_3)_2NCH(CHO)CH'-OH$ proceeds via a step-wise reaction requiring a hydrogen transfer before dissociation (Fig. 2 and 3). The m/z 422 and 404 ions are produced via eliminations of methanol from the m/z 454 ion and of $(CH_3)_2NCH=CHOH$ [87] from the m/z 491 (nogarene) ion. The positive CI mass spectrum consists mainly of three product ions: m/z 542, 510, and 492. Negative chemical ionization using isobutane leads to the formation of M^- due to resonance electron capture and its mass spectrum shows product ions of m/z 541, 523, 511, 509, and 491. These fragment ions produced in chemical ionization seem to be closely related to those in low energy EI mass spectra.

Nogarene The base peak is at m/z 116. The m/z 404 ion is formed via elimination of $(CH_3)_2NCH=CHOH$ [87] from the parent (m/z 491). The positive and negative CI mass spectra exhibit intensive peaks at m/z 491 and 492. The formations of m/z 404 and 116 ions are competitive in low energy EI mass spectra and result from fragmentation at the dimethyl amine end.

R-Nogarol The m/z 491 and 404 ions are produced via elimination of two water molecules from the A-ring and of $(CH_3)_2NCH=CHOH$ [87] from the m/z 491 ion. The formations of m/z 116, 491 and 404 ions are competitive in low EI energies.

7-Deoxynogarol The formation of m/z 493 ions is due to loss of a water from the parent. The m/z 424 ion is formed by elimination of $(CH_3)_2NCH=CHOH$ [87] from the

dimethyl amine end and the m/z 407 ion is produced via its successive loss of a water molecule.

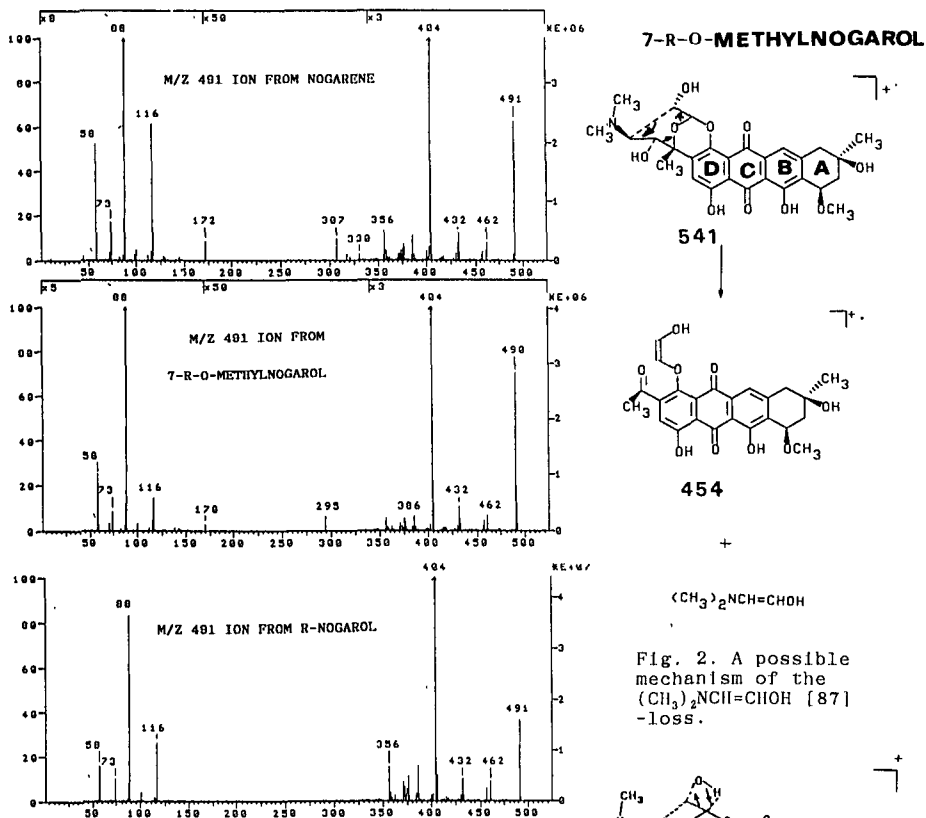


Fig. 1. CID MS/MS spectra of m/z 491 ions produced from various precursors.

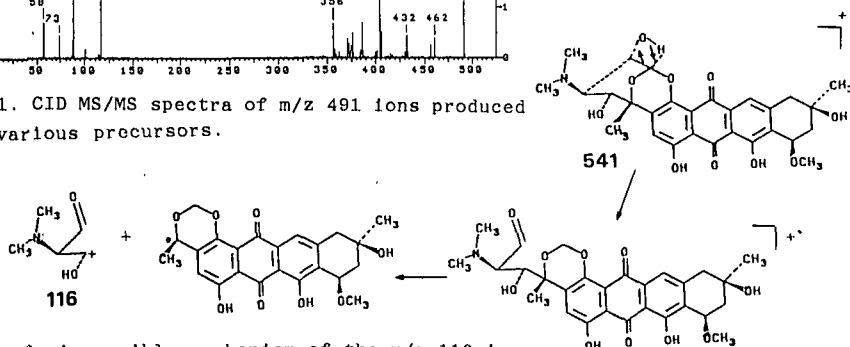


Fig. 3. A possible mechanism of the m/z 116 ion formation.

As a conclusion, major fragmentation pathways of the 7-R-O-Methylnogarol and its degraded analogues can be summarized as follows: (1) the formation of m/z 116 ions from the dimethyl amine end involving a hydrogen transfer before dissociation, and successive fragmentation of m/z 116 ions. (2) elimination of $(CH_3)_2NCH=CHOH$ [87] from the dimethyl amine end. (3) losses of neutral molecules (H_2O , CH_3OH) from the A-ring functionalities.

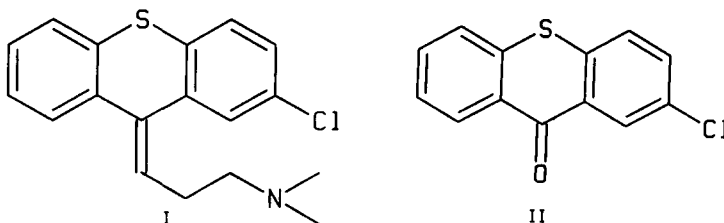
PHOTOCHEMICALLY-INDUCED REACTION OF CHLORPROTHIXENE WITH OXYGEN
UNDER METHANE NEGATIVE CHEMICAL IONIZATION CONDITIONS

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Chlorprothixene, 3-(2-chloro-9H-thioxanthen-9-ylidene)-N,N-dimethyl-1-propanamine, (I) has been observed to give rise to unusual ions in its methane negative chemical ionization (NCI) mass spectrum.



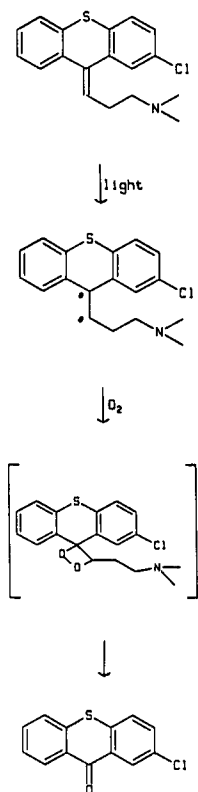
In particular, an unusual MH^- ion has been reported [P.S. Callery, W.A. Garland, and E.K. Fukuda, *Org. Mass Spectrom.*, in press]. This ion has been accounted for by electron capture followed by H $\cdot$ transfer from the reagent gas. In our studies with I, another unusual ion, m/z 246, has been observed and is presumably the molecular anion of 2-chloro-10-thioxanthone (II) formed by reaction of I with oxygen in the CI source. II is also observed to be a degradation product of I which forms only in the presence of air and light [A. Li Wan Po and W.J. Irwin, *J. Pharm. Pharmacol.*, **32**, 25, 1980]. This observation has been confirmed in our laboratories, as well as the observation that I undergoes rapid cis-trans isomerization in the presence of light.

To elucidate the mechanism of the reaction under methane NCI conditions, experiments with O_2 and $^{18}O_2$ were carried out. Under conditions where O_2 is minimized, the m/z 246 ion is not the base peak. When O_2 is admitted to the ion source, m/z 246 becomes the base peak. The methane NCI mass spectrum of 2-chloro-10-thioxanthone is very simple; only the molecular radical anion is observed at m/z 246 and m/z 248. When $^{18}O_2$ is admitted to the ion source, the m/z 248 ion increases relative to the m/z 246 ion confirming the thioxanthone structure. The chromatographic behavior of the m/z 246 ion shows considerable tailing which is consistent with a surface-mediated pathway.

A strict requirement of the exocyclic double bond was demonstrated by observing no m/z 246 in the methane NCI mass spectrum of reduced chlorprothixene (reduced at the exocyclic double bond).

N-desmethylchlorprothixene was derivatized with pentafluorobenzoylbromide in order to impart electron capturing ability to the side chain. The base peak in the methane NCI mass spectrum of this derivative was the $[M-HF]^-$ ion and no ions indicative of the possible aldehyde product were observed.

The proposed mechanism shown below involves the light-induced formation of a diradical from the exocyclic double bond, reaction with oxygen to form a four-centered intermediate and decomposition to II which captures an electron to form a radical anion.



This mechanism is consistent with the observation of the light-induced cis-trans isomerization of I observed in solution. The isomerization occurs via a diradical intermediate which has the proper symmetry to react with ground (triplet) state oxygen.

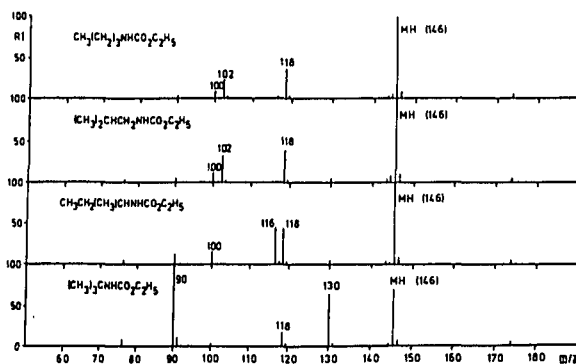
CHEMICAL IONISATION MASS SPECTRA OF URETHANES

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The methane chemical ionisation mass spectra of a range of 33 urethanes of general structure $\text{RNHCO}_2\text{C}_2\text{H}_5$ [$\text{R} = \text{C}_n\text{H}_{2n+1}$ ($n = 0 - 8$), $\text{CH}_2 = \text{CHCH}_2$, Ph , PhCH_2 , $\text{Ph}(\text{CH}_3)\text{CH}$ and PhCH_2CH_2] and $\text{R}_2\text{NCO}_2\text{C}_2\text{H}_5$ [$\text{R} = \text{C}_n\text{H}_{2n+1}$ ($n = 0 - 4$)] are reported and discussed. The spectra contain prominent $[\text{MH}]^+$ peaks (typical relative intensity 30 - 100%) accompanied by smaller adduct peaks, $[\text{M} + \text{C}_2\text{H}_5]^+$ (0 - 10%) and $[\text{M} + \text{C}_3\text{H}_5]^+$ (0 - 3%). Four main classes of fragment ions are also present in the spectra.

Two of these classes of fragment ions are associated with the O-ethyl group. Firstly, ions formed by the expulsion of ethene are of moderate abundance (10 - 60%). These ions are formed either from $[\text{MH}]^+$ ions initially protonated on the O-ethyl group or similar ions formed by proton transfer from the carbonyl group. Secondly, ions formed by ethanol loss from $[\text{MH}]^+$ are of significant abundance (5 - 25%) are present in the spectra. Although it is possible that these ions may be formed by H_2O loss from $[\text{MH} - \text{C}_2\text{H}_4]^+$ ions, this is unlikely on energetic grounds. Thirdly, ions formed by alkane loss from $[\text{MH}]^+$ are of analytical value. These ions are formed in a reaction involving elimination of the α -alkyl group together with a β -hydrogen atom from either the n-alkyl group or the NH hydrogen. Analysis of these $[\text{MH-alkane}]^+$ peaks (10 - 80%) can be used to identify most of the four butyl and eight pentyl isomers. Isomers differing beyond the α -carbon atom cannot be resolved. Finally, ions arising from elimination of an alkene from the N-alkyl group of $[\text{RNHCO}_2\text{C}_2\text{H}_5.\text{H}]^+$ have a mass-to-charge ratio of 90, and correspond to protonated O-ethyl carbamic acid $[\text{H}_2\text{NCO}_2\text{C}_2\text{H}_5.\text{H}]^+$. The abundance of this ion reflects the branching at the α -carbon atom of the N-alkyl group. When R is primary this ion is of low abundance (0 - 5%); it increases in importance when R is secondary (10 - 20%) and dominates the spectrum when R is tertiary (100%). This is clearly demonstrated in the spectra of the four butyl urethane isomers. The spectra differ greatly for the s-butyl and t-butyl isomers, but the n-butyl and i-butyl isomers give very similar spectra since they differ beyond the α -carbon atom of the N-alkyl group. This peak is also significant when the R-group can stabilise a positive charge i.e. aromatic groups such as $\text{Ph}(\text{CH}_3)\text{CH}$. This suggests that the mechanism for this reaction involves the formation of a putative

ion-molecule complex $[R^+ \cdots NH(HO)CO_2C_2H_5]$ which undergoes hydrogen transfer to give $(R - H)$ and $[H_2NCO_2C_2H_5 \cdots H]^+$. Such a mechanism would become increasingly favourable on progressing from primary to tertiary alkyl substituents.



Methane CIMS of the four isomeric butyl urethanes

REACTIONS OF BIDENTATE LIGAND ANIONS WITH LEWIS ACIDS AS PROBES OF
POTENTIAL ENERGY SURFACES

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Reactions of a wide variety of di-oxo and di-thio ligand anions with BF_3 and SiF_4 have been carried out in the gas phase using the flowing afterglow technique. These reactions can best be described by a generalized mechanism in which the anion first participates in a three-body addition step in which the coordination sphere of the Lewis acids is expanded; boron trifluoride goes from a trigonal planar neutral to a tetrahedral anionic species, while silicon tetrafluoride goes from a tetrahedral neutral to a trigonal bipyramidal anionic species. The presence of a second -OH or -SH chelating group on the bifunctional ligand anion presents the possibility of a second attack at the now saturated Lewis acid center, resulting in ring closure. This latter process can be viewed as a bimolecular substitution process in which a neutral HF molecule is ejected. Thirteen ligand anions of varying properties were selected in this study in order to assess the effects of certain ligand properties upon the reaction mechanism. These ligand anions included both substituted and unsubstituted aliphatic and aromatic 1,2-diols, 1,3-diols, and 1,4-diols as well as the unsubstituted, saturated 1,2-, 1,3-, and 1,4-dithiols and the aromatic 1,2-benzenedithiol. Rate coefficients and product branching ratios for this wide variety of reacting partners provide information on the influence of the following: (1) geometry about the central atom of the Lewis acid, (2) identity of the ligand anion donor atoms, (3) ligand flexibility, (4) ligand chain length, (5) substituent effects, (6) chelate ring size, (7) steric effects, (8) internal hydrogen-bonding in the Lewis acid-anion complex, and (9) internal hydrogen-bonding in the free ligand anion.

The reactions examined fall into three general classes: (1) those in which both monodentate and bidentate products are formed, (2) those in which only monodentate product is formed, and (3) those in which monodentate product and the oxide (BF_2O^- or SiF_3O^-) product are formed. The aromatic 1,2-diol anions and the saturated, unsubstituted 1,2- and 1,3-diol anions fall into the first category; these reactions result in formation of both monodentate addition product and bidentate chelation product. In marked contrast, none of the sulfur-donor ligand anions form bidentate product and, except in the case of the 1,2-ethanedithiol anion reaction, the sole primary products are the monodentate anion species, placing these reactions in the second category. Reactions of the aliphatic, substituted 1,2- and 1,3-diol anions as well as that of the anion of 1,4-butanediol belong to the third class in which the monodentate addition product and the oxide anion are formed. These reactions bear a striking similarity to reactions of the simple HO^- and CH_3^- reactions with BF_3 and SiF_4 .

For those reactions where both monodentate and bidentate anion products are formed, evidence indicates that the processes are occurring on a double-well potential energy surface in which the first well represents the monodentate anion and the second well represents the bidentate anion associated with the neutral HF molecule which subsequently is expelled. The height of the

barrier between the two potential minima is reflected in the branching ratios of the two product species. Results suggest that the geometry around the Lewis acid center as well as the size, flexibility, and degree of substitution of the ligand anion are factors which affect chelation and the extent to which it occurs. The ring closure mechanism appears to be facilitated by the presence of an intramolecular hydrogen-bond between the -OH proton and one of the fluorines of the Lewis acid in the adduct. Furthermore, inductive effects have a minimal effect on both the rate coefficients and the product branching ratios. Optimum geometries and single point energies for several of the Lewis acid/ligand anion pairs were obtained from *ab initio* calculations using extended basis sets. The results support this mechanistic picture and overlap population analyses point to hydrogen-bonded interactions as important.

Formation of the oxide anion product occurs where substituent effects or the length of the chain between donor groups in the ligand anion disfavor formation of an internal hydrogen-bond in the monodentate species; one such example is the reaction of the anion of 1,4-butanediol with both SiF_4 and BF_3 . The oxide anions BF_2O^- and SiF_3O^- produced in these reactions are the same products observed in the reactions of BF_3 and SiF_4 with both HO^- and CH_3^- , and indicate that, for this group of ligand anions, the effect of the second oxygen donor atom is negligible.

From our studies of this interesting group of reactions, the following summary points can be made: (1) hydrogen-bonding in Lewis acid-bifunctional anion complexes is important in subsequent formation of bidentate anion products, (2) where this hydrogen-bond is disfavored, either the monodentate species is the only product, or the oxide product is observed, (3) where both mono- and bidentate products form, the ratio of bidentate product to monodentate product is larger for the SiF_4 reactions relative to those for BF_3 indicating that the geometry about Si results in a less strained transition state, (4) steric hindrance and long chains may prevent hydrogen-bonding and thus orientation of the second attacking group, making anions with these properties behave like the simple hydroxide and methoxide anions, (5) rates of reaction do not vary with nucleophile, and these reactions occur at significant fractions (70 - 100%) of their corresponding collision rates, and (6) calculations show that the absence of bidentate anion formation in the reactions of the dithiolate anions may be due to energetic factors because reactions to form the bidentate anion plus HF are close to thermoneutral; however, the inability of sulfur to participate in strong hydrogen-bonding and its steric constraints as a result of its size may also be important factors.

PROTONATION ON PHENOXY OXYGEN IN METHANE CHEMICAL IONIZATION OF PROPOXYBENZENE

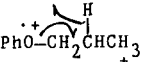
Richard W. Kondrat, Kyuseok Song, Dion A. Stams, and Thomas Hellman Morton

Department of Chemistry, University of California, Riverside, CA 92521

The predominant decomposition pathway for n-propyl phenyl ether under a variety of ionization conditions takes place via ion-molecule complexes. This is true from both electron-removal processes (Field Ionization Kinetics, Electron Impact, or Resonant Multiphoton Ionization, all of which generate $M^{+\bullet}$) and from chemical ionization. For the isotopically labelled compounds 1 and 2 the scrambling ratios (defined as the geometric mean of $PhOH^{+\bullet}/PhOD^{+\bullet}$ from 1 $^{+\bullet}$ and $PhOD^{+\bullet}/PhOH^{+\bullet}$ from 2 $^{+\bullet}$) are all greater than 2.0. Scrambling ratios for other



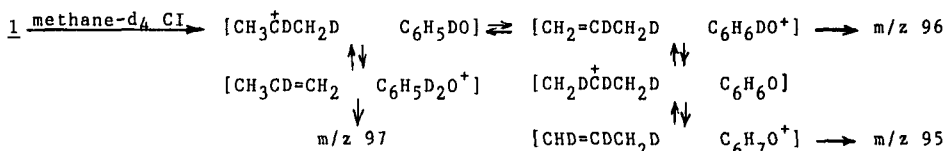
isotopically substituted variants are calculated from values in the literature, as summarized in the table. Scrambling ratios for CD_4 CI are also shown (the geometric mean of $C_6H_6DO^+/C_6H_5D_2O^+$ from 1 $+D^+$ and $C_6H_5D_2O^+/C_6H_6DO^+$ from 2 $+D^+$) as well as for other isotopic variants reported for other reagent gases.

Predicted for:	Scrambling Ratio	Observed for (this work):	Scrambling Ratio	k.i.e.
 $PhO-CH_2CHCH_3$ $[PhO^{\bullet}CH_2CHCH_3]$ scrambling no scrambling	0.0 2.5 5.0	RMPI (275.2 nm) RMPI (275.0 nm) RMPI (274.2 nm)	2.1 2.4 2.5	1.33 1.26 1.19
Reported for:		(RMPI values correspond to the 0,0 bands of the three conformational isomers observed in a supersonic jet)		
FIK (10^{-11} s) ¹	2.7			
" (10^{-8} s) ¹	3.0			
" (10^{-5} s) ¹	3.8			
EI (70 eV) ²	2.9	EI MIKES	4.3	1.15
CI (methane) ²	4.0	CI MIKES	3.6	1.38
" (isobutane) ²	4.9	(methane-d ₄ reagent gas)		

We find that CI MIKES and EI MIKES give nearly the same scrambling ratios, consistent with formation of an ion-molecule complex that contains isopropyl cation, which partially scrambles its hydrogens before transferring a proton to its neutral partner in the ion-molecule complex. The kinetic isotope effect for this proton transfer tells if it takes place to oxygen or to carbon. The data suggest it is to carbon; hence initial CI (as seen by MIKES) must be on oxygen.

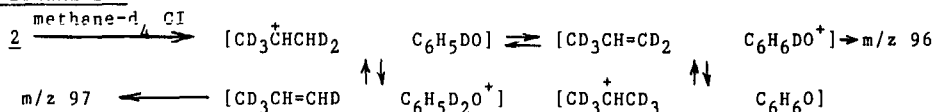
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SCHEME I



The CI MIKES spectrum of 1 with CD_4 reagent gas shows evidence for exchange of the D^+ deposited by the CI with the propyl sidechain. This exchange is revealed by the formation of $\text{m/z } 95$ with an intensity about 10% that of the $\text{m/z } 96$ intensity. This can be rationalized in terms of the interconversion of ion-molecule complexes (the species drawn in brackets) depicted in Scheme I above. One prediction of this scheme is that if the alkyl cation maintains the isopropyl structure without scrambling its hydrogens, then this interconversion cannot lead to exchange of D for H in the CD_4 CI of compound 2. As portrayed by Scheme II below, the formation of $\text{CD}_3\dot{\text{C}}\text{HCD}_3$ prevents formation of $\text{m/z } 95$.

SCHEME II



As predicted by this mechanism, no $\text{m/z } 95$ is observed from the CD_4 CI of 2 in the MIKES. Scheme I works equally well if the neutral $\text{C}_6\text{H}_5\text{DO}$ in the first ion-molecule complex is PhOD or if it has a cyclohexadienone structure with the D attached to the sp^3 carbon. On thermodynamic grounds we believe that the $\text{m/z } 96$ and 97 product ions are ring-protonated phenols. If the neutral partner in the ion-molecule complexes has the cyclohexadienone structure, this results from H^+ or D^+ transfer to an oxygen lone pair. If the neutral partner is PhOD , then the transfer is to an sp^2 carbon. Since the latter reaction has a rehybridization barrier, a larger kinetic isotope is to be expected. For comparison, k.i.e.'s for M^{++} decompositions (where the transfer occurs to the oxygen lone pair of $\text{PhO}^{\cdot}$, which has nearly the same proton affinity as phenol) have been measured. The k.i.e. from 70 eV EI is 1.30,² while we measure values less than or equal to that for a variety of electron removal conditions. The scrambling ratio measures the internal energy of the ion-molecule complexes,³ so EI MIKES and CI MIKES have nearly the same energy. The former k.i.e. is much larger than the latter.

Observation of Diazonium Ion-Crown Ether Molecular Complexes in the Gas Phase by Field Desorption (FD) and Fast Atom Bombardment (FAB) Mass Spectrometry. Khosrow Laali*, Department of Chemistry, Kent State University, Kent, OH 44242 and Robert P. Lattimer, the BF Goodrich R&D Center, Brecksville, OH 44141.

Although host-guest complexation between diazonium salts and crown ethers is quite well documented in solution through kinetic, thermodynamic and spectroscopic studies [1-4], the extent and mode of this interaction in the gas phase has not been addressed. We have recently shown that fragile carbocation and carbodication salts as well as onium ions can be ejected and intact mono-cations observed by desorption-ionization techniques [5]. Intact dication ejection was also achieved with several dication salts [6]. In relation to these studies, we utilized FD-and FAB-MS to study diazonium/crown complexation and to provide the first comparison with the condensed phase studies. The results are summarized in Table I.

Solution stability of diazonium/crown complex is strongly dependent on both electronic and steric factors. Whereas electron withdrawing groups increase the diazonio character and favor complexation, electron donating groups enhance the diazo character of the bond, hence, weaken complexation. The accepted host-guest model for $\text{ArN}_2^+/\text{crown}$ in solution is an insertion complex (IC), for which one x-ray structure is already available, whereas a charge transfer model (CT) was proposed for the anilinium ion/crown complex [2,7]. Kinetic studies on dediazonation of crown-complexed diazonium ions [8] showed that the mechanism of dediazonation remains the same i.e. a tight aryl cation/nitrogen molecule pair is involved. This intermediate is, however, too large to fit inside the cavity of the crown in an IC model; a CT model satisfactorily explains the kinetics. IR and UV band shifts observed upon complexation of diazonium ions with crowns is the opposite to the order established based on equilibrium constants for binding i.e. maximum band shifts are seen with larger, more flexible, crowns. This is explained through crown puckering and pi acid/sigma base interactions in a molecular cluster [4].

We have investigated crown complexation with various diazonium salts. Apart from $\text{ArN}_2^+/\text{Crown}$ 1:1 complexes, higher order complexes i.e. $\text{ArN}_2^+/\text{Crown}$ 1:2 and 1:3 were also observed. Neither the ortho steric crowding on the diazonium ion nor peri interactions prevented cluster ion detection. The mode of complexation is suggested to be via CT interactions. Pi acid/sigma base interactions between the oxygen lone pairs and the aromatic pi system could be responsible for higher order cluster ion formation, generating "encapsulated" diazonium ions. We utilized FAB/MS-MS (Table II) to examine the nature of this interaction in a 1:1 cluster with 18-Crown-6. The ions corresponding to the 1:1 complexes were mass selected and subjected to CAD analysis. Apart from ArN_2^+ and Ar^+ (impact dediazonation), ions corresponding to $(\text{crown}+\text{H})^+$ and $(\text{crown}-\text{H})^+$ were observed. Crown protonation is explained via a benzyne mechanism, and $(\text{crown}-\text{H})^+$ ion detection via hydride abstraction by N_2 (see schemes).

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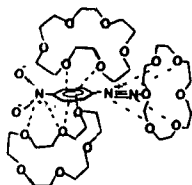
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Table I. Principal Ions in FD and FAB Mass Spectra of $\text{ArN}_2^+\text{BF}_4^-/\text{Crown Complexes}$

substit	diazonium (ArN_2^+)	crown	ion (m/z) and rel intensity	
			FDMS*	FAB-MS*
	PhN_2^+	18-C-6	105 (100) ArN_2^+ , 369 (100) $\text{ArN}_2^+(\text{crown})$, 633 (29) $\text{ArN}_2^+(\text{Crown})_2$	105 (100) ArN_2^+ , 297 (68) $(\text{ArN}_2^+)_2\text{BF}_4^-$, 369 (55) $\text{ArN}_2^+(\text{Crown})$
	PhN_2^+	24-C-8*	565 (100) $\text{ArN}_2^+(\text{crown})$	105 (70) ArN_2^+ , 565 (15) $\text{ArN}_2^+(\text{crown})$, 297 (12) $(\text{ArN}_2^+)_2\text{BF}_4^-$
o-Me	PhN_2^+	18-C-6	647 (100) $\text{ArN}_2^+(\text{crown})_2$, 383 (22) $\text{ArN}_2^+(\text{crown})$, 119 (18) ArN_2^+	119 (58) ArN_2^+ , 325 (14) $(\text{ArN}_2^+)_2\text{BF}_4^-$, 383 (5) $\text{ArN}_2^+(\text{crown})$
o-Me	PhN_2^+	24-C-8*	579 (100) $\text{ArN}_2^+(\text{crown})$	119 (22) ArN_2^+ , 325 (3) $(\text{ArN}_2^+)_2\text{BF}_4^-$, 579 (1) $\text{ArN}_2^+(\text{crown})$
2,4,6-Me ₃	PhN_2^+	18-C-6	147 (100) ArN_2^+ , 411 (52) $\text{ArN}_2^+(\text{crown})$	147 (90) ArN_2^+ , 381 (48) $(\text{ArN}_2^+)_2\text{BF}_4^-$, 411 (25) $\text{ArN}_2^+(\text{crown})$
2,4,6-Me ₃	PhN_2^+	24-C-8*	607 (100) $\text{ArN}_2^+(\text{crown})$	147 (39) ArN_2^+ , 381 (5) $(\text{ArN}_2^+)_2\text{BF}_4^-$
p-Br	PhN_2^+	18-C-6	183 (17) ArN_2^+ , 185 (18) ArN_2^+	449 (23) $\text{ArN}_2^+(\text{crown})$, 447 (22) $\text{ArN}_2^+(\text{crown})$, 185 (13) ArN_2^+ , 183 (12) ArN_2^+
p-Br	PhN_2^+	24-C-8*	643 (21) $\text{ArN}_2^+(\text{crown})$, 645 (19) $\text{ArN}_2^+(\text{crown})$	185 (11) ArN_2^+ , 183 (9) ArN_2^+ , 643 (tiny) $\text{ArN}_2^+(\text{crown})$, 645 (tiny) $\text{ArN}_2^+(\text{crown})$
p-NO ₂	PhN_2^+	18-C-6	414 (98) $\text{ArN}_2^+(\text{crown})$, 678 (100) $\text{ArN}_2^+(\text{crown})_2$, 150 (70) ArN_2^+ , 942 (4) $\text{ArN}_2^+(\text{crown})_2$	414 (53) $\text{ArN}_2^+(\text{crown})$, 150 (48) ArN_2^+ , 387 (14) $(\text{ArN}_2^+)_2\text{BF}_4^-$
m-NO ₂	PhN_2^+	24-C-8*	150 (18) ArN_2^+	150 (22) ArN_2^+ , 414 (20) $\text{ArN}_2^+(\text{crown})$
	anthracene-1- N_2^+	18-C-6	178 (tiny) $(\text{C}_{14}\text{H}_{10})^+$	205 (12) ArN_2^+ , 469 (5) $\text{ArN}_2^+(\text{crown})$
	anthracene-1- N_2^+	24-C-8*	178 (48) $(\text{C}_{14}\text{H}_{10})^+$, 665 (19) $\text{ArN}_2^+(\text{crown})$	178 (60) $(\text{C}_{14}\text{H}_{10})^+$, 205 (5) ArN_2^+

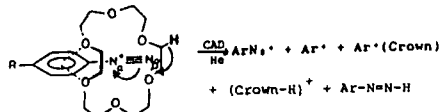
*Unless indicated otherwise, the base peaks were due to the corresponding protonated crowns or a crown fragment. In some cases a Na^+/Crown complex was also observed. *Dicyclohexano-24-crown-8.

Scheme I



An "Encapsulated" Diazonium Ion
With a 1:1 Complex

Scheme III



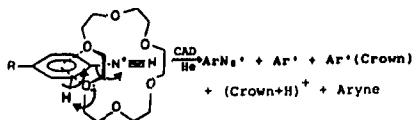
Collisionally Activated Dissociation of a
1:1 Complex [hydride abstraction by N_2
and formation of a $(\text{Crown-H})^+$ ion]

Table II

FAB CAD/MS-MS Spectra of $\text{ArN}_2^+\text{BF}_4^-/18\text{-Crown-6 Complex}$

Substituent	Mass Selected (M/z)	Products
O-Me	383 $\text{ArN}_2^+(\text{Crown})$	119 ArN_2^+
		91 Ar^+
		355 $\text{Ar}^+(\text{Crown})$
		265 $(\text{Crown-H})^+$
E-NO ₂	414 $\text{ArN}_2^+(\text{Crown})$	150 ArN_2^+
		122 Ar^+
		263 $(\text{Crown-H})^+$
E-NO ₂	387 $(\text{ArN}_2^+)_2\text{BF}_4^-$	150 ArN_2^+
		122 Ar^+
		139 (ArN_2^+)
E-Cl	403 $\text{ArN}_2^+(\text{Crown})$	141
		111 (Ar^+)
		113
		263 $(\text{Crown-H})^+$
E-OMe	399 $\text{ArN}_2^+(\text{Crown})$	135 (ArN_2^+)
		107 (Ar^+)
		263 $(\text{Crown-H})^+$

Scheme II



Collisionally Activated Dissociation of a
1:1 Complex [heterolytic dediazonium
and crown protonation via an aryne].

DIFFERENCES IN THE MECHANISMS OF PARENT-MOLECULAR ION FORMATION IN CHEMICAL IONIZATION AND FAST ATOM BOMBARDMENT MASS SPECTROMETRY

Qingmei Zha and Michel J. Bertrand

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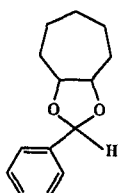
Fast atom bombardment (FAB) and chemical ionization (CI) are ideal techniques for the determination of the molecular masses of organic compounds because protonated molecular ions are usually generated under both conditions. Whether the protonation processes occurring in FAB and CI are the same has been a topic of interest since the development of FAB desorption-ionization. It was suggested initially¹ that FAB mass spectra are really produced by ion-molecule reactions in gas phase. FAB is a chemical ionization process with the matrix acting as the reagent gas. Alternatively, it was also postulated^{2,3} that ions observed in FAB mass spectra are the dissociation products of the precursors preformed in the matrix solution or of clusters ejected from the solution.

In our recent studies on the origin of predominant $[M-H]^+$ ions observed in both FAB and CI mass spectra of some derivatized sugars, it appears that simple hydride abstraction in gas phase can be used to describe the formation of $[M-H]^+$ ions in CI, but may not apply to FAB. Furthermore, the different mechanisms for the formation of parent-molecular ions occurring during FAB and CI conditions could be revealed from the exceptions, i.e. the major peaks observed in the molecular ion region are $[M-H]^+$ rather than $[M+H]^+$. We report in this paper on the different protonation processes observed in positively charged FAB and gas phase CI mass spectra for some acetal samples. The predominant peaks in the molecular ion region are $[M+H]^+$ in methane, isobutane and ammonia CI spectra while they are $[M-H]^+$ in the FAB spectra.

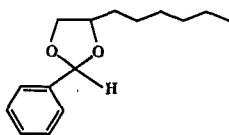
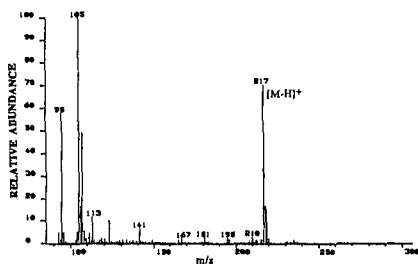
The FAB mass spectra of compound 1 and 2 are shown in Fig. 1. At first, these spectra caused confusion for the assignment of the molecular masses since the peaks in the molecular ion region were two mass units lower than expected from the protonated molecular ions. After confirming the structures of these compounds, it was realized that the observed parent-molecular ions were $[M-H]^+$. In order to determine the protonation properties of these compounds, their CI mass spectra were also recorded using methane, isobutane and ammonia as reagent gases. Interestingly, the CI mass spectra (Fig. 2) showed that the protonated molecular ions $[M+H]^+$ were observed to be the predominant peaks. The differences in parent-molecular ions observed in FAB and CI may imply that different mechanisms are involved in the generation of both types of mass spectra.

The major fragment ions observed in FAB and CI spectra of acetals are also different. An ion of m/z 107 is observed as the most abundant fragment ion in CI spectra of both samples. A plausible structure for this fragment ion is the benzyloxy ion $C_6H_5CH_2O^+$. This ion is, however, not observed as a major fragment ion in the FAB spectra. The most intense fragment ion under FAB conditions is m/z 105 whose structure is probably the benzoyl ion $C_6H_5CO^+$. Based on our observations, the major reaction pathways for sample 1 in FAB and CI are presented in Scheme 1. In CI, protonation occurs in gas phase by ion-molecule reactions and the protonated molecular ions in turns dissociate to form the benzyloxy ion (m/z 107). Protonation in FAB may differ from that in CI and may occur in solution through intermolecular hydrogen bonding. The protonated molecular ions, if formed in solution, are highly unstable in comparison with that in gas phase and readily form $[M-H]^+$ ions by elimination of hydrogens. This would explain why $[M+H]^+$ ions are essentially absent in the FAB spectra and that the predominant peaks correspond to $[M-H]^+$. The presence of benzoyl ions at m/z 105, produced by the dissociation of the $[M-H]^+$ ions, would also be consistent with the proposed reaction pathway.

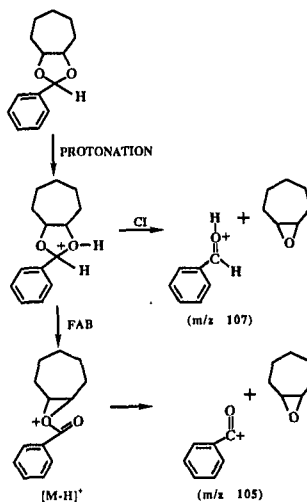
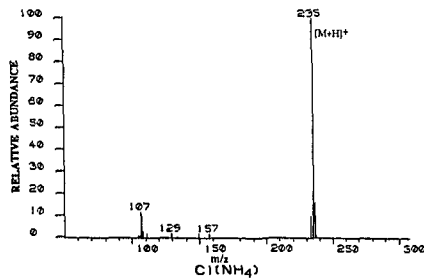
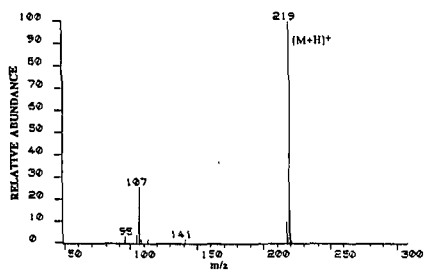
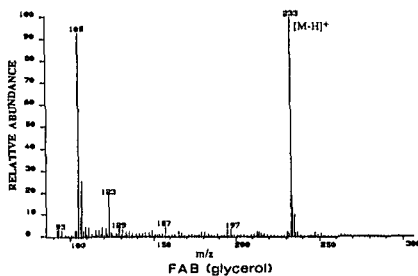
It is not difficult to reach a conclusion that acetal samples may undergo different protonation processes under FAB and CI ionization conditions. In both cases, there seems to be a competition between the formation of $[M+H]^+$ and $[M-H]^+$ ions. Ion formation and fragmentation in the gas or liquid phases may be the origin of different mass spectral behaviors. Acetals are easily protonated in CI by ion-molecule reactions occurred in the gas phase, and the fragmentation behavior depends on their internal energies since the $[M-H]^+/[M+H]^+$ ratio varies with different reagent gas. It is unlikely that the generation of FAB mass spectra of acetals can simply be explained by gas phase processes since they differ from the CI data both in the type of parent-molecular and fragment ions and appear to show a different energy behavior.



1. (Mol. wt 218)



2. (Mol. wt 234)



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MECHANISMS OF THERMALLY ANALOGOUS COLLISION-INDUCED CHARGE-REMOTE FRAGMENTATIONS

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Charge-remote fragmentations of closed-shell ions are a new class of gas-phase ion decompositions that are of special interest analytically because they provide a useful mass spectrometric technique for determining structures of a variety of large biomolecules. Recently, Adams and Gross¹ showed that metastable ion and collision-induced decompositions (CIDs) of some β -hydroxy olefin fatty acid ions undergo charge-remote fragmentations by mechanisms that are directly analogous to thermolytic decompositions of neutrals. Here, we report other thermally analogous mechanisms for charge-remote CIDs of monounsaturated fatty acid ions.

The formation of fragment ions *b* and *c* (Fig.1), representing cleavages allylic to the double bond, were investigated using deuterium labelling, MS-MS, and B/E linked scan experiments. CID spectra of $(M+2Li-H)^+$, $(M+Li)^+$, and $(M-H)^+$ ions of *cis*-9-octadecenoic acid, *cis*-9-octadecenoic-9,10-*d*₂ acid, *cis*-7-dodecenoic acid, *cis*-7-dodecenoic-4,4-*d*₂ acid, and *cis*-7-dodecenoic-11,11-*d*₂ acid were acquired by performing both B/E linked scans and MS-MS experiments (VG 70-S at Emory University and MS-50 Triple Analyzer at University of Nebraska).

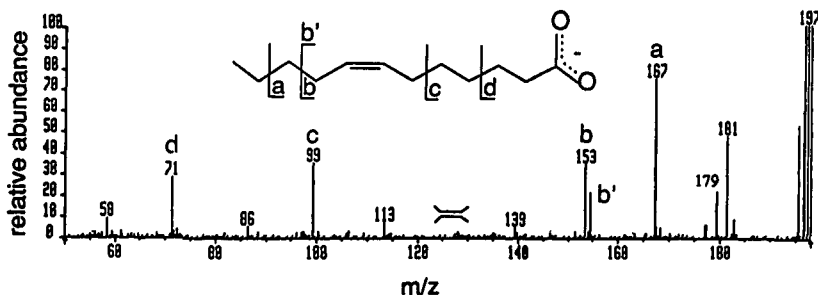
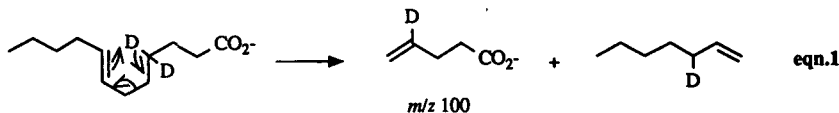


Fig.1 CID spectra of $(M-H)^+$ ions of *cis*-7-dodecenoic acid acquired by using B/E linked scans (VG 70-S, Emory).

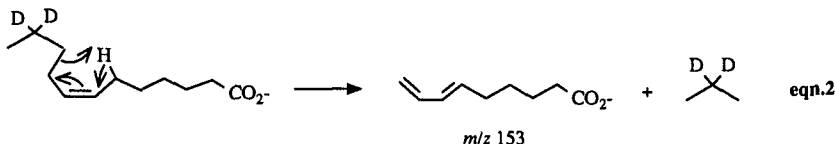
A 1,4-elimination of H_2 and $C_4H_{2n}^2$ was previously invoked by others³ to account for the formation of fragment ions *b* and *c* (Fig.1). However, this mechanism requires transfer of vinylic hydrogens that would be energetically less favorable than the 1,4-eliminations that occur elsewhere along the alkyl chain, and would thus not account for the higher relative abundances. In contrast, CID spectra of $(M-H)^+$ ions of *cis*-9-octadecenoic-9,10-*d*₂ acid *cis*-7-dodecenoic-4,4-*d*₂ acid provide evidence that allylic cleavage on the carboxylic side of the double bond arises by a retro-ene mechanism (eqn.1). This reaction is analogous to the thermal



retro-ene, which has first-order kinetics, large negative entropies⁴, and describes reactions such as the thermal isomerization of methyl vinyl cyclopropane^{4c}.

The CID spectra of $(M-H)^+$ ions of *cis*-7-dodecenoic-11,11-*d*₂ acid provide evidence that the cleavage allylic

to the double bond on the alkyl terminus occurs by a different mechanism. A 1,4-conjugate elimination (eqn.2) is proposed that accounts for the formation of the fragment ion of m/z 153. 1,4-Conjugate eliminations are classic pyrolytic decompositions that involve losses of H-X from X-C-C=C-C-H systems, and are believed to occur via a concerted (large negative entropies of activation) as opposed to a diradical mechanism^{4a,5}.



CID spectra of (M-H)⁻ ions of monounsaturated ions also show an odd-electron fragment ion, b' (Fig.1), which was previously suggested to be a distonic species and also was reported to occur by metastable ion decompositions³. However, here we do not observe this radical anion in metastable ion decomposition spectra acquired either by using the VG 70-S or by using the MS-50 Triple Analyzer. Deuterium labelling indicates that this ion arises by a homolytic bond cleavage, remote from the site of charge, to give a distonic species that contains a stable, allyl radical site. Such a charge-remote homolytic cleavage is a direct analogy to the thermolytic homolytic cleavage of 1-butene to give an allyl radical⁶.

Mechanisms proposed here for CIDs of closed-shell fatty acid ions invoke charge-remote eliminations that are analogous to thermolytic decompositions of neutral molecules. These elimination mechanisms are in accord with the charge-remote thermal-like mechanisms presented previously for metastable ion decompositions of β -hydroxy olefinic closed-shell ions¹. We do not understand the preferential occurrence of each mechanism on either side of the double bond. It could be a result of product ion stability or of indirect electronic effects of the charge-site through the alkyl chain.

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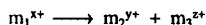
COLLISION-INDUCED DISSOCIATION OF MULTIPLY-CHARGED DIATOMIC CATIONS

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The ion translational spectroscopy of S_2^{3+} , Se_2^{3+} , Te_2^{3+} , SeS^{3+} , Cl_2^{3+} , Br_2^{3+} , I_2^{3+} , $BrCl^{3+}$, IBr^{3+} and the corresponding doubly-charged ions was investigated. In previous studies, the appearance energies of these ions were found to be in the region of 50 to 62 eV. The experimental work of this investigation was completed on a VG ZAB-2F mass spectrometer. Doubly-charged and triply-charged diatomic ions were produced by electron impact ionization at 100 eV ionizing energy. The acceleration potential was normally set at 8 kilovolts. When the ion intensity permitted, lower accelerating energies were used in order that product ions of very high translational energy could be transmitted through the E-sector. Helium was employed as the collision gas, and yielded the best collision efficiency of all other collision gases. Variable potential on the collision cell in the second field-free region of the instrument permitted authentication of the collision reactions.

All the data reported here was obtained using the mass-analyzed ion kinetic energy (MIKE) spectrometry technique. For a simple, direct fragmentation process such as:



the kinetic energy release T can be calculated from the ΔE_2 , the width of the CID peak corresponding to the observation of the m_2^{y+} ion by the relationship:

$$T = \frac{y^2 m_1^2 V_a}{16 \times m_2 m_3} \left(\frac{\Delta E_2}{E_0} \right)$$

where V_a is the accelerating potential and E_0 is the E-sector voltage transmitting the main beam of ions, m_1^{x+} . From the kinetic energy release, a charge separation distance $r(\text{\AA})$ can be calculated from the coulombic energy of repulsion $\phi(r)$ relationship:

$$\phi(r) = - \frac{e^2}{4\pi\epsilon_0 r}$$

where e is the electronic charge, 1.602×10^{-19} C, ϵ_0 is the permittivity of a vacuum, 8.854×10^{-12} J⁻¹ C² m⁻¹, and r is the interchange separation in meters. One can then calculate r directly from the relationships:

$$r(\text{\AA}) = \frac{14.40}{T} : \text{ for the doubly-charged ions.}$$

$$r(\text{\AA}) = \frac{28.80}{T} : \text{ for the triply-charged ions.}$$

where T is the kinetic energy release in electron volts.

Kinetic energy release and charge separation values for the doubly-charged and triply-charged diatomic ions in this study are given below:

	KER(eV)	r(Å)		KER(eV)	r(Å)
S ₂ ³⁺	11.60	2.49	S ₂ ²⁺	6.71	2.15
SeS ³⁺	10.75	2.73	SeS ²⁺	5.87	2.45
Se ₂ ³⁺	10.47	2.75	Se ₂ ²⁺	4.72	3.05
Te ₂ ³⁺	9.27	3.11	Te ₂ ²⁺	4.62	3.12
Cl ₂ ³⁺	12.76	2.26	Cl ₂ ²⁺	6.86	2.10
BrCl ³⁺	13.10	2.20	BrCl ²⁺	6.13	2.35
Br ₂ ³⁺	9.30	3.09	Br ₂ ²⁺	7.70	1.87
IBr ³⁺	9.28	3.10	IBr ²⁺	5.31	2.71
I ₂ ³⁺	9.10	3.16	I ₂ ²⁺	5.04	2.86

One of the most significant results obtained in this study was the absence of any observable unimolecular fragmentation of the triply-charged ions. Other triply-charged ions were sought from combinations of atoms in the Group VI and Group VII elements i.e.: SCl, SeBr, TeI, etc. No triply-charged ions for these combinations were found. Atom superposition electron delocalization (ASED) calculations were completed for all the ions of study and for the Group VI - Group VII combinations. ASED predicts bond distances for the Group VI - Group VII combinations to be smaller than to the comparable Group VI or Group VII homonuclear diatomics. It is believed that the Group VI- Group VII diatomics encounter a dissociative level before undergoing triple ionization.

THE ROLES OF GAS-PHASE BASICITY AND SURFACE ACTIVITY IN THE FORMATION OF FAB MASS SPECTRA^{*}, T. Keough and M.P. Lacey, The Procter and Gamble Co., Miami Valley Laboratories, PO Box 398707, Cincinnati, OH 45239-8707.

It has been previously shown, for equimolar two-component mixtures, that the component with the higher gas-phase basicity (GB) generally dominates the static FAB mass spectrum¹. This observation has led to the development of a gas-collision model²⁻⁴ which states that initially desorbed ions and neutrals participate in gas-phase reactions, such as recombination and proton transfer, that ultimately determine which ions are detected in FABMS. On the other hand, it is well-known that FABMS response is strongly influenced by condensed-phase phenomena such as analyte surface activity (SA)^{5,6} and pre-forming ions in solution⁷⁻⁹. The purpose of this study is to gain some perspective on the relative importance of some of the factors effecting FABMS response.

Experimentally, we analysed equimolar mixtures of monoalkylammonium salts (A). The long alkyl chain largely determines the SA while the head group



largely determines the GB. Trimethylammonium chlorides (R's=CH₃, TMAC's) of varying chain length were used to study the effects of SA. GB differences are virtually eliminated because the TMAC's have identical head groups with no labile protons and no basic sites capable of accepting protons. Dodecylammonium chlorides were used to study the effects of GB. By varying the surfactant head group from -NH₃Cl to -N(CH₃)₃HCl, the GB of the corresponding free amines increase by about 12.5 kcal mol⁻¹. However, the SA's remain nearly identical since the long alkyl chain length is held constant. Variation in relative FABMS response as a function of analysis time, preference factors¹ and suppression factors¹ were experimentally determined for analytes of both low and high SA. Furthermore, some competitive experiments were conducted to directly assess the relative importance of SA and GB effects.

The TMAC's are very surface active as evidenced by their relatively large preference factors, Table 1. Analysis of an equimolar mixture (0.1 mol%) of C₈, C₁₂ and C₁₆TMAC showed that SA differences can lead to large variations in relative response as a function of analysis time. At first, the most SA component of the mixture (C₁₆TMAC) is preferentially detected. As it is depleted, the responses of the less SA components increase. This should be contrasted with the much more modest variation in relative response, as a function of analysis time, that is produced by a difference in GB of about 12.5 kcal mol⁻¹, Figure 1. The FAB spectra of an equimolar (0.1 mol%) mixture of dodecylammonium chloride and dodecyldimethylammonium chloride show only a minor time-dependent variation that may result because of slight differences in the SA's of the head groups. Similarly, the FABMS preference factors (Table 1) show a strong dependence on SA. The pfs increase by a factor of almost 70 from C₈ to C₁₆TMAC. However, in the absence of SA effects, a GB difference of 12.5 kcal mol⁻¹ changes the relative pfs by less than a factor of 2 (Table 1, hexyl and dodecylammonium chlorides). FABMS suppression factors also show a strong dependence on SA. The suppression factor between C₈ and C₁₆TMAC is 50-fold while that between dodecylammonium chloride and dodecyldimethylammonium chloride is only a factor of 3. These results suggest that, for very surface active compounds, SA effects can dominate GB effects on FABMS response. This conclusion is reinforced by the results obtained in some competition experiments summarized in Table 2. The first mixture establishes the suppression factor between two alkylammonium salts differing in alkyl chain length (SA) by six -CH₂- groups. Mixture 2 shows that the corresponding suppression factor, resulting from a difference in GB of 12.5 kcal mol⁻¹, is an

order of magnitude smaller. Mixtures 3 and 4 illustrate that the effects are cooperative. Note that in mixture 4, hexyldimethylammonium chloride is suppressed even though its GB is greater than that of dodecylammonium chloride.

The previously reported¹ suppression factor of ammonium chloride by aniline hydrochloride is $R=3.7$. We have attempted to estimate the contribution of SA effects to this overall suppression by analysis of a series of equimolar solutions of tetramethylammonium chloride and phenyltrimethylammonium chloride, Figure 2. From the solution containing 10 mol% of each analyte, we calculate a suppression factor of 2. We conclude that even for analytes of low surface activity, SA effects may still be as important as GB effects. In general, it will be difficult to accurately assess the effect of any single factor on FABMS response. All factors, including GB, SA, relative solubilities, sampling efficiencies, etc. will have to be evaluated.

*This work is dedicated to the memory of David E. Chapman.

Table 1 Summary of FAB/MS preference factors

Compound	X	Preference Factor
NX_4Cl	H	20 ¹
	CH_3	42 ^a
PhNX_3Cl	H	37 ¹
	CH_3	88 ^a
C_6TMAC		2.772 ^b
C_{12}TMAC		12.664 ^b
C_{14}TMAC		39.683 ^b
C_{16}TMAC		191.511 ^c
$\text{C}_6\text{NH}_3\text{Cl}$		862 ^d
$\text{C}_6\text{N}(\text{CH}_3)_2\text{HCl}$		1095 ^d
$\text{C}_{12}\text{NH}_3\text{Cl}$		11,985 ^d
$\text{C}_{12}\text{N}(\text{CH}_3)_2\text{HCl}$		17,009 ^d

a. Analyte concentration 0.5 mol% b. Analyte concentration 0.005 mol% c. Analyte concentration 0.0005 mol% d. Analyte concentration 0.1 mol%

Table 2. Summary of suppression factors obtained in the SA and GB experiments.

Mixture	Analyte 1 An1	Analyte 2 An2	Experiment	$R(\text{An2}/\text{An1})^a$
1	$\text{C}_6\text{NH}_3\text{Cl}$	$\text{C}_{12}\text{NH}_3\text{Cl}$	ΔSA	29
2	$\text{C}_{12}\text{NH}_3\text{Cl}$	$\text{C}_{12}\text{NC}_2\text{HCl}$	ΔGB	3
3	$\text{C}_6\text{NH}_3\text{Cl}$	$\text{C}_{12}\text{NC}_2\text{HCl}$	$\Delta\text{SA} + \Delta\text{GB}$	82
4	$\text{C}_6\text{NC}_2\text{HCl}$	$\text{C}_{12}\text{NH}_3\text{Cl}$	$\Delta\text{SA} - \Delta\text{GB}$	20

a. Determined from the glycerol solutions containing 0.10 mol% of each analyte

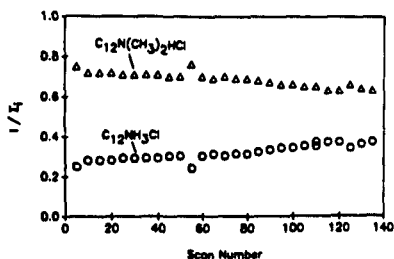


Fig. 1. Time dependence of the FAB spectra of the indicated mixture.

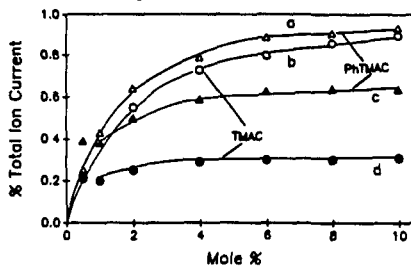


Figure 2. (a) and (b) Variation in intensities of the analytes as function of conc. from separate solutions containing each analyte. (c) and (d) Variation of analyte ions from equimolar solutions of both analytes.

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UNIMOLECULAR DISSOCIATION OF METASTABLE $\text{Mn}_2(\text{CO})_{10}^+$ IONS
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INTRODUCTION: Metastable $\text{Mn}_2(\text{CO})_{10}^+$ ions have been studied using tandem mass spectrometry. The unimolecular dissociation rate constants of $\text{Mn}_2(\text{CO})_{10}^+$ produced under different ionization/excitation conditions are reported. The RF-only quadrupole ion guide of a tandem mass spectrometer is shown to be suitable for studying the kinetics of unimolecular dissociation of metastable ions. It provides a variable and relatively long ion residence time (10^{-5} - 10^{-3} sec). It is possible to correlate the unimolecular dissociation rate constants with the internal energy distribution of metastable ions by theoretical calculation using the threshold energy obtained from collision-induced dissociation (CID).

EXPERIMENTAL: The $\text{Mn}_2(\text{CO})_{10}^+$ ions were generated by electron ionization of $\text{Mn}_2(\text{CO})_{10}$ vapor. Mass-selected ions were injected into an RF-only quadrupole ion guide via a series of retardation lenses. The ion kinetic energy on entering the RF-only quadrupole can be adjusted such that the ion resident time is varied. The translational energy distribution of the ion beam was calculated from the first derivative of the curve of total ion current vs E_c , where $E_c = V_s - V_c$, where V_s and V_c are the potentials of the ion source and collision quadrupole. The peak of distribution curve was chosen as the zero-point of ion kinetic energy. The RF level of the quadrupole ion guide was carefully controlled to minimize its effect on primary ion transmission efficiency. The RF level is proportional to $E_T^{1/2}$ ($E_T = V_c - V_{c0}$) under optimized tuning. Argon gas was used as the collision gas in the CID experiment. The number density of the gas inside collision cell was calculated from the gas pressure outside the quadrupole chamber and the gas conductance through the collision cell apertures. The effective length of quadrupole ion guide was taken as 12.5 cm. No compensation for E_T was made.

RESULTS AND DISCUSSION: An ion beam has a translational energy distribution function $i(V_c)$. When such an ion beam passes through the ion guide, the first-order kinetics become

$$i(V_c) = i_0 \exp \{ (V_c - V_{c0})^2 / 2\gamma^2 \} \quad (1)$$

$$I_p / (I_p + \Sigma I_{Fi}) = \{ \int_{V_{c0}}^{\infty} i(V_c) \exp(-k_T t) dV_c \} / \{ \int_{V_{c0}}^{\infty} i(V_c) dV_c \} \quad (2)$$

where i_0 and γ are empirical constants and I_p and I_{Fi} are the ion intensities.

Within the time window from 10^{-5} to 10^{-4} sec, the translational energy spread of the ion beam has little effect. The rate constants can be calculated from a plot of $\ln(I_0/I_p)$ versus t and I_{Fi}/I_0 versus $\{1 - \exp(-k_T t)\}/k_T$.

TABLE 1. Unimolecular Dissociation Rate Constants of $\text{Mn}_2(\text{CO})_{10}^+$							
Rate constants (s^{-1})	423K			473K			523K
	30 eV	50 eV	70 eV	30 eV	50 eV	70 eV	30 eV
Total	2300	2200	2400	2600	3300	3500	3400
→ $[\text{Mn}(\text{CO})_5]^+$	2100	2000	2300	2400	3000	3300	3100
→ $[\text{Mn}_2(\text{CO})_9]^+$	160	89	190	170	160	200	200
→ $[\text{Mn}(\text{CO})_6]^+$	78	83	72	100	210	160	130

The value of unimolecular dissociation rate constant increases as the temperature increases. This indicates that the internal energy of the ions reflects the temperature of the neutral parent compound. The ionizing electron energy also has an effect on the dissociation rate constants. Within the electron energy range shown above this is smaller than temperature effect.

COLLISION-INDUCED DISSOCIATION: With argon gas in the quadrupole ion guide, the collision-induced dissociation (CID) of $\text{Mn}_2(\text{CO})_{10}^+$ can be studied. Under ideal conditions, the CID cross section $\sigma(E)$ should have the energy dependence described by

$$\sigma(E) = \sigma_0 (E + E_{\text{int}} - E_0)^n / E \quad (3)$$

where E_0 is the threshold energy for CID and E_{int} is the internal energy of the ions.

Figures 1 and 2 show the energy dependence of the CID cross section for $\text{Mn}_2(\text{CO})_{10}^+$. The value $(E_{\text{int}} - E_0)$ for the fragments was obtained by fitting the experimental data to a theoretical model. The ions were produced using 12 eV ionizing electrons and $T = 473\text{K}$.

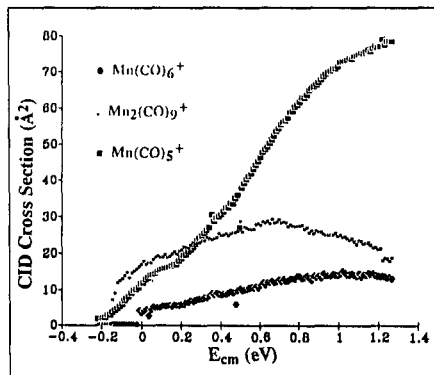


Fig. 1: Energy Dependence of the CID cross section for $\text{Mn}_2(\text{CO})_{10}^+$

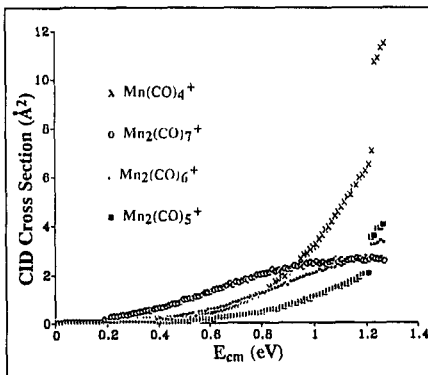


Fig. 2: Energy Dependence of the CID cross section for $\text{Mn}_2(\text{CO})_{10}^+$

INTERNAL ENERGY: An RRKM calculation was performed in order to correlate the unimolecular dissociation rate constant with the internal energy of the ions at the point at which they enter the quadrupole ion guide. The basic expression of $k(E)$ given in RRKM theory is

$$k(E) = \alpha G(E^+)/hN(E^*) \quad (4)$$

The sum of states $G(E^*)$ in the transition state is evaluated by direct computer counting since an analytical approximation has a large error when E^* is small. The density of states $N(E^*)$ of excited ions were calculated using a Whitten-Rabinovitch approximation. The vibrational states of $\text{Mn}_2(\text{CO})_{10}^+$ were assigned to be the same as its neutral analog. The vibrational frequencies of the transition states were chosen as the mean of initial reactants and final products. We assumed that the internal energy distribution of ions is narrow enough that only a discrete value of E_0 contributes to the unimolecular dissociation and CID significantly so that

$$k_{\text{uni}} = \langle k(E) \rangle = k(E_{\text{int}}) \quad (5)$$

When the unimolecular dissociation rate constant and energy dependence of the CID cross section were measured under the same ion beam conditions, the apparent CID threshold value E_0' can be used in an RRKM calculation. Hence, the internal energy of the ions can be obtained. Once the internal energy of the ions was known, the CID threshold energy E_0 of the ions can be obtained.

TABLE 2. Results of RRKM Calculations

	$\text{Mn}(\text{CO})_5^+$	$\text{Mn}(\text{CO})_6^+$	$\text{Mn}_2(\text{CO})_9^+$
$k_i (\text{s}^{-1})$	221.7	7.06	-
$E_{\text{int}} (\text{eV})$	0.76	0.84	-

TABLE 3. Dissociation Thresholds

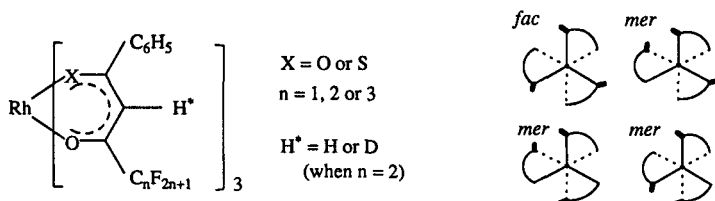
Fragment	$E_0' (\text{eV})$	$E_0 (\text{eV})$
$\text{Mn}(\text{CO})_4^+$	0.32	1.12
$\text{Mn}(\text{CO})_5^+$	-0.20	0.60
$\text{Mn}(\text{CO})_6^+$	-0.05	0.75
$\text{Mn}_2(\text{CO})_5^+$	0.53	1.33
$\text{Mn}_2(\text{CO})_6^+$	0.39	1.19
$\text{Mn}_2(\text{CO})_7^+$	0.19	0.99
$\text{Mn}_2(\text{CO})_9^+$	-0.15	0.65

CONCLUSIONS: The unimolecular dissociation of metastable $\text{Mn}_2(\text{CO})_{10}^+$ was observed in the RF-quadrupole ion guide of tandem mass spectrometer. Unimolecular dissociation rate constants were reported for the ions produced using various ionization/excitation conditions. Dissociation kinetics (ion resident time = 10^{-5} to 10^{-3} sec) have been examined. The translational energy spread of the ion beam was shown to be responsible for a nonlinear deviation when $t > 10^{-4}$ sec. Collision-induced dissociation of $\text{Mn}_2(\text{CO})_{10}^+$ was investigated using tandem mass spectrometry. The energy dependence of the CID cross section was used to obtain the threshold energy ($E_0 - E_{\text{int}}$) which then was used in a RRKM calculation. RRKM theory was used to relate the unimolecular dissociation rate constant to the internal energy of metastable ions. The CID threshold energies corrected for ion internal energy were reported.

MASS SPECTROMETRY OF RHODIUM CHELATES: HYDROGEN TRANSFER DURING ION DECOMPOSITION.

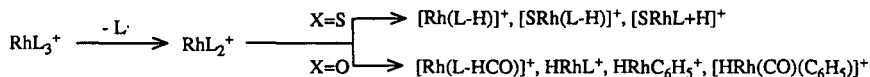
John B. Westmore and Gary A. Stern, Department of Chemistry,
University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Introduction. Bidentate β -ketoenolate and monothio- β -ketolate ligands (L) react with rhodium(III) to form stable, neutral complexes (RhL_3) that are soluble in organic solvents and can be sublimed. These d^6 complexes are diamagnetic, kinetically inert, and have structures based on a metal-centred octahedron. Our ^{19}NMR studies show that the β -ketoenolates are formed as a nearly statistical mixture of *fac* and *mer* isomers and confirm a *facial* octahedral structure for the thio complexes. Positive ion electron ionization (EI) mass spectra of the listed complexes, which have both perfluoroalkyl and phenyl substituents attached to the chelate rings, exhibit several decompositions involving hydrogen transfer. The origin of the hydrogen transferred in these reactions was determined by deuterium-labelling at the chelate ring carbon.



Experimental. Positive ion EI mass spectra of complexes, introduced on the solids probe, were recorded with a VG-7070E-HF double-focussing mass spectrometer of EB geometry, at an electron beam energy of 70 eV, a source temperature of 200°C, and an ion acceleration voltage of 6 keV. Ionic decompositions (collisionally assisted, if necessary) in the first field-free region were identified by linked scanning at constant E/B (daughter ion spectra). Exact masses of ions were measured at a spectrometer resolution of $M/\Delta M = 5000$, or higher if necessary to resolve isobaric ions. PFK was used as the mass calibrant.

Results and Discussion. The EI mass spectra are strongly influenced by the presence of S in the chelate ring, with different ion decomposition pathways being followed for $X = O$ and $X = S$. Here we focus on those reactions that involve H transfer, the most prominent of which are summarized by:

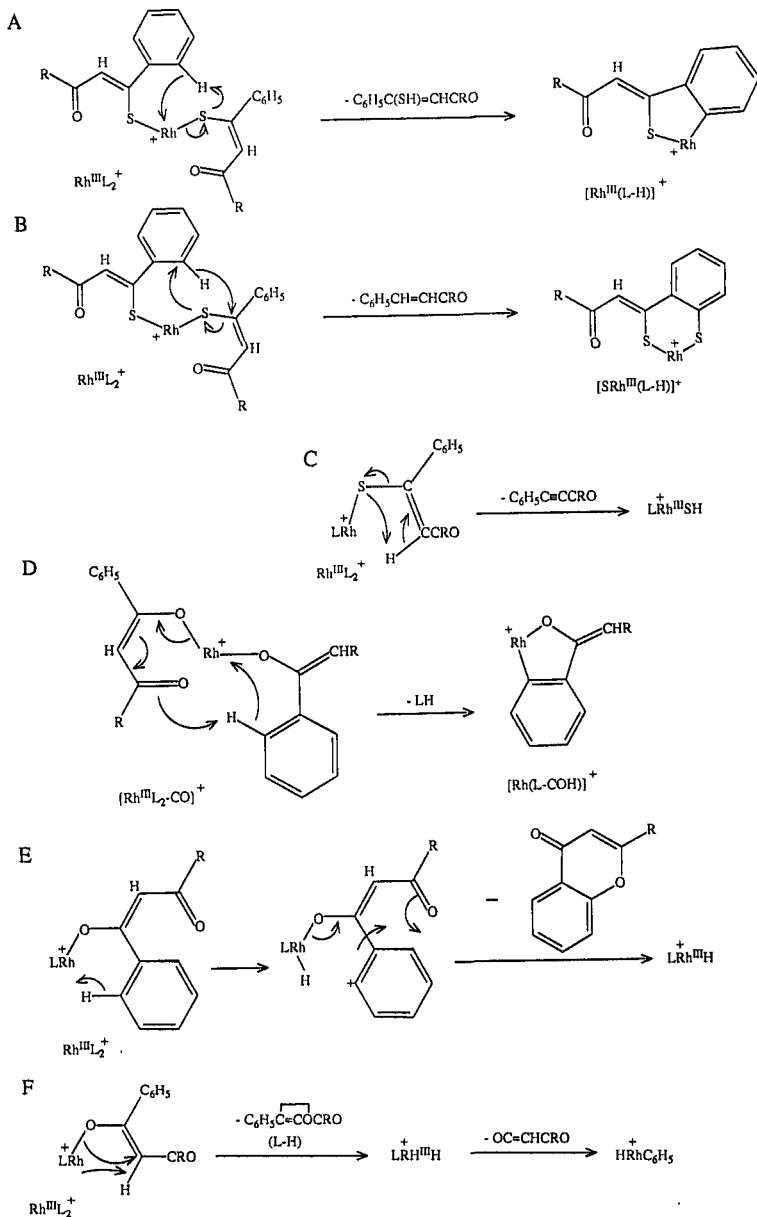


The origins of the hydrogens involved in the transfer reactions, for $n = 2$, were calculated by assuming that isotope effects in the transfer reactions are negligible. The results are summarized in the Table. Note that, in addition to the different ion species formed, there is also a dichotomy with respect to the hydrogen transfer behavior. When sulfur is present the hydrogen *lost* originates mainly from the phenyl group and the hydrogen *retained* comes mainly from the chelate ring. When sulfur is absent these preferences are reversed.

TABLE. Source (%) of hydrogen transferred in the formation of the indicated ions for complexes with $n = 2$.

Ion species	Lost from ion		Retained in ion	
	phenyl	chelate ring	phenyl	chelate ring
$X = S$				
$[Rh(L-H)]^+$	~93	~7		
$[SRh(L-H)]^+$	~82	~18		
$[SRhL+H]^+$			20±10	80±10
$X = O$				
$[Rh(L-HCO)]^+$	~42	~58		
$HRhL^+$			~65	~35
$[HRhC_6H_5]^+$			~82	~18
$[HRh(CO)(C_6H_5)]^+$			~79	~21

In the mechanisms proposed below for some of these reactions, we observe a greater tendency for H migration to Rh when X = O (schemes D - F) than when X = S (schemes A - C).



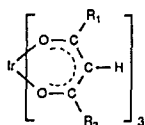
MASS SPECTRAL STUDIES OF IRIIDIUM COMPLEXES OF
2,4-PENTANEDIONE, 1,1,1-TRIFLUORO-2,4-PENTANEDIONE
AND 1,1,1,5,5,5-HEXAFLUORO-2,4-PENTANEDIONE

Gary A. Stern and John B. Westmore

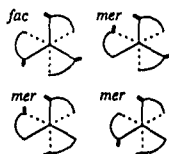
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INTRODUCTION

With β -ketoenolate ligands (L) iridium forms stable, neutral complexes (IrL_3) that are soluble in organic solvents and can be sublimed. The IrL_3 (d^6) complexes are diamagnetic, kinetically inert and have a structure based on a metal-centered octahedron. The three titled complexes are hereinafter referred to as $\text{Ir}(\text{acac})_3$, $\text{Ir}(\text{tfa})_3$ and $\text{Ir}(\text{hfa})_3$. We have shown by ^{19}F NMR spectrometry that $\text{Ir}(\text{tfa})_3$, which has unsymmetrical ligands, is formed as a nearly statistical mixture of *mer* and *fac* isomers. There is only one possible geometrical isomer for $\text{Ir}(\text{acac})_3$ and $\text{Ir}(\text{hfa})_3$ since each has symmetrical ligands.



	R_1	R_2
$\text{Ir}(\text{acac})_3$	CH_3	CH_3
$\text{Ir}(\text{tfa})_3$	CH_3	CF_3
$\text{Ir}(\text{hfa})_3$	CF_3	CF_3



EXPERIMENTAL

Synthesis. All three iridium chelates were prepared by a modified procedure of Davignon *et al*.^{1,2} On purification by fractional sublimation, yellow $[\text{Ir}(\text{acac})_3]$ and $[\text{Ir}(\text{tfa})_3]$ and orange $[\text{Ir}(\text{hfa})_3]$ crystalline products were obtained. These were characterized by mass, ^1H and ^{19}F NMR spectrometries. Deuteration of Hacac at the bridging carbon atom was achieved by mixing it with CH_3OD containing DCl . From this the iridium complex was then prepared as above except that all necessary reagents were replaced by their deuterated analogues.

Mass spectrometry. Positive ion electron ionization mass spectra of complexes, introduced by the direct insertion probe, were obtained with a VG-7070E-HF double-focussing mass spectrometer of EB geometry at an electron beam energy of 70eV, a source temperature of 200°C and an ion acceleration voltage of 6 KeV. Ionic decompositions (collisionally assisted, if necessary) in the first field free region were identified by linked scanning at constant E/B (daughter ion spectra). Exact masses of ions were measured at a spectrometer resolution of $M/\Delta M=5000$ or higher if necessary to resolve isobaric ions. PFK was used as the mass calibrant.

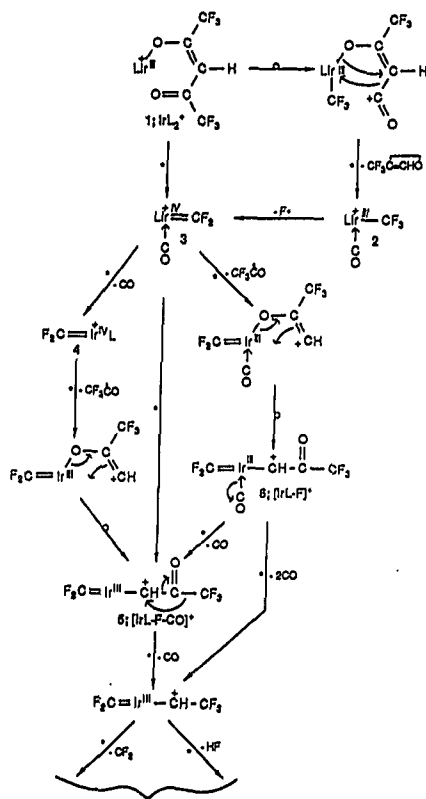
RESULTS AND DISCUSSION

The major metal-containing ions are IrL_3^+ and IrL_2^+ ; the former corresponds to the base peak for $\text{Ir}(\text{acac})_3$ and $\text{Ir}(\text{tfa})_3$, the latter for $\text{Ir}(\text{hfa})_3$. Ions of all three complexes show an overwhelming tendency to decompose by losses of even-electron neutral species, i.e. CO, CF_2 , HF, and CF_2CO for $\text{Ir}(\text{hfa})_3$; CO, CH_4 , C_2H_4 and CH_2CO for $\text{Ir}(\text{acac})_3$, and a combination of these for $\text{Ir}(\text{tfa})_3$. These preferred decompositions are attributed to iridium's preference for the +III and +IV oxidation states and of its ability to form single and multiple bonds with carbon. These consecutive losses can only be explained by initial scission of an Ir-O bond and formation of an Ir-C bond (schemes 1 and 2). Migration of a $\cdot\text{CF}_3$ or $\cdot\text{CH}_3$ group to iridium, evident by formation of ions such as LiIrCF_3^+ , $[\text{IrL}+(\text{CO})(\text{CF}_3)]^+$, LiIrCH_3^+ and $[\text{Ir}+(\text{CO})(\text{CH}_3)]^+$ would also facilitate loss of a carbonyl group. Losses of odd-electron fragments, such as $\cdot\text{CF}_3$, $\text{CF}_3\dot{\text{C}}\text{O}$, $\cdot\text{CH}_3$ and $\text{CH}_3\dot{\text{C}}\text{O}$ do occur, but almost exclusively after oxidation of Ir(III) to Ir(IV) .

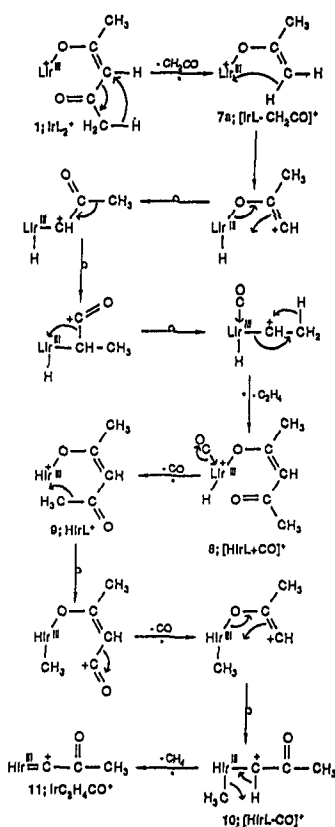
To illustrate, decomposition pathways from $[\text{Ir}(\text{hfa})_2]^+$, 1, are shown in scheme 1. Formation of Ir- CF_3 and Ir-CO bonds, and loss of the even-electron fragment $\text{CF}_3\dot{\text{C}}=\text{CHO}$

yields $[\text{IrL}+(\text{CO})(\text{CF}_3)]^+$, 2. Although unconfirmed by metastable evidence, loss of F $\cdot$ from 2 would yield the fluorocarbene ion $[\text{IrL}+(\text{CO})(\text{CF}_2)]^+$, 3, containing iridium (IV). From 3, consecutive losses of CO and CF_3CO (loss of this odd-electron species indicates reduction to iridium(III)) yield LiIrCF_2^+ , 4, and $[\text{IrL}-\text{F}-\text{CO}]^+$, 5, respectively. Alternatively, when loss of CF_3CO precedes loss of CO the ions $[\text{IrL}-\text{F}]^+$, 6, and once again 5, result. This ion and its daughters undergo numerous decompositions that involve losses of even-electron species exclusively, i.e. CO, CF_2 and HF.

Scheme 2 shows decomposition pathways from $[\text{Ir}(\text{acac})_2]^+$, 1. Initial loss of CH_2CO yields 7a, which then undergoes sequential losses of C_2H_4 , CO, CO and CH_4 . The formation of the resulting ions, 8-11, is rationalized by several rearrangements, supported by the mass spectrum of the deuterated analogue of $\text{Ir}(\text{acac})_3$, that include migration of H and CO to Ir.



Scheme 1



Scheme 2

1. L. Davignon, A. Dereigne and J.M. Manoli, *J. Less Common Metals*, 21(3), 345 (1970).
2. L. Davignon, J.M. Manoli and A. Dereigne, *J. Less Common Metals*, 21(3), 341 (1970).

STRUCTURAL IMPLICATIONS REGARDING FRAGMENTATION OF 12-CROWN-4 FROM PHOTOIONIZATION MASS SPECTROMETRY.

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We have investigated the photoionization of the crown ether, 12-crown-4 ($C_8H_{16}O_4$). Structural information regarding fragment ions is inferred from appearance energies when combined with known thermochemical data. The fragmentation of 12-crown-4 molecular ion will be discussed in light of these results.

The photoionization mass spectrometer has been described previously (1). The hydrogen many-line spectrum, generated from a dc discharge using low pressure hydrogen, serves as the light source for these experiments. The relative photoionization efficiency (PIE = ion count/transmitted photon count) is calculated at a variety of wavelengths and plotted as a function of photon energy to produce the PIE curve.

PIE curves for twelve fragment ions were obtained and are shown in Figures 1 and 2.

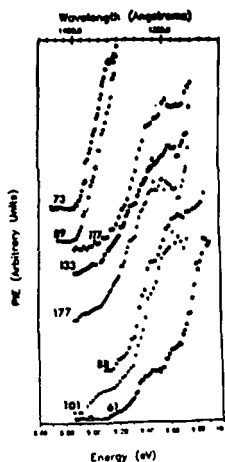


Figure 1. Photoionization efficiency curves for daughter ions of 12-crown-4.

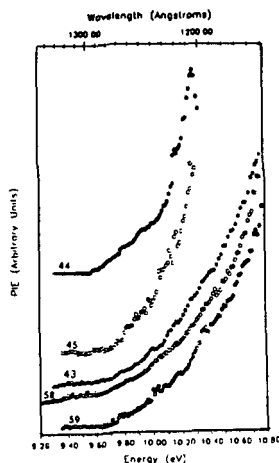


Figure 2. Photoionization efficiency curves for daughter ions of 12-crown-4.

Their appearance energies (AE) and suggested chemical compositions for ionic products are given in Table 1. The molecular ion at m/z 176 was never observed in these experiments. Heats of formation for the ions formed were obtained by combining their AEs with heats of formation of likely neutral products, when available. The results are summarized in Table 2.

Table 1. Ionization and appearance energies (298 K) of 12-crown-4 and its fragment cations.

m/z	Ion	IE or AE, eV
176	$C_8H_{16}O_4^+$	8.60 ^a
133	$C_6H_{12}O_3^+$	8.90
117	$C_6H_{12}O_2^+$	8.95
101	$C_4H_8O_2^+$	8.92
89	$C_4H_8O^+$	8.86
88	$C_4H_8O^+$	9.14
73	$C_4H_8O^+$	8.90
61	$C_2H_4O_2^+$	9.10
59	$C_2H_4O^+$	9.70
58	$C_2H_4O^+$	9.63
45	$C_2H_4O^+$	9.70
44	$C_2H_4O^+$	9.58
43	$C_2H_4O^+$	9.70

^a Inferred from PES, N. Kajitani, et al., Bull. Chem. Soc. Japan, 32 (1979) 2199.

Table 2. Thermochemical data relevant to the ionization and dissociation of 12-crown-4.

m/z	ion	neutral(s)	ΔH_{298}° kJ mole ⁻¹
176	$C_{12}H_{24}O_4^+$	$C_{12}H_{24}O_4$	-631 ^a
133	$C_6H_{12}O_2^+$	CH_3CO	252 ^c
117	$C_6H_{12}O_2^+$	CH_3COOH	-24 ^a
101	$CH_3C(OH)CHC(OH)CH_3$	$C_2H_5O_2$	474 ^b
89	(p-dioxane)H ⁺	$C_4H_8O_2$	277 ^a
88	$C_4H_8O_2^+$	p-dioxane	-47 ^c
73	$C_2H_4OCHCH_3^+$	$C_2H_4O_3$	403 ^a
61	$C_2H_4O_2^+$	$CH_3CO+c-C_4H_8O$	-179 ^c
59	$CH_3CH_2OCH_2^+$	$C_2H_5O_3$	564 ^c
58	$C_2H_4O^+$	1,3,6-trioxacyclooctane	-316 ^a
45	$C_2H_4O^+$	p-dioxane+CH ₃ CO	521 ^b
44	$C_2H_4O^+$	2,4,6-trimethyl-1,3,5-trioxane	-293 ^c
43	$C_2H_3O^+$	p-dioxane+CH ₃ CHOH	455 ^c
			-208 ^a
			593 ^a
			-288 ^a
			765 ^c
			-467 ^d
			645 ^c
			-385 ^a
			924 ^c
			-632 ^d
			687 ^c
			-382 ^a

^aS.G. Lias, J.E. Bartmess, J.F. Liebman, J.L. Holmes, R.D. Levin and W.G. Mallard, J. Phys. Chem. Ref. Data, 17 (1988), Supplement No. 1.

^bJ.J. Butler, M.L. Fraser-Monteiro, L. Fraser-Monteiro, T. Baer and J.R. Hass, J. Phys. Chem., 86 (1982) 747.

^cThis Work.

^dT.B. Pedley, R.D. Naylor, S.B. Kirby, Sussex-N.P.L. Computer Analyzed Thermochemical Data, 1986.

A comparison of reported mass spectra (2-4) of similar compounds including catechol polymethylene diether derivatives, aromatic crown ethers and saturated crown ethers suggests that the dissociation constraints, and therefore that fragmentation need not necessarily produce the most thermodynamically stable daughter ions. The thermodynamic implications of the AEs reported here support kinetic control of the

photolionization/fragmentation of 12-crown-4. The dissociation mechanism of the 12-crown-4 molecular ion can be easily explained on the simple mechanistic basis shown in Figure 3. Presumably removal of a nonbonding electron from an oxygen atom in 12-crown-4 leads to prompt fragmentation of the molecular ion. It appears that some fragments, of the type $[C_2H_4O]_n^+$ ($n=1,2$) are formed by immediate dissociation of this structure. For other molecular ions an intramolecular H-shift appears to occur, which catalyzes ring-opening. This leads to ions of the type $[C_2H_4O]_nH^+$ ($n=1-3$).

Trends in appearance energies of the investigated photofragments, plus structural implications derived from thermochemical analysis and results from EI mass spectra of crown ethers (3) suggest that the fragmentations observed follow simple mechanisms, but that the fragment ions do not necessarily exist in their most stable isomeric forms.

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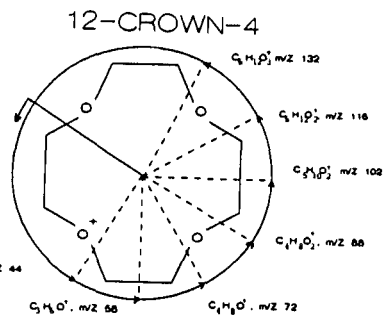


Figure 3. Dissociation mechanism of 12-crown-4.

A COMPETITION BETWEEN TWO ISOMERIZATION PROCESSES IN UNIMOLECULAR DISSOCIATION

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One of the advantages of the photoelectron photoion coincidence (TPEPICO) time-of-flight mass spectrometry is to determine the decay rate constants for unimolecular dissociations. The rates observed in the TPEPICO experiments are indicated by the TOF distributions. A symmetric peak shape implies that the decomposition is relatively fast with the rate constant greater than 10^7 s^{-1} . On the other hand, metastable decomposition ($< 10^7 \text{ s}^{-1}$) is reflected by asymmetric TOF distributions. For the later case, the rate constants can be numerically calculated by analyzing the TOF distributions.

It is well established that the analysis of asymmetric TOF distributions for the first generation fragment ions gives only the total decay rate of the parent ion. Therefore, in a unimolecular dissociation involving two simple cleavages the rate constants calculated from TOF distributions should be identical for the two competitive processes. The decay rate constant for each fragment process is then obtained from the total rate constant and relative abundance of ions in the breakdown graph at a given internal energy. This is described by Equation 1 where k_T is the total rate constant, $B(m_i, E)$ is the branching ratio of mass m_i at the internal energy E .

$$k(m_i, E) = k_T B(m_i, E) / \sum B(m_i, E) \quad [1]$$

Recently, the formation of m/z 28 (C_2H_4^+) and m/z 56 ($\text{C}_2\text{H}_4\text{CO}^+$) in the unimolecular dissociation of ethyl formate ions at low internal energies were found to have different rate constants with a single-component feature (Figure 1). The rate constants calculated from the TOF distributions for the formation of m/z 28 are higher than those for m/z 56 by an order of magnitude. This observation, however, cannot be simply explained by a competition between two direct fragmentation or a competition between a direct fragmentation and a prior isomerization¹. A plausible explanation may be that the fragmentations occur from intermediate isomers specific to each fragment ion (Figure 2). It is likely that m/z 28 is formed via a stepwise McLafferty rearrangement² which is characterized by the formation of a stable isomeric structure, the distonic radical. It is also postulated that the m/z 56 is formed from its specific isomer, the enol ion of propionic acid. The potential energy surface for the formation of m/z 56 is summarized in Figure 3.

To determine the rate for the system shown in Fig. 2, one needs to determine the solutions of the determinant of a 5×5 matrix.

$$\begin{vmatrix} (k_1 + k_3) - k_i & k_2 & k_4 & 0 & 0 \\ k_1 & -(k_2 + k_5) - k_i & 0 & 0 & 0 \\ k_3 & 0 & -(k_4 + k_6) - k_i & 0 & 0 \\ 0 & k_5 & 0 & -k_i & 0 \\ 0 & 0 & k_6 & 0 & -k_i \end{vmatrix}$$

It will yield five solutions for k_i : one real, two imaginary and two zero solutions. The general solutions for I_1 and I_2 are given by linear combination of three non-zero solutions simplified by Euler equation. Thus, the rates for P_1 and P_2 will exhibit multi-component behavior.

Since the rate constants observed for the formation of both m/z 28 and m/z 56 in the unimolecular dissociation of ethyl formate ions are characterized by single-component behavior, it seems that a steady-state approximation could be applied to this specific case. Thus, the total rate constants can be given by Equation 2:

$$k_T = (k_1 + k_3) - k_1 k_2 / (k_2 + k_5) - k_3 k_4 / (k_4 + k_6) \quad [2]$$

which is generally satisfied under conditions of Equation 3:

$$k_2(k_4 + k_6) \frac{dI_1}{dt} + k_4(k_2 + k_5) \frac{dI_2}{dt} \ll [k_1 k_4 k_5 + k_1 k_5 k_6 + k_2 k_3 k_6 + k_3 k_5 k_6] \exp(-k_T t) \quad [3]$$

The rates for the two final products formed from their corresponding precursors are determined by Equations 4 and 5.

$$dP_1/dt = k_1 k_5 / (k_2 + k_5) \exp(-k_T t) \quad [4]$$

**HIGH MASS CARBON CLUSTERS FORMED FROM POLYCYCLIC AROMATIC HYDROCARBONS
OBSERVED BY LASER MASS SPECTROMETRY; LINEMAN, D.N., VISWANADHAM, S.K.,
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15260**

EXPERIMENTAL: All samples were analyzed with the Leybold-Heraeus LAMMA-1000 laser TOF MS, using a Nd:YAG laser (266nm). All samples were deposited on zinc foil for analysis, using a variety of organic solvents.

DISCUSSION: Laser irradiation of Polycyclic Aromatic Hydrocarbons (PAH) has been found to generate carbon clusters from C₁⁺ through C₆₀₀⁺. Unlike earlier research by Kaldor(1), Smalley(2), and others concerning laser vaporization of graphite, in which two cluster types were observed (low mass and high mass), laser vaporization of PAH's yields four different cluster types. The appearance of each type is strongly influenced by laser energy and focus conditions. Figure 1 shows the LMS spectrum of each cluster type and the cluster's corresponding formula. Carbon clusters observed at the laser focal point are Type IA. Type IA clusters are of the form C_nH_m⁺, where: n = 1-30 (approx.) and H = 0-3. By defocusing 100 microns, cluster Types IA, IB, II and III can be observed. Type IB clusters are of the form C_nH_m⁺, where: n = 3-50 and m = 2-10. Type II clusters are of the form [xM - yH]⁺ where: M = Mol. Wt. of the PAH, x = 1 to 5, and yH is a compound dependent loss of H. Type III clusters are the so called "high mass" carbon clusters. These clusters are composed only of carbon, and are observed for even numbered carbons only. Type III clusters are of the form C_{2n} where n = 20 to 300. Type III clusters have been generated from a variety of organic compounds, very diverse in structure. Compounds such as pyrene, tetraphenylbenzene, phenazine, polyimide, and even coal all produce Type III clusters which are identical. Upon examination of the structures of these compounds, one finds that the commonly occurring moiety in each is the benzene ring. Of all the compounds we have analyzed to date, only those containing aromatic rings formed Type III "high mass" carbon clusters. The ease with which Type III clusters are formed is directly proportional to the compounds aromaticity. Those compounds which contained a high number of conjugated rings (coronene) formed Type III clusters most easily, while those which contained aromatic rings connected through single bonds (tetraphenylbenzene) or aromatic rings separated by heteroaromatic rings (phenazine) were most difficult to form Type III clusters. Tripyridyltriazine, a compound composed entirely of heteroaromatic rings formed no Type III clusters. The combustion process plays an interesting role in Type III cluster formation. Soot generated by PAH's will also yield Type III clusters upon laser irradiation. While Type III clusters are easier to generate from conjugated PAH's than compounds with nonconjugated aromatic rings, the combustion products of both types of compounds yield Type III clusters with approximately equal efficiencies. However, only those compounds which contain some degree of aromaticity will form combustion products capable of generating Type III clusters; an example of this can be seen in figure 2. Soot formed from esterone (aromatic) will generate Type III clusters, while soot formed from testosterone (non-aromatic), will not. Type III clusters have been seen in both positive (through C₅₈₄⁺) and negative (through C₂₀₀⁻) ion mode, as shown in figure 3.

CONCLUSION: The structures and mechanisms of formation of the four carbon cluster types detected are not yet clearly understood. There is however evidence which seems to indicate possible relationships between the cluster types, which will give insight into the processes involved in the formation of "high mass carbon clusters". Work is currently being done in this area.

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FIGURE 1

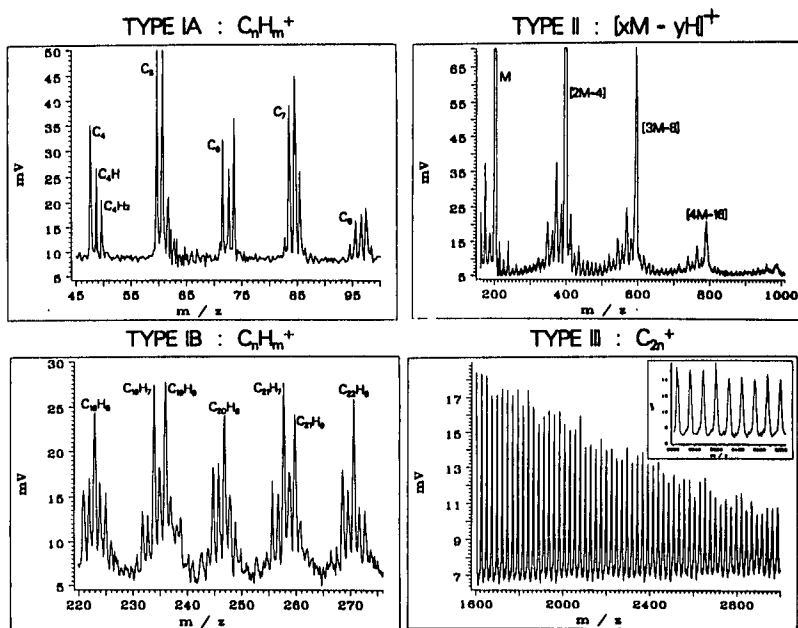


FIGURE 2

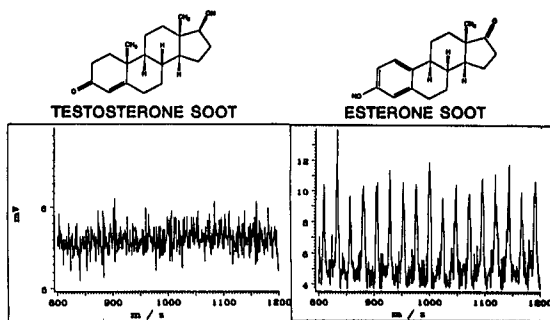
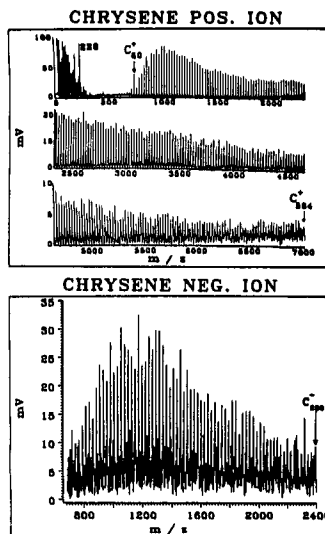


FIGURE 3



THE KINETIC ENERGIES OF IONS FORMED BY RESONANT AND
NON-RESONANT MULTIPHOTON IONIZATION

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Measurement of the translational energy of product fragment ions is a method which has been used to obtain information on ionic fragmentation mechanisms (1). In this study the translational energies of fragment ions produced by both resonant and non-resonant MPI have been measured to provide answers to the following questions: are the fragmentation mechanisms different in the case of resonant and non-resonant MPI when the spectra are superficially similar? And, are the fragmentation mechanisms the same for two different non-resonant MPI cases? The system chosen for study was fluorobenzene. 70 eV, 266 nm, 532 nm and 355 nm MPI spectra are shown (Fig. 1).

All experimental data were obtained using a laser/time-of-flight mass spectrometer in which it was possible to alter the time between the laser light crossing the source and the application of the draw-out pulse (time-lag) on the microsecond time-scale. Experimental time-of-flight profiles were obtained at different time-lag values. Agreement with computer generated profiles, which allowed input of different ion energies and time-lag values, was very good (see Fig. 2). At long time-lag values ion-loss effects, which are dependent on ion energy, become important. Comparison of experimentally obtained and computer generated ion-loss curves allowed the determination of ion energies (Fig. 3). Table 1 summarizes the experimentally determined kinetic energies of the fluorobenzene ions for the three wavelengths and Table 2 summarizes ion energies, calculated using equations from (1) for different fragmentation mechanisms.

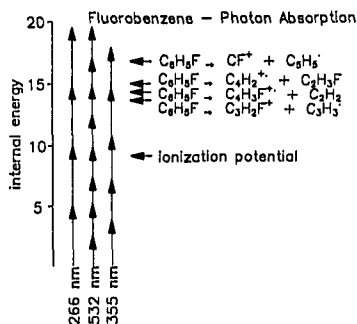
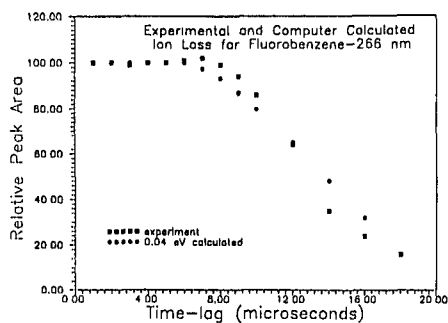
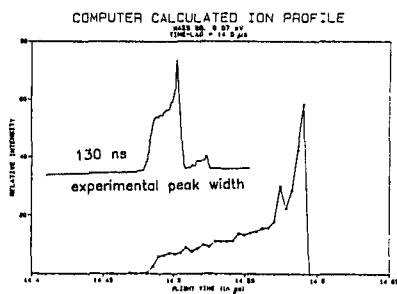
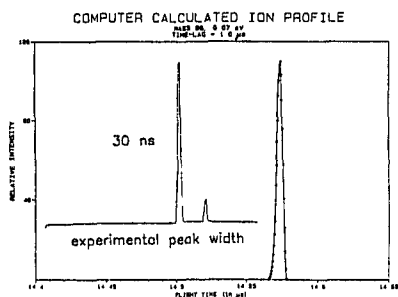
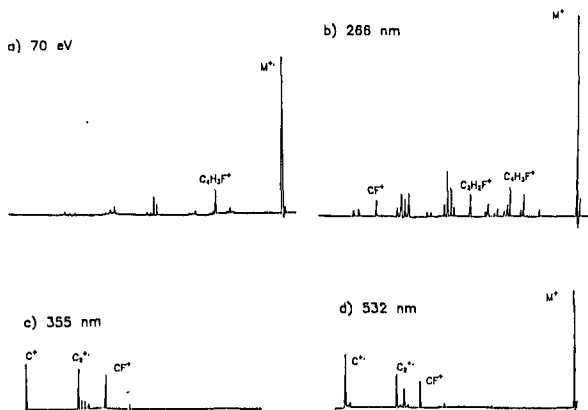
Table 1 Experimental K.E.'s of Fluorobenzene Ions in eV.

m/z	ion	266 nm	355 nm	532 nm
96	$C_6H_5F^+$	0.04	not obs	0.07
70	$C_4H_3F^+$	0.12	"	not obs
57	$C_3H_2F^+$	0.18	"	"
31	CF^+	0.15	2.8	0.40
24	C_2^+	not ms'd	2.2	0.35

Table 2 Calculated K.E.'s of Fluorobenzene Ions in eV.

Reaction	Threshold(eV)	Number of Photons*	KE(eV)	*For 266 nm MPI, double this number for 532 nm.
$C_6H_5F \rightarrow C_4H_3F^+ + C_2H_2$	14.1	4	0.10	
$C_6H_5F \rightarrow C_3H_2F^+ + C_3H_3$	13.6	3	0.05	
		4	0.22	
$C_6H_5F \rightarrow CF^+ + C_5H_5$	16.8	4	0.11	
		5	0.23	
		6	0.35	
$C_4H_3F^+ \rightarrow CF^+ + C_3H_3$		1	0.24	
$C_3H_2F^+ \rightarrow CF^+ + C_2H_2$		1	0.39	

The data indicate that at 266 nm $C_4H_3F^+$, $C_3H_2F^+$ and CF^+ are formed via a direct ionic fragmentation route after the absorption of four photons by the neutral molecule. At 532 nm the best fit for CF^+ production is for further photon absorption by $C_3H_2F^+$ indicating an ionic ladder switching mechanism. The high energies observed for CF^+ and C_2^+ at 335 nm fit neither of these schemes but might result from coulombic repulsions in the formation of more than one ion from a single neutral molecule.



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FRAGMENTATION OF $M^{+\bullet}$ IN METHYL KETONES IN THE ION TRAP:
VARIATION OF MECHANISM WITH CHAIN LENGTH

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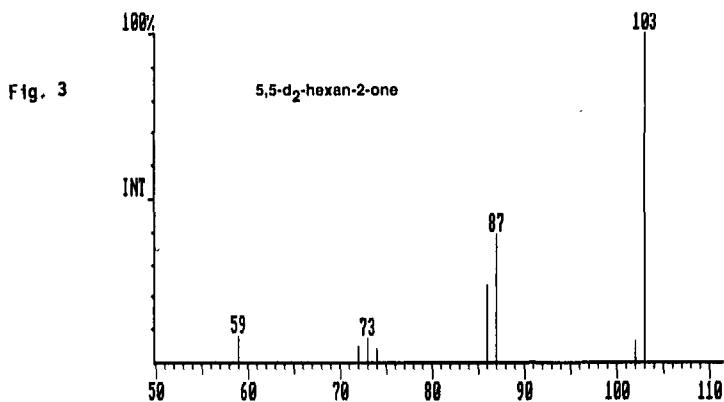
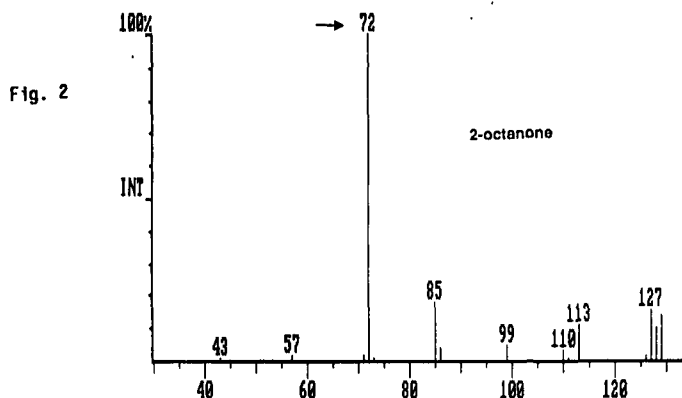
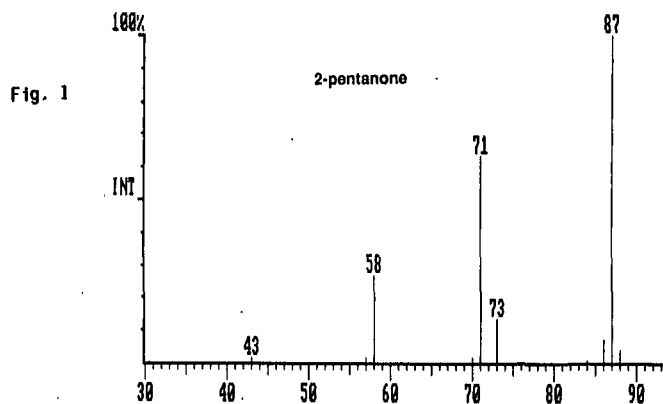
The collisionally activated dissociation of the molecular ion in a series of methyl ketones ranging from 2-butanone to 2-octanone has been studied using a Finnigan Ion Trap Mass Spectrometer. Helium was used as the buffer gas and the tickle voltage was 400mv in each case, resulting in about 10 e.v. excitation energy. Under these conditions only a few fragment ions were produced, mainly $M^{+\bullet}$ -alkyl and McLafferty-type ions at m/z 58, 72 and 86, depending on chain length.

Loss of methyl from 2-pentanone (Fig. 1) then methyl and ethyl from 2-hexanone and 2-heptanone suggests displacement of the alkyl group first from the beta, then gamma positions. In higher ketones, loss still occurs from the gamma position (m/z 85) but further cleavage is also observed (m/z 99).

McLafferty-type ions are observed where they are feasible (m/z 58 in 2-pentanone) but a similar ion is seen one methylene unit more at m/z 72 in higher members of the series. Eventually this ion represents the most important cleavage pathway as seen in 2-octanone (Fig. 2).

An attempt was made to determine the source of the migrating proton in these peaks using 2-hexanone labelled in positions both alpha and gamma to the carbonyl. The gamma position was the source of the proton for the m/z 58 (Fig 3.), but scrambling had also occurred as evidenced by the ion at m/z 86. Interestingly, self-CI (m/z 103) involved none of the deuterium atoms.

Further experiments with specifically labelled compounds will be carried out in an effort to delineate these processes more completely.



Mass Spectrometric Techniques Applied to Assignment of Structures
in Three or Four Step β -Lactam Syntheses

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Cincinnati, Ohio 45267

The pharmaceutically useful β -lactams are currently made from fermentation derived penicillins. Typically a penicillin G salt is produced by fermentation and then deacylated enzymatically to give 6-aminopenicillanic acid (APA). This synthon, APA, is then chemically modified to give the currently used semi-synthetic penicillins and cephalosporins. Direct chemical synthesis of β -lactams has been achieved several times by means of multiple step syntheses--typically 10-25 steps from a simple precursor. We have found a four step procedure from glycine to APA--the first direct synthesis of this synthon--by means of Leuchs anhydride intermediates as depicted in Figure 1 (1). Mass spectrometry played an important role in confirming the structures of intermediates in part because of the inherent lability of Leuchs anhydrides; the anionic intermediates Na⁺ FLAG and LATZ are somewhat stabilized as anion derivatives.

The first crucial intermediate was GLA reported in Houben-Weyl (2) to have a m.p. of 100°; instead, we find that it decomposes over a wide temperature range, but gives a clean DI mass spectrum with the molecular ion m/z

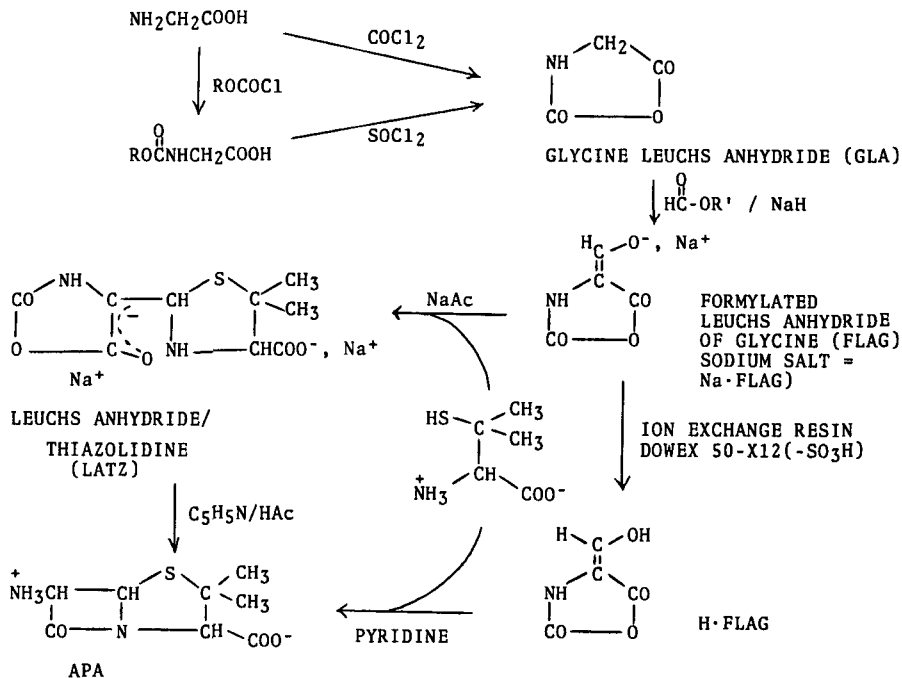


FIGURE 1. A short, Leuchs Anhydride Based Direct Synthesis of 6-Amino Penicillanic Acid (APA).

RAPID IDENTIFICATION OF URINARY METABOLITES OF ADINAZOLAM (U41123)
IN HUMANS USING TANDEM MASS SPECTROMETRY

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One of the objectives of our laboratory is to critically evaluate the utility of tandem mass spectrometry, interfaced with various sample introduction/ionization techniques, in elucidating drug metabolite structures without the use of radioisotopes. Adinazolam (U-41,123F, 8-chloro-1-[(dimethylamino)methyl]-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine) is a psychoactive drug demonstrating pharmacological activities in animals characteristic of both benzodiazepine anxiolytics and tricyclic antidepressants. The major metabolic products of adinazolam present in human urine have previously been determined using radio-isotope labeled drug and various physico-chemical techniques. This particular compound represents a challenging test case in that most of the drug is excreted in urine as one metabolite and the other metabolites present represent less than 10% of the total. The present study has examined the utility of FAB MS-MS analysis of crude urinary fractions and GC-MS-MS analysis of an organic solvent extract in elucidating the structures of metabolites of adinazolam.

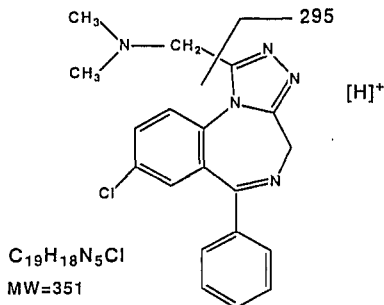
Sample preparation: ODS FRACTIONS: Urine (50 ml) was extracted using a C-18 ODS solid phase extraction cartridge and crude separations of this complex mixture were obtained by elution with 5 ml washes of increasing amounts of methanol in water (0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, and 100% methanol). The crude extracts were then concentrated and analyzed by FAB-MS. SOLVENT EXTRACTION: Urine (50 ml) was combined with 10 ml 2 M K₂HPO₄ and extracted with 10 ml of butyl chloride. Following centrifugation the organic phase was washed with 10 ml 1 M NaOH, dried under a stream of nitrogen and redissolved in 250 µl CH₂Cl₂. Instrumentation: Finnigan MAT TSQ-70 triple quadrupole mass spectrometer, Hewlett-Packard 5890 gas chromatograph and Ion Tech FAB source. Analysis Conditions: GC-MS: GC column, 20 m DB-1, J&W Scientific, 0.25 mm I.D., 0.1 µm film thickness; methane reagent gas at 1 Torr. FAB: 2-Hydroxyethyl disulfide matrix; xenon at 8 kV. MS-MS: Argon collision gas, 1 mTorr.

All metabolites of adinazolam previously observed resulted from metabolic changes occurring in the 1-[(dimethylamino)methyl] moiety. The structures of these metabolites and % of total dose excreted as extractable metabolites in urine were observed as follows: Unmetabolized drug, 0.7%; 8-chloro-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine (estazolam), 0.8%; 1-[(methylamino)methyl]-estazolam, 53.4%; 1-hydroxymethyl-estazolam and formamide, N-[(8-chloro-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine-1-yl)methyl]-N-methyl-, 4.0%; acetamide, N-[(8-chloro 6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepinyl)methyl]-, 3.7% and 1-aminomethyl estazolam, 1.2%. At collision energies less than 50 eV the daughter ion mass spectrum is dominated by cleavage products originating from the (dimethylamino)methyl side chain and triazolo ring system. Performing parent scans of these ions would be an obvious choice to detect metabolic transformations occurring at this side chain (knowing nothing about the metabolism of this compound). When the solvent extracted sample was analyzed by GC-MS-MS using parent ion scans for m/z 295 (the 8-chloro-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine ring system, estazolam) the chromatogram was observed to be dominated by m/z 338, the N-demethyladinazolam metabolite. Daughter ion mass spectra of m/z 295, 338 and 352 confirmed the presence of adinazolam, N-demethyladinazolam and estazolam. The metabolite structures could have

been easily deduced without the availability of reference compounds. Detection and confirmation of other metabolites was complicated by lower concentrations and lack of chromatographic resolution from the N-demethyl metabolite when enough sample was injected to obtain daughter ion mass spectra.

An intense signal at m/z 338 was also observed for FAB-MS-MS analysis (again, parents of m/z 295). The daughter ion mass spectrum of m/z 338 confirmed the presence of the N-demethyl metabolite (again, this structure could easily have been deduced without the availability of reference compound). Identification of parent drug and other metabolites was complicated by the presence of large amounts of the major metabolite (for example, m/z 295 is observed in the FAB mass spectrum of the N-demethyl metabolite) and by sample suppression of ionization. This latter observation was demonstrated by spiking the ODS eluate samples with 0.5 μ g of adinazolam and observing the response compared to adinazolam alone. Greater than a 100-fold reduction in signal was observed. Summary: 1. Using information obtained from the positive ion CAD mass spectrum obtained for MH^+ of adinazolam, FAB-MS-MS and GC-MS-MS analysis easily identified the major early formed metabolite of this drug in human urine. 2. GC-MS-MS analysis identified parent drug and two other minor metabolites. This particular example represents a special case in that drug metabolites, especially urinary, are usually not amenable to GC analysis without chemical derivatization. 3. The failure of FAB-MS-MS analysis of crude fractions eluted from an ODS extraction cartridge to identify parent drug and minor metabolites was related to both the presence of other compounds in the sample and the absence of any chromatographic separation from the major metabolite.

U-41123



8-chloro-1-[(dimethylamino)methyl]-6-phenyl-
4H-s-triazolo[4,3-a][1,4]benzodiazepine).

EFFECT OF PHTHALATE IMPURITIES ON PEAK AREAS DURING NEGATIVE ION METHANE GC/MS ANALYSES OF NABILONE AND DEUTERATED NABILONE IN HUMAN PLASMA SAMPLES. P. H. Dhahir, R. Barton, R. L. Wolen, and A. P. Breau, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

INTRODUCTION: Cesamet® (nabilone hydrochloride, Lilly) is a synthetic cannabinoid used in the treatment of nausea and vomiting secondary to oncologic chemotherapy. To facilitate the development and evaluation of oral formulations, we have utilized the technique of coadministration of a suspension of deuterated nabilone (D6) together with individual formulations of the unlabeled drug. The time related concentrations of both the labeled and unlabeled drug in human plasma are quantitated by negative ion GC/MS to permit the direct comparison of various formulations while minimizing individual subject variability.

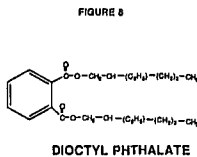
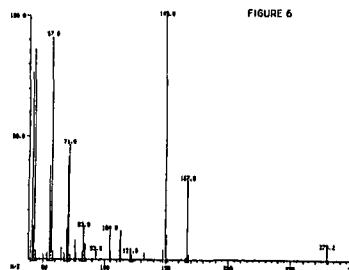
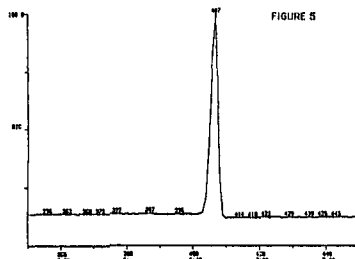
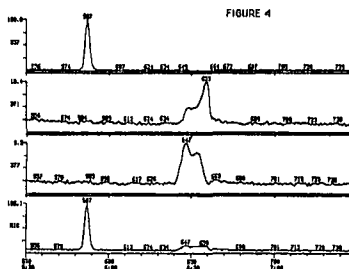
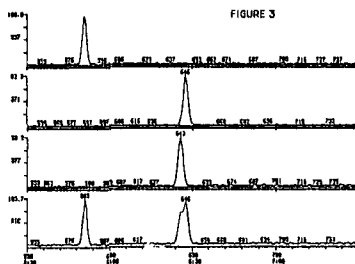
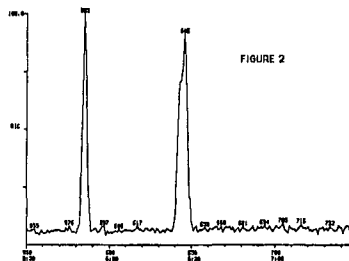
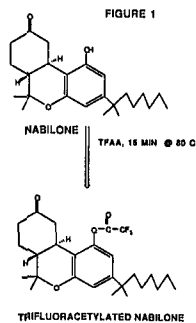
In the course of the analyses an abrupt loss of signal was observed in some samples. Full scan GC/MS analyses indicated that a co-eluting contaminant, dioctyl phthalate, was present in these samples which quenched the ionization of nabilone. This work demonstrates the ability of certain plasticizers to influence negative ion CI which can result in quantitation errors.

MATERIALS AND METHODS: Plasma Extraction--A fixed amount of internal standard was added to a 500 μ l plasma sample and the protein precipitated with acetonitrile. The supernatant liquid was recovered following centrifugation of the sample and the acetonitrile removed with nitrogen gas. The aqueous residue was made alkaline with sodium hydroxide solution extracted into butyl chloride which was washed with dilute hydrochloric acid. The organic extract containing nabilone and internal standard was dried under nitrogen, reconstituted in hexane and derivatized by the addition of trifluoroacetic anhydride (TFAA) (Figure 1). The derivatized extract was dried under nitrogen and reconstituted in hexane for analysis.

INSTRUMENTATION: The samples were analyzed in the spitless mode on a Finnigan 4500 gas chromatograph/mass spectrometer in the negative ion CI mode. Helium carrier gas head pressure was maintained at 10 psi. A 15 meter DB-1 capillary column (0.25 μ film thickness) was utilized with initial temperature at 150°C. The oven temperature was programmed at 100/min at 250°C. The derivatized internal standard eluted at 5.83 minutes and derivatized nabilone at 6.5 minutes (Figure 2). The methane reagent gas was maintained at 0.6 torr in a 150°C ion source. The filament was held at 70 eV with an emission current of 0.25 mA. The electron multiplier voltage was held at 1450 V. The nabilone and deuterated nabilone negative ions were monitored at m/z values of 371 and 377, respectively. The internal standard, an analog of nabilone, was monitored at $m/z=357$ (Figure 3). Monitoring rate was 0.3 seconds/scan.

RESULTS: The reconstructed ion chromatograms for most samples displayed normal gaussian shaped peaks (Figure 3) which were quantitated by AutoQuant. An occasional sample produced peaks of non-gaussian shape that abruptly terminated (Figure 4). Reanalysis of the aberrant samples by EI GC/MS revealed a co-eluting contaminant (Figures 5 & 6), not present in the unaffected samples (Figure 7). The co-eluting material was the common plasticizer dioctyl phthalate (Figure 8). The source of the contamination was traced to tubing that came in contact with the trifluoroacetic anhydride reagent.

DISCUSSION: The ability of the phthalate material to quench negative ion chemical ionization emphasizes the need for care in avoiding contact with plastics. Although the mechanism of this interesting observation has not been defined it is speculated that the phthalate structure may either charge exchange with the analyte or compete more favorably for the slow thermal electrons needed to produce a stable negative ion. The work demonstrated the need to complement single ion monitoring procedures with full scan spectra to insure the purity of observed peaks. The need for visual review of data is also emphasized by this finding in order that problems of this type can be identified and corrected without undue delay.



HYBRID MS/MS of AROMATIC DIAMIDINO ANALOGS

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Introduction: Aromatic diamidino compounds such as pentamidine have been shown to be effective chemotherapeutic agents in the control of *Pneumocystis carinii* pneumonia, complications of which are a leading cause of death in AIDS patients. A systematic investigation of structure/activity relationships of pentamidines included mass spectrometric characterization of reference materials, new synthetic mixtures, and mixtures of biological origins such as human urine and blood extracts. These studies required the development of MS/MS techniques to enhance specificity and provide structural information on novel compounds.

Experimental: Compounds were synthesized using proprietary techniques and verified by FT-NMR, FTIR, UV, and mass spectrometry. All mass spectra were acquired using a VG 70-250SEQ tandem hybrid MS/MS of EBQ geometry, operated either in the EI or liquid secondary ionization (LSIMS) mode, with the following conditions: EI: 200 °C source, 70eV I.P., $V_{acc} = 8$ kV, 200mA standard source configuration; resolution = 1000 (10% valley) or >25,000 (accurate mass confirmations); LSIMS: Cs ion gun at 30 kV, $V_{acc} = 8$ kV, 1000 resolution, glycerol matrix. Collision experiments were conducted using 2×10^{-4} mbar Ar (guage) in the rf-only collision cell (q). Parents were focused using MSI, with 50% main beam attenuation at CE = 30eV (lab reference). All source tuning conditions were held constant during MS/MS experiments, minimizing collision energy variation between experiments.

Results and Discussion: Ring-closed compounds proved to be amenable to EI MS/MS analysis and were characterized by hydride loss followed by ring cleavage to stable nitriles (fragmentations shown in Fig. 1.) Subsequent losses included fragments of amidine-phenols, sequential alkyl-chain cleavages, and numerous hydrogen rearrangements. For one lot of suspected low purity, the MS/MS spectra of sequentially selected parents allowed determination of a free amidino impurity in the presence of ring-closed propamidine, since no daughter scans produced a signal for the putative M^+ of the impurity and the impurity gave a MS/MS spectrum unlike that of the principal analyte. Ring-open diamidino compounds were thermally labile and highly dependent upon initial conditions, thus requiring LSIMS for production of molecular species with few structurally informative fragments, necessitating MS/MS (Fig. 2). The effect of collision energy on the appearance of amidino CAD spectra was investigated by selecting the $[MH]^+$ ion with MSI (EB) and scanning MSII (Q) at either a pre-determined CE or by collecting CAD spectra sequentially in 5 eV increments over the CE range 20-80 eV. The ion abundance recorded showed higher mass species favored at lower energies, with greater collection of lower mass collision products at higher CE (Fig. 3). This observation may reflect both the actual production of lower mass daughters in the collision experiment and the ion-energy acceptance range of the quadrupole analyzer, although optimization of MSII (Q) ion energy acceptance at any given CE did not significantly change the observed abundances. The use of LSIMS, MS/MS, and selection of optimum CE allowed the determination of small amounts of compound spiked into urine (Fig 4), by selection of a low S/N signal (m/z 313, MH^+) using MSI followed by CAD to yield an MS/MS spectrum highly correlated to that of an authentic reference standard. Application of the MS/MS techniques to qualitative and quantitative analysis of amidino compounds and metabolites is presently under investigation.

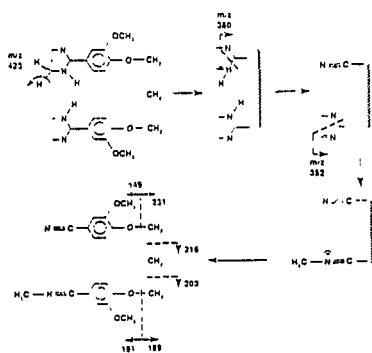


FIG. 1

Relationship of Fragment Intensity to CAD Collision Energy

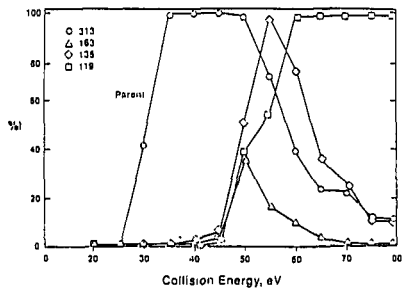


FIG. 3

FIG. 2

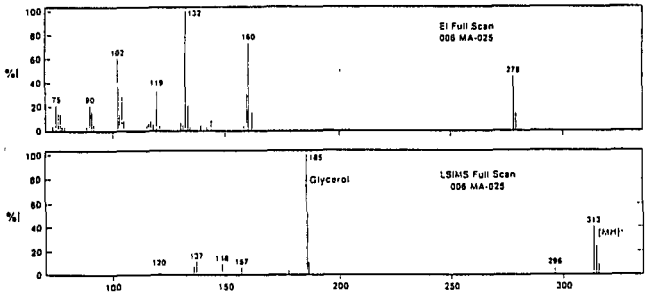
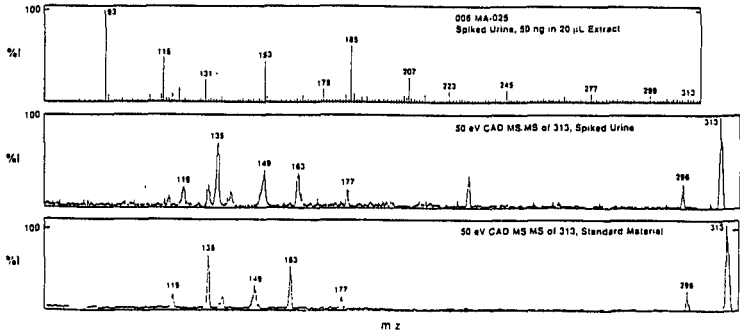


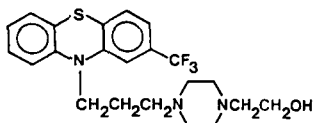
FIG. 4



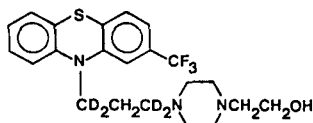
A SELECTED CAD-DAUGHTER ION RECORDING ASSAY FOR FLUPHENAZINE
AND A DEUTERIUM LABELLED ANALOG IN PLASMA

R.W. Edom, G. McKay, E.M. Hawes and K.K. Midha
College of Pharmacy, University of Saskatchewan, Canada S7N 0W0

Fluphenazine (FLU) is a piperazine-type phenothiazine drug widely used in the therapy of schizophrenia. In order to evaluate the single dose pharmacokinetics of this drug in patients undergoing chronic therapy, it was necessary to develop an assay with sufficient sensitivity and specificity to allow simultaneous quantitation of FLU and a stable isotopomer (d_4 -FLU) in plasma.



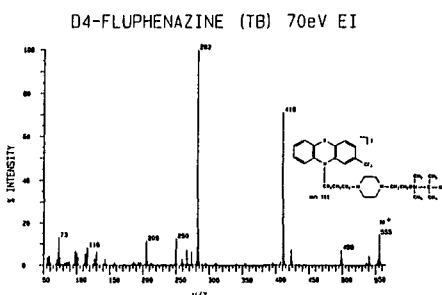
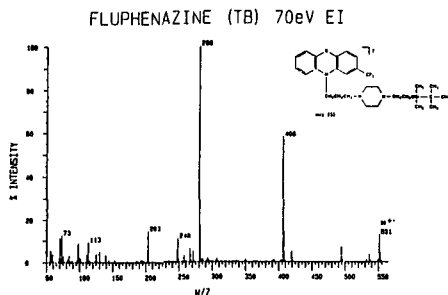
Fluphenazine



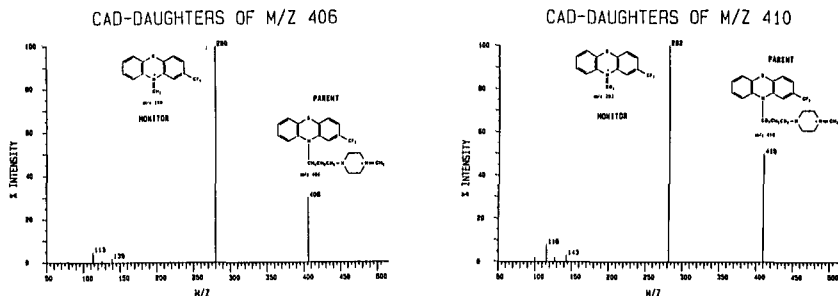
d_4 -Fluphenazine

The d_4 -FLU was synthesized in our laboratories (Sardesai et al, J. Labelled Compd. Radiopharm. 23: 317, 1986). The deuterium labels were strategically placed in the propyl portion of the side chain where it is currently believed little metabolism occurs. To investigate the isotopic effect of the deuterium substitution in d_4 -FLU, it was co-administered with equimolar doses of FLU via oral and IV routes to three purebred Beagle dogs. Plasma and urine samples were collected serially over 56 hours. The new assay procedure was developed and applied to this study.

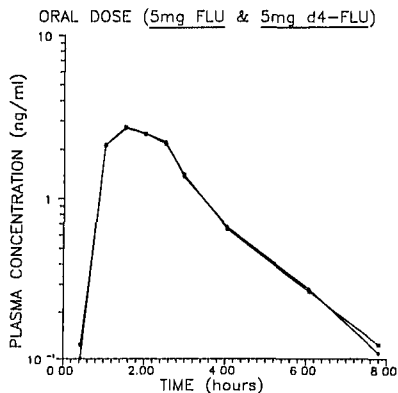
Quantitation of FLU is challenging because of the low plasma concentrations observed with therapeutic oral doses. In addition, GC-MS analysis is difficult due to its tendency to adhere to non-silanzed surfaces, and LC-MS cannot provide the required sensitivity. Therefore the assay was developed using quantitative EI-MS/MS on a tandem hybrid mass spectrometer (VG-70SQ). Selected ion recording was used to monitor daughter ions arising from specific parent ions in FLU and d_4 -FLU via collisionally activated dissociation (CAD). Samples were introduced on the direct probe with the selectivity of MS/MS obviating the need for chromatography. A solvent extraction using 5% isopropanol in n-pentane, and derivatization with MTBSTFA (producing the tert-butyl dimethyl silyl ether) was necessary to improve the sensitivity of the assay.



The parent ions for FLU and d₄-FLU (m/z 406 and 410 respectively) were chosen for their intensity and for their tendency to yield primarily one intense daughter ion with CAD. Shown below in the MS/MS spectrum for each parent ion are the daughter ions monitored for quantitation.



Using 2 mL plasma samples the assay can be applied through the range of 100 to 0.1 ng/mL. The recovery from plasma averages 50.7% for FLU and 50.4% for d₄-FLU. Replicate analysis of samples showed an average coefficient of variation $\leq 8\%$, and quality control samples showed an average accuracy of $\geq 94\%$.



Above is the plasma concentration vs. time profile for a dog given a single oral dose containing equimolar amounts of FLU and d₄-FLU (≈ 5 mg each). Also typical of the IV doses, the profiles for each isotopomer are virtually coincident. In this example, the half-life is 1.44 hours for FLU and 1.54 hours for d₄-FLU. AUC values are also very similar: 7.644 for FLU and 7.654 for d₄-FLU. Each isotopomer had identical values for T_{max} (1.53 hours) and C_{max} (2.70 ng/mL). We conclude this evidence demonstrates the lack of measurable isotopic effect caused by the deuterium substitution in d₄-FLU.

Supported by the Medical Research Council of Canada (P.G.34).

PURIFICATION OF INHIBITORS OF OUABAIN BINDING FROM BOVINE
POSTERIOR PITUITARIES USING HIGH PRESSURE LIQUID
CHROMATOGRAPHY AND MASS SPECTROMETRY.

P.S. McQuade^{1,2}, F.S. LaBella² and K.G. Standing¹

Departments of Physics¹ and Pharmacology and Therapeutics²,
University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

In all cells maintenance of sodium and potassium concentrations within the cell involves $\text{Na}^+\text{-K}^+$ ATPases (Stahl, 1986). Since these ions are so vital to the processes within the cell, many endogenous modulators have been proposed to regulate $\text{Na}^+\text{-K}^+$ ATPase function. A convenient means of detecting this modulation involves monitoring ouabain binding.

The previous use of high mass mass spectrometric techniques has focussed on confirmation of the sequence of various bulk isolated proteins. To date fewer examples of the use of these techniques to identify various minor neuropeptides are extant. In one example the authors confirmed the existence of Substance P in a sample isolated from the cat spinal cord (Lisek et al., 1989). In a second example, this technique was used to identify various proopiomelanocortin peptides in the bovine intermediate-posterior pituitary (Feistner et al., 1988). Finally, a previously unknown endogenous neuropeptide modulating calcium channels was further characterized by mass spectrometry (Callewaert et al., 1989).

We employed the high pressure liquid chromatograph to isolate various compounds from the bovine posterior pituitary, characterized them using ouabain binding and further identified them using mass spectrometry. The mass spectrometer thus confirmed the presence of a distinct material, clarified the purity of the isolated material, aided in structural identification and identified any inorganic ions which may also be present.

Experimental

Freeze dried bovine posterior pituitaries were extracted with acetone; defatted using petroleum ether and the peptides were dissolved using 0.2 M acetic acid. This extract was chromatographed on a Sephadex G-25 column and that fraction corresponding to material of from 1,000 to 5,000 atomic mass units (a.m.u.) was further chromatographed on a Bio Rad AG-50 W column to remove sodium and potassium. The peaks of material from both columns were determined using the Lowry protein assay. Following removal of the salts, the material was assayed for ouabain binding inhibitory activity. Those fractions with activity were pooled and further purified using a Waters high pressure liquid chromatography system. Fifty-seven milligrams of extract were chromatographed on a μ Bondapak reversed phase column using a linear gradient of acetonitrile (from 0 to 100% over 60 minutes). The initial peak had the greatest activity and also the greatest amount of material (32.8 mg). Using a shallower acetonitrile gradient (0 to 100% acetonitrile over 120 minutes) the initial sample was resolved into 5 regions of ouabain inhibiting activity, with the initial peak weighing 18 mg compared to 0.4 mg for peak 2, 4.9 mg for peak 3, 4.0 mg for peak 4 and 4.2 mg for peak 5. The initial peak reduced ouabain binding to 65% of control. This material also had potency in an in vitro potency test. The tension on a canine heart muscle preparation was reduced by 200 nanograms of this material. Using nitrocellulose, this initial peak was examined using both plasma desorption (courtesy of Dr. P. Roepstorff) and cesium ion desorption. In both cases a major peak at 1085 a.m.u. was observed. This peak was identified as arginine vasopressin (Feistner et al., 1988). Arginine vasopressin failed to have activity in the ouabain binding assay. A second smaller peak of mass 401 a.m.u. was also observed.

The mixture was resolved further using an analytical μ Bondapak column and a very shallow solvent gradient (0 - 20% acetonitrile). The arginine vasopressin was separated from several other peaks. A late peak had activity over several fractions while a very sharp division between

activity and lack of it was observed in fractions 77 and 78. Fraction 77 had only 40% of the control activity in the ouabain binding assay. Upon examination in the time-of-flight mass spectrometer (Tang et al., 1988) fraction 77 had a strong mass at 391 a.m.u. and a smaller one at 550 a.m.u. (Figure 1). Fraction 74 also was examined; this fraction did not have those masses observed in Fraction 77. This pattern was very similar to that observed for leucine enkephalin (Gaskell et al., 1989). Another sample appearing in fraction 70 (i.e. 70 minutes into the HPLC run) appeared to have two larger masses associated with activity i.e. 1473.5 a.m.u. and 999.5 a.m.u. These masses were not observed in those fractions not having activity.

Conclusions

- 1) Mass spectrometry identified a neuropeptide as having activity
- 2) Confirmed that activity was not due to ion contamination
- 3) Provided some structure and identity information
- 4) Confirmed purity
- 5) These results suggest that within the posterior pituitary there exists a family of related peptides with modulatory properties toward Na^+ , K^+ ATPase and sodium-potassium homeostasis.

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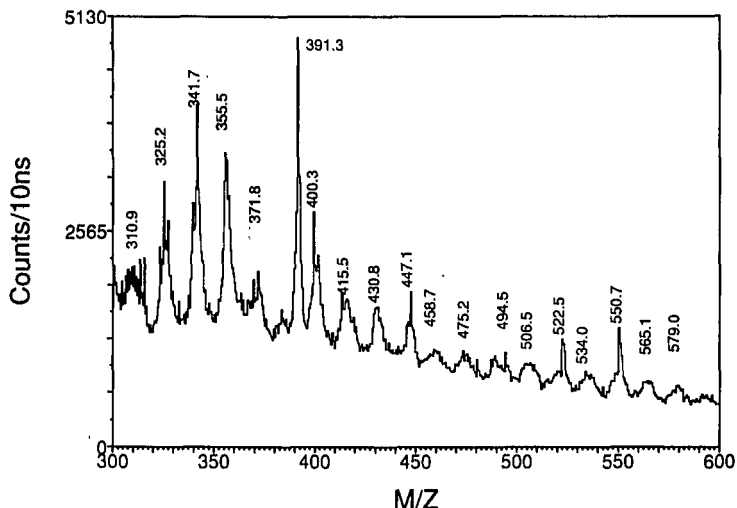
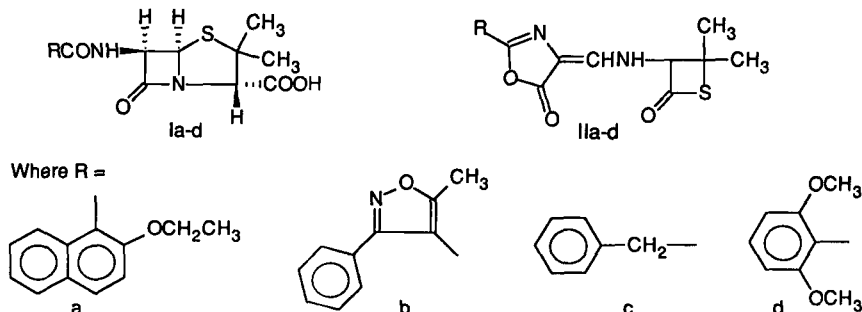


Fig. 1: Fraction 77

THE STRUCTURE ELUCIDATION AND MASS SPECTROMETRY OF PENICILLINIC ACID THIOLACTONES

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The degradation pathways and products of penicillins in various aqueous media are studied by the pharmaceutical industry to determine product potency, shelf-life, and safety. Recent investigations of the degradation of nafcillin (**1a**) and oxacillin (**1b**) in aqueous solutions revealed one minor component of unknown identity for each drug. The unknown components were isolated in small quantities, and their structures elucidated by means of infra-red (IR), nuclear magnetic resonance (NMR), and mass (MS) spectrometries.



Initial IR, ^1H -NMR, and MS results on the oxacillin and nafcillin degradants revealed that the spectra did not match the spectra of any previously known aqueous degradants. However, these techniques gave structural information which was used to postulate possible structures for the degradants. For example, the C=O stretch of the β -lactone, normally found in the IR spectrum at 1763 cm^{-1} for the sodium salt of nafcillin or at 1789 cm^{-1} for the free acid, was missing in the IR spectrum of the nafcillin degradant. Also missing were the characteristic absorbances for the free or cationated carboxylic acid of nafcillin. However, absorbances consistent with the structure of a penicillenic acid derivative (e. g., δ -lactone C=O stretch at 1732 cm^{-1}) were present.

The ^1H -NMR spectra indicated that the R-groups from both the nafcillin and oxacillin (the ethoxy-substituted naphthalene and the phenyl-substituted isoxazole, respectively) were present in the corresponding degradants. The proton spectra for both degradants were similar when the specific resonances for the naphthalene and isoxazole groups were removed. These data suggested that identification of one degradant would provide the structure of the other degradant.

Electron ionization (70 eV) mass spectrometric analyses of the unknown components confirmed the similarity between the two structures and provided further structural information. Potential molecular ions were found at m/z 383 and 396 for the degradants of oxacillin and nafcillin respectively, corresponding to 18 amu less than the molecular weights of the parent penicillin free acids. Apparent losses from the molecular ion of 28, 55, 60, and 88 daltons were also observed in the spectra of both degradants. Accurate mass fast-atom bombardment mass spectrometry provided an elemental composition of $\text{C}_{19}\text{H}_{18}\text{N}_3\text{O}_4\text{S}$ for the protonated molecular ion of the oxacillin degradant at m/z 384. This experiment was followed by further electron impact studies which provided the elemental compositions of the molecular ion of the nafcillin degradant ($\text{C}_{21}\text{H}_{20}\text{N}_2\text{O}_4\text{S}$) and all major fragment ions in the spectra of both degradants. Thus, the elemental compositions of the unknowns were determined to be the same as those of dehydrated (one water molecule) forms of the corresponding parent penicillins. The losses of 28 and 60 were found to be losses of CO and COS, respectively, indicating the presence of a thioester.

Although substantial structural information was available regarding specific moieties, subgroup connectivity remained unknown. ^{13}C NMR experiments provided the final information needed to postulate structures for the two unknowns. Unambiguous determination of proton

To further confirm the proposed structures, the thiolactones (IIb,c) of the penicillenic acids of oxacillin (Ib) and Penicillin-G (Ic) were synthesized and characterized by mass, NMR, and IR spectrometries. The synthesis consisted of converting the parent penicillin to the penicillenic acid,¹ followed by dehydration of the penicillenic acid with DCC (1,3-dicyclohexylcarbodiimide). The nafcillin penicillenic acid was not synthesized, but enough was isolated from degradation experiments to allow small crystals to be grown. X-ray crystallographic analysis of a selected crystal provided the definitive structure proof of the penicillenic acid thiolactone structure. Although a literature search at the time of structure elucidation did not yield any references to penicillenic acid thiolactones as known penicillin degradants, Fujii et al. have since reported the corresponding penicillenic acid thiolactone (IIa) as a degradant of methicillin (Id).²

intermediate structure is especially interesting, and is under further investigation.



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²Fujii R · Mizushima N · Kubota A · Ohuchi F · Machida Y · Nagai T. 1988. *Yakuzaiigaku*

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MASS SPECTROMETRY OF MITOMYCIN C AND SELECTED ANALOGS

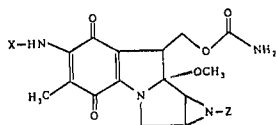
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Mitomycin C (MMC) is of clinical interest as a potent anti-cancer alkylating agent (1). The mass spectral behavior of MMC and several of its analogs were studied. Using MMC as a model (2,3) analogs substituted at the 7-amino and/or 1a- positions were examined to determine the role of these side chain substituents on fragmentation. Another set of analogs with potential clinical utility as anti-tumor agents are the nucleoside and nucleotide adducts of MMC (4). These were examined using FAB-MS with the objective of applying this fragmentation information to the study of other MMC analogs as well as MMC-DNA adducts.

Work done with MMC using various ionization methods such as EI, CI, DCI, and FAB have confirmed the earlier EI results of Van Lear and Hodges(2,3): the major fragmentation pathways involve the mitosene moiety with the most abundant ions arising from loss of the carbamate and/or 9a-methoxy side chain. Metastable studies using EI and CI have shown new fragmentation pathways previously not reported. For example, ions from the decomposition of m/z 334 include m/z 291, 261, and 242, in addition to the m/z 302 and 273 peaks reported by Van Lear (2). Although there was some variation in the EI fragmentation exhibited by the 7-amino and 1a- substituted analogs of MMC, the basic pathways shown by MMC are observed. All the analogs showed a molecular ion in EI. A difference generally noted with substitution at the 7- position is the significant increase in the relative intensity of M^+ (with the exception of the propargyl derivative 4) with respect to MMC and 2 (see Table). Other differences observed in the spectra of the 7-amino analogs include an increase in the relative intensity of the M^+-HCNO ion (except for 4) and a decrease in the relative intensity of ions a and b. The base peaks with EI generally involve loss of the 7-amino side chain. In fact, the 7-aminofurfuryl side chain is the base peak (m/z 81) for 5. The FAB-MS (+) spectra of MMC and 8 gave good results using 3-nitrobenzyl alcohol (NBA) as the matrix (less sample-related peaks were observed using glycerol). Intense molecular ion-related peaks are observed, as well as some structurally significant fragmentation. This is of particular use in identifying metabolites of MMC in addition to the more polar, less volatile analogs.

Preliminary work has been done on MMC-nucleoside and nucleotide adducts. This includes FAB-MS (+) of the adduct of MMC and 6-mercaptapurine riboside, an important anti-tumor agent. Ions are observed which represent both the intact adduct (MH^+ and MH -carbamate) as well as fragmentation arising from both components. For example, $B+2H$ at m/z 153 from the nucleoside portion and ions at m/z 302 and 242 from the mitosene portion are seen. Also, the positive and negative ion (Fig. 1) FAB-MS has been done on MMC-uridine-5'-monophosphate (MMC-UMP). The negative ion spectra provides useful molecular weight information ($M-H^-$), while the positive ion spectra yields much more structurally significant information, such as mitosene+H, mitosene - carbamate, and UMP+H. Examples of MMC adducts in which the anti-cancer nucleotides 5-fluorouridine-2'-deoxy-5'-monophosphate (FdUMP) and arabinosyl cytosine-5'-monophosphate (araCMP) are complexed with MMC have also been done. Work is in progress on elucidating the MS behavior of the MMC-tide and side adducts, including metastable and high resolution studies.

MMC Analogs



Cmpd	X	Z	MW
1	H	H	334 (MMC)
2	H	CH ₃	348
3	C ₂ H ₅	CH ₃	388
4	CH ₃ C≡CH	CH ₃	386
5	CH ₃ C ₆ H ₅ O	CH ₃	428
6	CH ₃ C ₆ H ₅ O	H	418
7	CH ₃ C ₆ H ₅ O	CH ₃	432
8	CH ₃ CH ₂ OCH ₃	H	392

CHARACTERISTIC IONS OF CMPDS 1-8 (% RI)

Assignment	1	2	3	4	5	6	7	8
M ⁺	334 (2)	348 (5)	388 (56)	386 (1)	428 (53)	418 (26)	432 (27)	392 (2)
M-HCNO	291 (25)	305 (1)	345 (11)	---	385 (4)	375 (38)	389 (13)	349 (10)
M-H ₂ NCO ₂ (a)	273 (60)	287 (97)	327 (7)	325 (1)	367 (11)	357 (63)	371 (68)	331 (17)
a-OCH ₃ (b)	242 (100)	256 (83)	296 (21)	294 (39)	336 (45)	326 (58)	340 (39)	300 (73)
OTHER		272 (100) (a CH ₃)	268 (53) (b C ₂ H ₅)	293 (98) (b H)	81 (100) (c ₁₀ H ₉ O)	242 (100) (b C ₆ H ₅ O)	268 (100) (b C ₆ H ₅ O)	254 (76) (b ⁺ H C ₁₀ H ₉ O)
			70 (100) (c ₁₀ H ₉ O)	272 (100) (a HCNO CH ₃ OH C ₁₀ H ₉)			256 (93) (b C ₆ H ₅ O)	

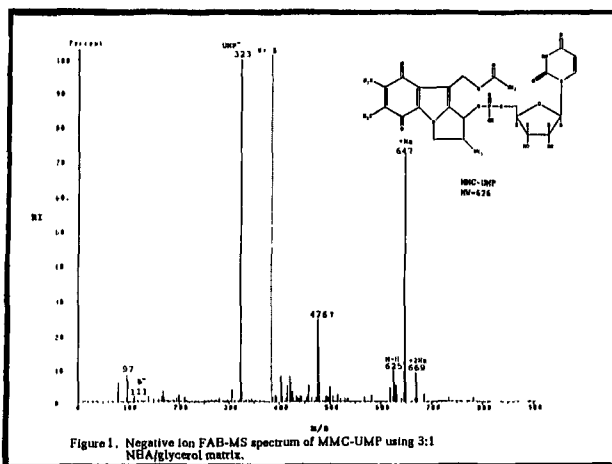


Figure 1. Negative ion FAB-MS spectrum of MMC-UMP using 3:1 NEA/glycerol matrix.

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ACKNOWLEDGMENTS

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MASS SPECTROMETRY IN PREHISTORIC RESEARCH

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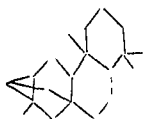
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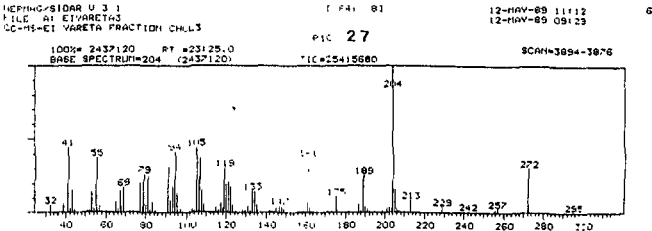
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Analyses were performed using NERMAG Sidar mass spectrometer. We used GC/MS EI+ and we obtained 40 peaks in the spectra of chloroform extract of the plant "yareta". Our work is a demonstration of computer assisted structure determination from 10 µg of sample. We identified 7 long chain aliphatic acids, 6 hydrocarbon, 2 aromatic substances, 6 terpenes and 2 terpenes like products.



TRACHYLOBANE



ANALYSIS OF BLOODROOT EXTRACT USING TLC/SIMS,
HIGH RESOLUTION MS, AND MS/MS

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The benzophenanthradine alkaloids (see structure 1) are a pharmacologically important class of natural products, with nematocidal, antitumor, and cytotoxic activity. The extract of the bloodroot plant contains several of these alkaloids, and this extract has been used in various medications for over 100 years. The extract is a crude mixture of alkaloids, as seen in the glycerol FAB spectrum (Figure 1). The diversity of the benzophenanthradine alkaloids is indicated in Table 1, which tabulates the number of candidate structures corresponding to each of the prominent ions in the FAB mass spectrum. The purpose of the present work was to employ a combination of mass spectrometric techniques, including TLC/SIMS, high resolution mass spectrometry, and tandem mass spectrometry, to identify some of the components of a crude plant extract, and resolve uncertainties about the composition of the extract. By utilizing the complementary information provided by the different mass spectrometric techniques, the amount of sample manipulation could be kept to a minimum.

Twenty eight grams of bloodroot rhizomes were dried, pulverized, and extracted with chloroform. The extract, (dry weight 0.46 grams), was diluted into 5 ml of chloroform, and 5 μ l aliquots were used in the ensuing analyses, without further purification. TLC/SIMS analysis was performed on a custom-built instrument using cesium ions at 6 kV and a quadrupole mass analyzer. High resolution work was done on a ZAB-HF instrument, operating with a resolution of 12,000 at 8 kV. Tandem mass analysis was performed on a Finnigan TSQ-70, using argon as the collision gas and a collision energy of 30 eV.

A discrepancy was noted in a literature survey of previous work on bloodroot extract. A crystal structure study (2) found the primary component in the extract to be oxysanguinarine, (molecular weight of 348), whereas an HPLC analysis (3), determined sanguinarine to be the most abundant. The latter study identified six components in the extract, only one of which, chelerythrine, had a molecular weight on 348.

Using the above-mentioned techniques, we determined the material at m/z 332 to be sanguinarine, based on R_f in TLC, mass spectrum from TLC, tandem mass spectrometry (Figure 2), and exact mass measurement. Two components were found to be present at nominal mass 348, in a 10:1 ratio. Based on exact mass measurements (Figure 3), these components were tentatively identified as oxysanguinarine and chelerythrine. Certified, purified standards for these materials were not available for comparison of response factors in TLC. Identification of the higher mass components in the extract was similarly hindered by the absence of standards. However, exact mass measurement and MS/MS reduce the possibilities to positional isomers. This information can rapidly be collected on the components of a complex mixture, without any sample purification, owing to the molecular specificity of mass spectrometry and the complementary nature of the various techniques.

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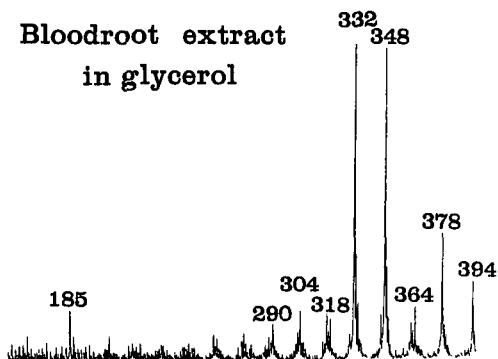


Figure 1

Tandem MS

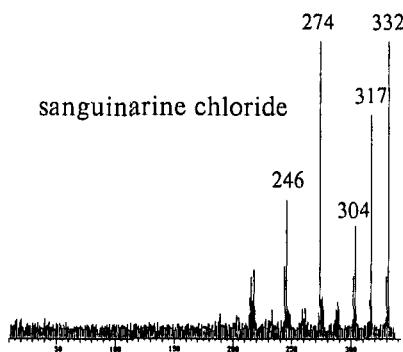
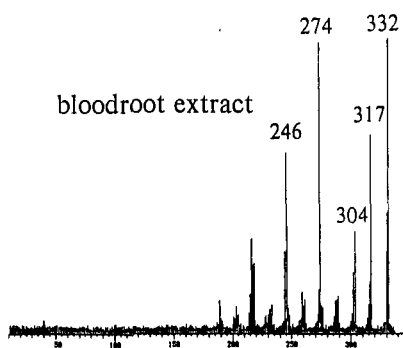
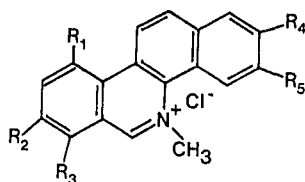


Figure 2



$R_1 = \text{H or OCH}_3$
 $R_2 + R_3 \text{ and } R_4 + R_5 = \text{OCH}_2\text{O}$
or $R_2, R_3, R_4, \text{ and } R_5 = \text{OCH}_3$

Structure 1

Prominent Analyte Ions

m/z	benzophenanthridine alkaloids	<u>Paper.</u> <u>Sang.</u>	<u>Paper.</u> <u>Sang.</u>
332	2	1	1
348	5	2	2
364	5	0	3
378	4	2	4
394	3	1	2

Table 1
from reference (1)

High-resolution MS

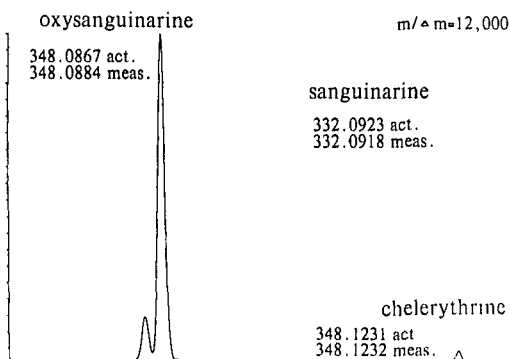


Figure 3

DERIVATIZATION OF PUTATIVE BRADYKININ
METABOLITES FOR GC-MS ANALYSIS

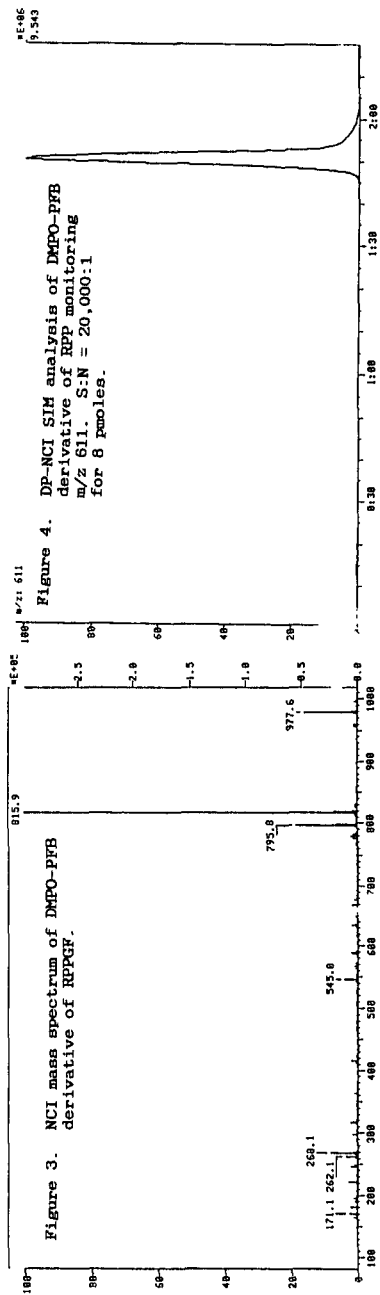
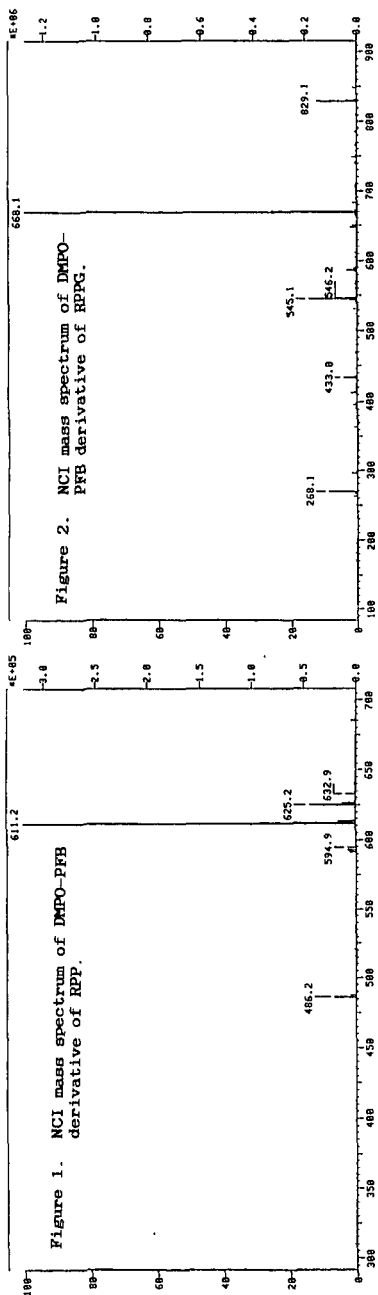
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The long-term objective of this research project is to identify and quantify metabolites of bradykinin (BK; RPPGFSPFR) in human urine and/or plasma. Metabolic peptide fragments that have been reported to be present in plasma following I.V. administration of BK include RPP, RPPG and RPPGF. We have attempted to develop GC-MS procedures to quantitate these three peptides. One problem with this approach (GC-MS) is the poor yields observed for many of the derivatization reactions used to form products amenable to GC analysis. This is especially true for amounts totaling approximately 10 nmol or less. For this reason we chose to 1) examine derivatives amenable to negative ion chemical ionization (NCI) and 2) synthesize stable isotope labeled internal standards. Peptides were converted to dimethylpyrimidylornithine pentafluorobenzyl (DMPO-PFB) derivatives by first condensing with 2,4-pentanedione followed by reaction with pentafluorobenzyl bromide (PFBB).

Samples were dried under nitrogen at R.T., 5 μ l H₂O, 10 μ l ethanol, 5 μ l triethylamine and 10 μ l 2,4-pentanedione added and heated at 100 °C for one hour. Following drying under N₂, 25 μ l of 1 M acetic acid was added and the samples were heated at 100 °C for 10 min. Samples were extracted 2 times with approximately 1 ml. of ether and dried as above. Pentafluorobenzyl derivatives were formed by reaction with PFBB for 30 min. at R.T. (30 μ l CH₃CN, 20 μ l 20% PFBB in CH₃CN and 10 μ l diisopropylethylamine in CH₃CN). Reagents were removed as above and samples were dissolved in toluene.

The RPP-, RPPG- and RPPGF-DMPO-PFB derivatives were observed to be amenable to GC (methyl silicone bonded phase capillary, 5 m) although RPPGF-DMPO-PFB eluted at relatively high temperatures (> 310 deg C) and the approximate relative responses (GC-NCI-MS) of RPPGF:RPPG:RPP were 1:10:100. NCI mass spectra all exhibited M⁻ - 181 (M⁻ - PFB) as the base peak (m/z 611, 668 and 815 respectively, Figures 1-3). The decreased response for RPPG and RPPGF could be related to thermal decomposition in the injection liner. For this reason direct probe -NCI (DP-NCI) was also evaluated and proved satisfactory since equal amounts of each peptide derivative gave a similar response. Using selected ion monitoring (SIM) GC-NCI analysis of the RPP derivative gave a S:N of 10,000:1 for 40 pmole (2.7 nmoles derivatized) and DP-NCI analysis gave a S:N of 20,000:1 for 8 pmole (2.7 nmoles derivatized, Figure 4). We also synthesized ¹⁸O₂-internal standards by exchange in acidic H₂¹⁸O (100 °C, 18 hr.). Preliminary data obtained using this derivative and an ¹⁸O-labeled internal standard suggested the presence of RPP in human urine, however, we now believe this observation to be at least in part an artifact of the purification procedure.



APPLICATION OF AUTOMATED GC/MS/MS FOR THE QUANTITATIVE DETERMINATION OF NE-11740 IN PLASMA

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INTRODUCTION

The primary objectives of this work were: 1) to develop a sensitive and selective assay for NE-11740 in plasma; and 2) to evaluate the utility of GC/MS/MS for the routine trace-level assay of drugs in biological matrices. The work described here goes beyond initial method development and validation studies by demonstrating the high efficiency and reliability of quantitative GC/MS/MS methodology over 22 months of routine application.

NE-11740, 1-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-5-hexyn-1-one, is being evaluated for anti-inflammatory/anti-rheumatic indications. In support of investigatory pharmacokinetic studies, an automated GC/MS/MS method for the determination of ppb-level NE-11740 in plasma was developed. The need for rapid turnaround for thousands of samples placed stringent requirements on this methodology. In addition to high sensitivity and selectivity, short analysis time and high reliability were also vital.

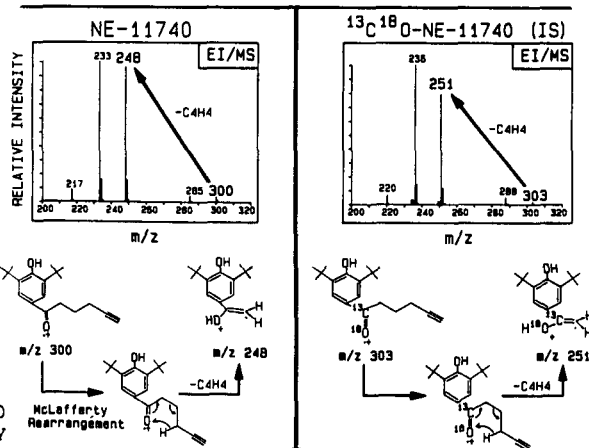
EXPERIMENTAL

Plasma Sample Preparation. Plasma samples (0.1 to 2 g) were spiked with 50 ng $^{13}\text{C}_{18}$ -labeled NE-11740. Proteins were precipitated by addition of acetonitrile and the supernatant extracted with ethyl ether. The organic layer was taken to dryness under nitrogen and reconstituted in 100 μl of 4-decanone. Typically, 2 μl splitless injections were employed.

Automated GC/MS/MS Approach. Analyses were conducted on a Finnigan TSO-45 using automated GC/(EI)MS/MS with collisionally activated dissociation (CAD) and selected reaction monitoring (SRM). Below are shown EI mass spectra for NE-11740 (MW= 300) and the $^{13}\text{C}_{18}$ -NE-11740 internal standard (MW= 303), along with corresponding ion reactions that were used in the MS/MS SRM detection scheme. Daughter ions were formed using CAD with an 11.7 eV ion collision energy and a collision gas pressure of 2.2 mtorr Ar.

The GC/MS/MS analysis was automated via Macro programs that allowed computer control of all assay steps, from sample injection via the Varian 8080 autosampler, to final report printout. Cycle time was only 6-7 min. By running the GC/MS/MS assay in an unattended manner, 70 real samples were typically assayed in a single day.

The selectivity of the MS/MS detection scheme employed here was such that the only GC eluents detected from plasma extracts were NE-11740 and $^{13}\text{C}_{18}$ -NE-11740. This "ultimate" selectivity redefined the role of the GC as simply a reproducible means of sample introduction. Thus, a combination of short column (< 15 m), high initial temperature (180°C), and rapid temperature ramp (25°C/min), minimized GC retention time (1.5 min).



By eliminating interferences, this MS/MS methodology provided excellent low-level detectability for NE-11740, with minimal sample preparation and short analysis time. The quantitation limit ($S/N > 10:1$) was typically 1 ng/sample (20 pg on column). The SRM (m/z 300-to-248) chromatogram for an extract of a 2 ml plasma sample containing 1.4 ng NE-11740 (right) shows a strong signal over a background consisting only of electronic noise.

RESULTS

Initial Validation. High sensitivity of the GC/MS/MS detection scheme, combined with the use of an M+3 stable-isotope-labeled internal standard resulted in a 1-to-3000 ng/sample (20 pg to 60 ng on column) linear dynamic range. A typical standard analytical curve (right) shows the linearity of this method, with correlation coefficients of > 0.999 for both the entire range and a 1-to-50 ng/sample subset, which also have nearly equal slopes.

Accuracy and precision of the overall method was determined through assay of NE-11740-spiked 1 ml plasma aliquots. Results are summarized below.

N	ng SPIKED	ng DETERMINED	% RECOVERY
4	2.00	2.08 $\pm$ 0.20	104 $\pm$ 9.6
12	20.0	20.8 $\pm$ 1.7	104 $\pm$ 8.1
19	100.	98.7 $\pm$ 6.4	99 $\pm$ 6.5
11	200.	205. $\pm$ 14	103 $\pm$ 6.9
5	2000	1980 $\pm$ 130	99 $\pm$ 6.7

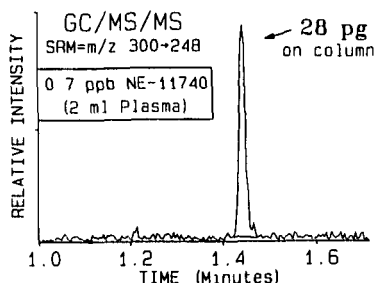
Long-Term Performance. Method accuracy was continually assessed by analyzing NE-11740-spiked plasma samples. Data accumulated over 22 months for 20 to 270 ng NE-11740-spiked plasmas ($N = 215$) showed a 100.05 ± 5.24 % analytical recovery, indicating no bias in the method.

A measure of method precision was based on duplicate (parallel) assays of real plasma samples (right). Duplicates were analyzed on the same day, but with 2-6 hr between GC/MS/MS runs. From these data, an overall 6.2 % ($N = 350$) method relative standard deviation was calculated.

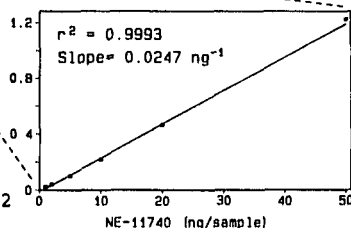
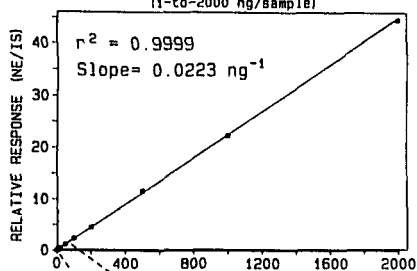
SCOPE OF APPLICATIONS/CONCLUSIONS

A 1-to-3000 ng/sample working range for 0.1 to 2 g samples allowed access to an effective 0.5-to-30,000 ppb NE-11740 range in plasma. This, along with method automation, facilitated its application to > 3000 real plasma samples (5000 GC/MS/MS runs). The method was equally applicable to mouse, rat, dog, monkey, and human (clinical) plasma. With minor variations in sample work-up, it was also applied to the assay of NE-11740 in urine.

We have found this GC/MS/MS assay to be very forgiving of instrumentation wear (column degradation, dirty ion volumes, etc.). This is due largely to its high selectivity and use of a stable-isotope-labeled internal standard.



TYPICAL STANDARD ANALYTICAL CURVE
(1-to-2000 ng/sample)



DUPLICATE ASSAYS

N	NE-11740 RANGE	AV. DEV. FROM MEAN
36	1.0 to 9.9 ng/g	4.9 %
82	10.0 to 99.9	3.0 %
198	100. to 999.	4.7 %
34	1000 to 3000	2.0 %

**CHARACTERIZATION OF DERIVATIVES OF CHIRAL ANTIARRHYTHMIC DRUGS
AND THEIR METABOLITES USING FAB-MS**

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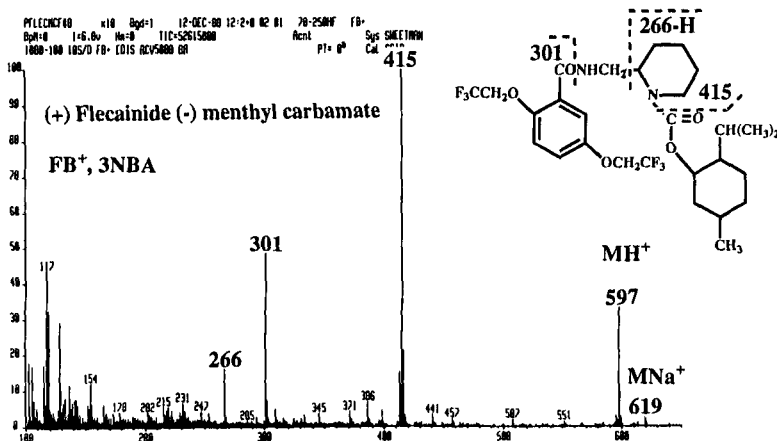
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Optimal drug therapy for conditions associated with disturbances of cardiac rhythm is often complex and requires detailed knowledge of the pharmacology of the drugs used. Many antiarrhythmic drugs are chiral molecules, normally given as racemic mixtures, where each enantiomer may show its own spectrum of pharmacological activities. Therefore, rational drug therapy would be assisted greatly by preparation and pharmacological testing of individual enantiomers. There is also a need for methodology to easily detect enantioselective drug metabolism by resolving enantiomers of drugs and their metabolites which have been isolated from biological fluids or tissues. In this regard we have prepared the (-) menthyl chloroformate derivatives of propranolol, propafenone, encainide, flecainide and several of their pharmacologically-active metabolites and resolved the resultant diastereomers using HPLC (C_{18} reversed phase column methanol containing 15-20% water as solvent system.) The products were characterized using positive ion FAB-MS with 3-nitrobenzyl alcohol (3-NBA) as matrix since electron impact ionization could not be used to verify molecular weight because of the lability of these molecules. By contrast, the FAB spectra each showed intense peaks due to MH^+ and $(MH^+ - \text{menthyloxycarbonyl})$ together with several structurally-significant fragment ions. We have also examined the spectra of other diastereomeric derivatives of propafenone such as propafenone glucuronide, a major circulating metabolite of propafenone, which was synthesized via its triacetyl methyl ester derivative. HPLC was carried out one of two separate systems. System A used an ultrasphere ($5\mu m$, 4.6mm X 250mm, Altex) silica column at ambient temperature and a mobile phase of ethyl acetate/hexane/triethylamine (15:85:1, v/v) at a flow rate of 1 ml/min. System B and a C_{18} reversed phase column with methanol containing 15-20% water as elution solvent.

Conversion of chiral drugs to their menthyl carbamate derivatives was effected as follows: The sample containing drug (10ng to $5\mu g$) was dissolved in acetonitrile containing N,N'-diisopropylethylamine ($50\mu l$, 90:10v/v) and to this mixture was added (-) menthyl chloroformate in acetonitrile ($50\mu l$, 10:90v/v). The solution was heated for 2 hr. at 60° and the acetonitrile was evaporated. The residue was dissolved in the initial mobile phase prior to analysis by HPLC.

FAB-MS spectra were obtained using a VG 70-250 HF instrument equipped with a standard unheated VG FAB ion source and a standard saddle field gun (Ion-Tech Model B11N) producing a beam of xenon atoms at 8 keV and 1mA. The mass spectrometer was adjusted to a resolving power of 2500 and spectra were obtained at 6 kV and at a scan speed of 10 sec/decade. 3-NBA or glycerol was applied to the stainless steel stage via a glass rod and was spread to a thin continuous layer. Compound dissolved in an appropriate solvent at a concentration of $1-5\mu g/\mu l$ and $1.5\mu l$ was applied to the center of the stage.

The FAB⁺ spectra of the menthylcarbamate derivatives of chiral, antiarrhythmic drugs using 3-NBA as matrix are typified by the spectrum of (+) flecainide (-) menthyl carbamate shown below which shows an intense peak for MH^+ together with several structurally significant fragment ions.



In summary, conversion of chiral, antiarrhythmic drugs and their metabolites containing a phenolic or secondary amine function to their menthyl carbamate derivatives using (-) menthyl chloroformate and analysis of the resultant mixture of diastereomeric derivatives using reversed phase HPLC with methanol containing 15-20% water was solvent system provides an effective means for evaluating the possibility of enantioselective drug metabolism. These derivatives have been prepared for the chiral drugs encainide, propranolol, propafenone and flecainide. This methodology has been used to demonstrate the enantioselective metabolism for each of these drugs. Derivatization of encainide is interesting in that it was observed that (-) menthyl chloroformate reacts with the tertiary (piperidine) nitrogen to give two menthyl carbamate diastereomers which could be resolved easily on normal phase HPLC on a silica column. Positive ion FAB-MS using 3-nitrobenzyl alcohol as matrix provides an excellent means for the characterization of the menthyl carbamate derivatives formed. For each derivative an intense peak corresponding to MH^+ is observed together with a peak arising by loss of the carboxy-menthyl group and other fragment ion information. Negative ion FAB-MS provides a useful means for the characterization of glucuronide conjugates of drugs. This was illustrated using the glucuronide of propafenone. Choice of FAB matrix is quite critical. Despite the fact that the free glucuronide is freely-soluble in either glycerol or 3-nitrobenzyl alcohol, vastly superior spectra of this and other free glucuronides are obtained using glycerol as matrix. However, when the glucuronide is converted to the less polar per-acetyl methyl ester derivative, superior results are obtained using 3-nitrobenzyl alcohol as matrix. These observations extend to other poly-hydroxylic compounds such as oligosaccharides and phosphatidyl inositols. The utility of this methodology has been demonstrated by studying the metabolism of (+) encainide using rat liver microsomes where it was observed that (-) encainide underwent O-demethylation at a greater rate than (+) encainide. No such enantioselectivity was observed for N-demethylation. The methodology was further applied for the simultaneous determination of propranolol enantiomers in human urine after administration of (+) propranolol where it was shown that (+) propranolol was metabolized at a greater rate than (-) propranolol. This methodology should have wide applicability in investigations involving the pharmacology of chiral drugs. Supported by NIH grants GM31304 and RR01688.

FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF PLATINATED OLIGONUCLEOTIDES

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The reaction of *cis*-diamminedichloroplatinum (II) (cisplatin), an important anticancer agent, and ribonucleic acids are well known, on the molecular level. Numerous studies have shown that the anticancer activity of cisplatin is due to platination of DNA resulting in adducts that interfere with cell functions. Cisplatin hydrolyzes to the very active diaquo and to the slightly less reactive monoquo forms. The water ligands are good leaving groups and allow the platinum to form two coordinate bonds to DNA. The primary site of this coordination is the N7 of guanine and to a lesser extent O9, although similar reactive sites on other nucleotides, such as adenine, are known. 1. Cisplatin can bind to one base on a single strand of DNA, bind to two bases on a single strand, or bind to two bases on different (duplexed) strands.

The modes of binding have been observed by numerous analytical methods, primarily ^{195}Pt and ^1H NMR (2), X-ray crystallography(3), all coupled with standard biochemical digestion and separation techniques. The negative ion fast atom bombardment (FAB) mass spectrometry of natural oligonucleotides has been described by Grotjahn and others (4). The present study attempts to establish a methodology to purify and analyze by mass spectrometry small quantities of platinated oligonucleotides. Fast atom bombardment mass spectrometry will be used to complement other analytical methods in the characterization of cisplatin complexes with the DNA tetramer, d(GpCpGpC).

Procedures and Results

Platination of 100 μg aliquots of nucleotide was carried out using 10 equivalents of cisplatin in water at 37°C for 3 to 6 days. The platination reaction resulted in two DNA products that were separated by open bed gel filtration monitored at 254 nm; these corresponded to a single diammineplatinum adduct and a bis-diammineplatinum adducts. FAB mass spectra were obtained using a JEOL HX-110HF mass spectrometer at 10 kV accelerating potential. Approximately 4 μg of each sample were analyzed in 2 μl of triethanolamine matrix. Negative ion FAB mass spectra contained deprotonated molecular ions, $[\text{M-H}]^-$, and fragment ions corresponding to the elimination of all associated ammonia ligands. No doubly stranded adducts were detected.

B/E linked scanning with collisional activation was carried out on the $[\text{M-H}]^-$ ions of the single stranded nonplatinated tetramer (m/z 1173), the single diammineplatinum adduct (m/z 1401), and on the bis-platinum adduct $\text{d(GpCpGpC)}\cdot(\text{Pt})_2$ (m/z 1561). The most abundant fragment ions of the unplatinated molecule corresponded to $[\text{d(GpC)}]^-$ (m/z 635), loss of guanine (249) and the resulting deguanylated fragment (m/z 1023) (Figure 1). Similar fragmentation was observed in the daughter ion scan of the mono-platinated oligonucleotide except that ions corresponding to phosphodiester cleavages were absent (Figure 2). Fragmentation of the bis-platinated oligonucleotide molecular ion show loss of one and two platinum atoms, as well as loss of cytidine (289) and the resultant ion $[\text{M-Cp}]^-$ (m/z 1271), although at very low relative abundance (Figure 3).

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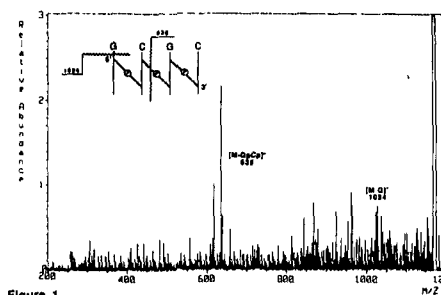


Figure 1.

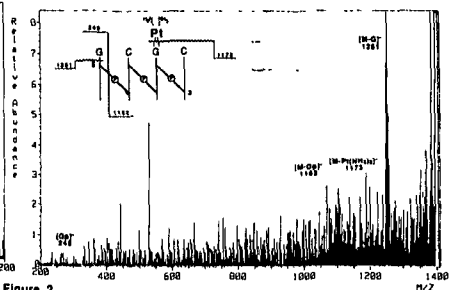


Figure 2.

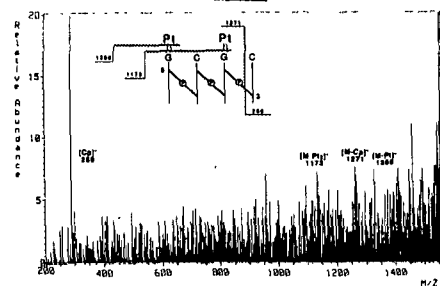


Figure 3.

Conclusions

Negative ion FAB mass spectrometry confirms that reaction of high levels of cisplatin with self complementary, double-stranded small oligonucleotides results in denaturation and release of single strand DNA significant structural changes that have measureable effects on the mass spectrometry of DNA. Cisplatin then binds to active sites on the unduplexed d(GpCpGpC) strand. The presence of both mono- and bis-platinated species indicates than cisplatin is doubly coordinated to the N7 and O9 of guanine, since addition to cytosine would be highly unfavorable. Cisplatin stabilized the phosphate backbone in the immediate vicinity of binding. In the mono-platinated species, binding most likely occurred at the 3' guanine, and fragmentation at the 5' guanine. In the bis-platinated species, the two platinum atoms were probably bound to guanine bases. This study indicates that FAB mass spectrometry is a useful method for the characterization of metal-oligonucleotide complexes.

GAS CHROMATOGRAPHY-ION TRAP MASS SPECTROMETRY DETERMINATION OF ARECOLINE IN HUMAN PLASMA. H.U. Shetty, N.H. Greig, T.T. Soncrant and S.I. Rapoport. Laboratory of Neurosciences, Bldg. 10, Rm. 6C 103, National Institute on Aging, NIH, Bethesda, MD 20892., and Athena Neurosciences Inc., 800F-Gateway Blvd., S. San Francisco, CA 94080.

Alzheimer's disease is accompanied by an impairment of the forebrain cholinergic system. Despite selective regional reductions of presynaptic terminals, the postsynaptic muscarinic receptors remain unaffected¹. Stimulation of these receptors with cholinergic agonists may compensate for the neurotransmitter deficit and, thereby, improve the cognitive functions of these patients. The muscarinic cholinergic agonist, arecoline, has been reported to enhance memory in normal healthy subjects². However, its value in treating patients with Alzheimer's disease remains inconclusive³ due, potentially, to a failure to achieve and maintain a pharmacologically adequate concentration. We describe, for the first time, a sensitive and specific assay for arecoline in human plasma that we are presently using to define the therapeutic window and evaluate the clinical potential of this agent in the treatment of Alzheimer's disease.

A Finnigan Mat 700 Ion Trap Detector (ITD) was interfaced with a Perkin-Elmer Sigma 2000 Gas Chromatograph (GC), *via* a transfer line. The GC setup featured a splitless inlet system with a narrow bore glass insert and a J&W DB-5 fused-silica capillary column of 30 m x 0.25 mm dimension and 0.25 μ m film thickness. Helium was used as the carrier gas. Typically, a column flow rate of 1.6 ml/min, a split vent flow of 33 ml/min and a septum purge flow of 3 ml/min were maintained. Samples were analysed by "cold trapping" and, for this, the injector temperature was set to 170°C and the initial oven temperature to 50°C; then, 30 sec after injection, the oven temperature was ramped at 20°C/min up to 130°C and, thereafter, by 5°C/min to a final temperature of 150°C. The split vent valve was opened 60 sec after injection. The transfer line temperature was maintained at its default value of 200°C. The ITD was tuned manually for high sensitivity detection and its data acquisition was set in the multiple ion detection mode. Prior to analysis, 1,3-diphenyl-1,1,3,3-tetramethyldisilazane was injected (0.5 μ l, twice) into the GC at 200°C. An internal standard, homoarecoline, was prepared by esterifying arecaidine with ethanol. It was purified by distillation, converted to HBr salt, and NMR and MS data were obtained. Buffer solutions, ranging between pH 2.0 and 12.0, were spiked with arecoline, equilibrated with ether and the quantity partitioned to this layer was determined by GC with Nitrogen Phosphorus Detection. Homoarecoline (free base) was used as an external standard in this experiment to determine the optimum pH for arecoline extraction. For the standard curve, human plasma (1 ml) was spiked with arecoline HBr (100-0.78 ng free base) followed by the internal standard (100 ng free base). The pH was adjusted (100 μ l, 0.5M borate buffer, pH 9.0) and the plasma sample then was extracted with a mixture of pentane and ether (80:20, v/v). The solvent layer was transferred to silanized tapered tubes, subjected to centrifugal evaporation (40 mm Hg pressure at room temperature), the residue then was reconstituted in n-hexane containing 1% triethylamine (25 μ l), and 1-1.5 μ l volumes of this, finally, were injected into the GC. In additional studies, male Fischer-344 rats were administered arecoline (5 mg/kg, i.v.), blood samples were withdrawn at different time intervals and plasma separated. The plasma profile of arecoline then was determined, using 250 μ l of plasma.

The mass spectra for arecoline and homoarecoline, acquired by ITD, are shown in Figure 1. The prominent fragment ion m/z 140, common to both, was chosen for selected ion monitoring. A plot of percentage recovery of arecoline versus pH of the aqueous phase, from three independent determinations, indicated a pH range of 8.0 to 10.0 is optimal for recovery, and a higher pH promoted hydrolysis. Consequently, pH 9.0 was chosen for the extraction. A representative ion chromatogram, obtained by spiking 12.5 ng of arecoline and 100 ng of

homoarecoline, the internal standard, into 1 ml human plasma, is shown in Figure 2. Retention times of 8 min 12 sec and 9 min 25 sec were noted for arecoline and homoarecoline, respectively. The standard curve exhibited linearity between 100 ng and 0.78 ng of arecoline per ml of plasma ($r = 0.9988$). The applicability of the assay procedure was demonstrated in animal studies: Figure 3 shows the plasma concentration-time profile of arecoline in rats. Arecoline rapidly disappeared with a half-life of 3.5 min. In summary, this selected ion monitoring assay takes into account the inherent volatility, adsorptive nature and sensitive functionality of the molecule and, thereby, provides adequate sensitivity and specificity. The methodology is presently being used to monitor the plasma profile of arecoline in treated Alzheimer's patients.

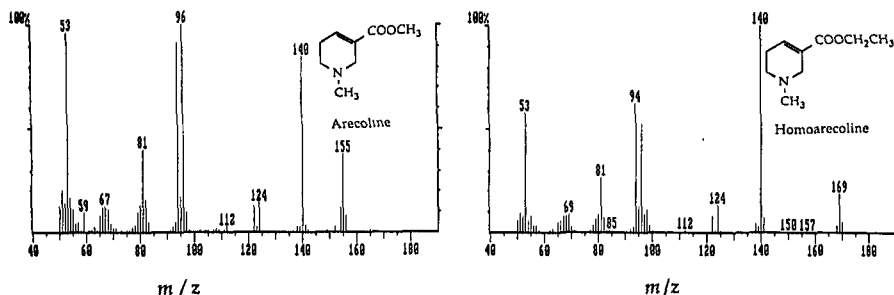


Figure 1. Mass spectra of arecoline and homoarecoline.

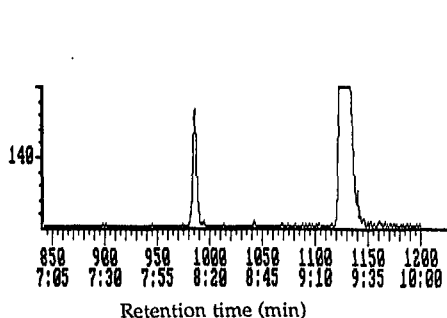


Figure 2. Selected ion chromatogram from plasma spiked with arecoline (12.5 ng) and the internal standard (100 ng).

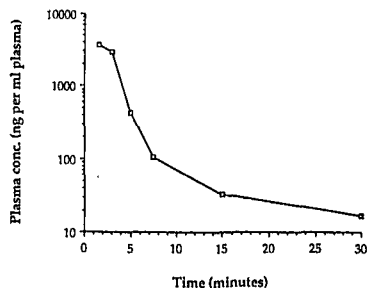


Figure 3. Plasma concentration-time profile of arecoline in rats.

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ECNI - SELECTED REACTION MONITORING FOR QUANTITATION OF DEXAMETHASONE IN PLASMA Kathleen A. Kayganich*, Timothy G. Heath*, and J. T. Watson*+; Departments of Chemistry* and Biochemistry+, Michigan State University, East Lansing, MI 48824

The dexamethasone suppression test (DST), used for years to diagnose Cushing's Syndrome, has been proposed for diagnosing endogenous depression in psychiatric patients.¹ After administration of 1 mg of dexamethasone the plasma levels of cortisol are normally suppressed for a 24-hour period. In patients with endogenous depression, cortisol levels return to normal within 3 to 4 hours. In order to make a proper diagnosis it is necessary to determine the dexamethasone plasma levels at 8, 16, and 24 hours following drug administration. Plasma levels of dexamethasone will normally diminish from 4 to 0.1 ng/ml during this 24-hour period. Such analyses require sensitive and selective methodology.

Previous results² from an assay based on oxidation of dexamethasone to a highly electrophilic species with detection by electron-capture negative ionization (ECNI) GC-MS indicated that the limit of detection (LOD) was ca. 1 ng/ml of dexamethasone in human plasma and the limit of detection of a pure standard of oxidized dexamethasone was ca. 30 pg injected on-column using an HP5985 GC-MS modified for negative ion detection. Recently, with the acquisition of a Finnigan triple stage quadrupole, TSQ70, we have evaluated the extent to which selected reaction monitoring (SRM) might increase the selectivity and sensitivity of the oxidation-based ECNI-SIM (selected ion monitoring) assay.

Dexamethasone is a synthetic corticosteroid drug which contains one fluorine atom. Shown in figure 1 is the oxidation reaction of dexamethasone as well as the ECNI mass spectrum of the oxidized product. Upon collisionally activated dissociation (CAD), the molecular anion of the oxidation product of dexamethasone undergoes the loss of HF. Interfering substances from the plasma matrix are unlikely to contain any fluorine atoms and therefore will not undergo a neutral loss of 20 u.

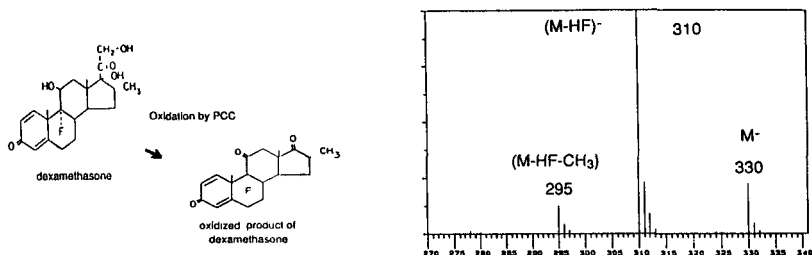


Figure 1. a) oxidation of dexamethasone b) ECNI spectrum of oxidized dexamethasone

Monitoring the reaction of m/z 330 (M^-) to m/z 310 ($M-HF^-$) using low energy CAD eliminates interference from the plasma matrix. The improved selectivity of SRM over SIM is illustrated in Figure 2 which compares SRM and SIM for the detection of oxidized dexamethasone after extraction from a plasma sample. Ca. 30 pg of oxidized dexamethasone and 400 pg of the oxidized isotopically labelled ($M+9$) internal standard was injected onto the column.

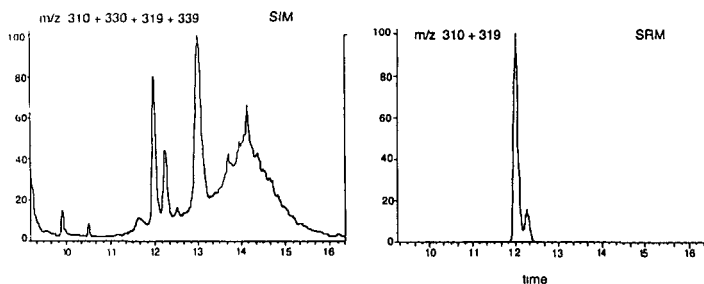


Figure 2. reconstructed ion current profiles obtained by a) SIM and b) SRM

Experimental

Sample processing consisted of extraction using a 200 mg C18 PrepSep column conditioned with 5ml of 8M urea, 5 ml methanol, and 10 ml distilled water. After loading 1.5 ml of plasma that had been spiked with 50 ng of $^{13}C_6$ - $2H_3$ -dexamethasone internal standard, the column was rinsed with 4 ml H_2O and 4 ml of 15% MeOH- H_2O . The dexamethasone was eluted with 4 ml of 70% MeOH-hexane. After solvent removal, the extract was

oxidized in 1 ml CH_2Cl_2 with pyridinium chlorochromate. Anhydrous sodium acetate was added to buffer acid liberated from the oxidant. After 6 hours the reaction mixture was passed through a 500 mg silica solid phase extraction column that had been conditioned with CH_2Cl_2 . The column was then rinsed with 1.5 ml CH_2Cl_2 and the oxidized dexamethasone was eluted with 4 ml of 5% acetone in CH_2Cl_2 . The eluate was reconstituted in 100 μl of ethylacetate; 2 μl was taken for analysis by both SIM and SRM.

Conditions for mass spectral analysis were as follows: 300 μA , 100eV, pressure of NH_3 ca. 1 torr (9300 mtorr on convection gauge situated on the gas inlet line), EM = 1200V for SIM and 1400V for SRM, source temperature 120° and 100°C for SIM and SRM, respectively. GC conditions: 30m x 0.25mm DB-5, 11 psi, 60°-260°C @ 40/min then 260°-280°C @ 4/min, splitless injection. A study of the effects of collision gas pressure and collision energy, E_{lab} , indicated that the optimum argon pressure and E_{lab} value for the production and transmission of m/z 310 from m/z 330 are 2.5 mtorr and 3 eV, respectively.

Results

The LOD for a pure standard of oxidized dexamethasone under CAD conditions by SRM is about the same as that obtained by SIM ($S/N = 4$ for ca. 160 fg of pure standard). At this low collision energy, autodehydration of the electron apparently does not occur to any appreciable extent. Therefore, the absolute sensitivity of the SRM and SIM assays are expected to be the same. The limits of detection for dexamethasone in plasma, however, may differ due to lower background in SRM. A criterion for the limit of detection is the concentration of analyte that produces a signal greater than the signal from the blank (containing internal standard) by at least three times the standard deviation of a blank value.³ Based on this criterion, the LODs for the SIM and SRM assays are 0.31 and 0.26 ng/ml, respectively.

A number of plasma samples taken at various times following drug administration to patients undergoing the DST were analyzed using both SIM and SRM. The quantitative results are shown in table 1. Given the high selectivity of negative ion detection in combination with MS/MS, as well as the selectivity imparted by the chemical oxidation, simplification of the procedure through the use of the direct probe (DIP) rather than the GC for sample introduction was also investigated. The results obtained by DIP-ECNI-SRM are also shown in table 1.

Triplicate analysis of duplicate spiked plasma samples gave relative standard deviations of 3.6% by SRM and 9.1% by SIM for the procedure. In spite of this variation, most of the concentrations of dexamethasone found in the real plasma samples by SIM and SRM are quite similar. The concentrations obtained by DIP-SRM vary more from those obtained by GC-SRM and GC-SIM. This may be due to the presence of interfering ion current at either m/z 310 or m/z 319.

Table 1. Concentrations of dexamethasone in ng/ml of plasma

Sample	SIM	SRM	DIP-SRM	RIA (n=10)
P2	0.64	0.61	0.81	0.66
P5	0.64	0.87	0.50	0.42
P11	2.97	2.75	2.35	0.91
P18	27.4	33.8	49	20.5
P3	0.18	0.14		0.26
P4	0.088	<LOD		0.24
P14	0.045	0.093		0.12
P15	0.097	0.018		0.08
P16	0.175	<LOD		0.10
P17 (blank)	<LOD	<LOD		0.07
7A*	2.09(s=.11)	1.88(s=.10)		
7B*	1.83(s=.13)	1.82(s=.04)		

*duplicate spiked plasma sample (2 ng/ml), measured in triplicate

Conclusions

The limit of detection of the SIM assay is comparable to the LOD of the SRM assay, however, the precision for the SRM assay appears to be better. The limits of detection of the SIM and SRM assays both appear to be limited by the high blank values, rather than the chemical or instrumental procedures. The presence of unlabelled dexamethasone in the isotopically labelled internal standard is probably responsible for these high values. Adding a structurally similar compound to act as the carrier during the sample preparation would allow the addition of a smaller quantity of internal standard, resulting in lower blank values and probably a lower LOD. The fact that no appreciable increase in selectivity or sensitivity is gained by using MS/MS suggests that substantial discrimination against the biochemical background and in favor of the analyte is provided by the chemical oxidation and by negative ionization of the oxidized product.

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Negative Chemical Ionization Versus Electron Impact Ionization Detection of Scopolamine by Gas-Chromatography/Mass Spectrometry in Human and Rat Plasma. J. Deutsch, T.T. Soncrant, N.H. Greig and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, N.I.H., Bethesda, MD 20892.

INTRODUCTION

Scopolamine, a natural alkaloid, has a broad spectrum of biological activities. Being an antagonist of the muscarinic receptor, it has been found to significantly impair memory and, in some cases, to mimic the memory impairment of normal aging^{1,2}. Due to the time consuming nature and poor sensitivity of existing assays, previous pharmacodynamic studies of scopolamine were carried out without extensive understanding of its in vivo pharmacokinetics. We report here an improved GCMS assay, using negative ion chemical ionization together with electron impact detection, that is suitable for quantitation of scopolamine in various biological tissues.

EXPERIMENTAL

The GCMS measurements were carried out on either a Finnigan 4500 GCMS, equipped with an electron impact and a chemical ionization ion source, or a HP 5970 bench top electron impact GCMS. The reactive gas for the chemical ionization was CH₄ and the ions were monitored on either positive or negative ionization mode. The samples were separated on the GCMS with a DB5 capillary column, 30m x 0.25mm. Samples were run in a temperature programming mode at 145 °C for 3 minutes followed by a 10°C/min increase for 5 minutes, with the injector at 200 °C and the detector line at 280 °C. The retention time of the heptafluorobutyl scopolamine derivative (Fig. 1b) was 4.7 minutes at 5 psi He pressure at the top of the column. The multiple ion monitor was set to detect ions m/z 331 and m/z 334 for the negative ion chemical ionization, and at m/z 138 and m/z 141 for the electron impact ionization.

Extraction procedure: Plasma (0.5 ml) or homogenized tissue (1.0 g) was spiked with trideuterated scopolamine.HBr (50-200 ug), treated with 1M Na₂CO₃ solution (0.5 ml), and extracted twice with n-butyl chloride (2.0 ml). Following centrifugation, the organic layer was separated and the solvent was removed by speedvac. To the remaining material, 5M NaOH solution (0.5 ml) was added and heated at 50 °C for 20 minutes. The alkali solution then was diluted with water (1.0 ml) and extracted with methylene chloride (2.5 ml). The organic phase was washed twice with water (1.0 ml) and dried on anhydrous CaCl₂. After removal of the solvent, the extracted material again was dissolved in methylene chloride (100 ul), treated with heptafluorobutyric anhydride (10ul) and heated at 40 °C for 20 minutes. Following removal of the solvent, the remaining material was dissolved in acetone (20 ul). 1-5 ul of the acetone solution then was injected into the GCMS for quantitation.

RESULTS AND DISCUSSION

It is well established, that for GCMS determination³, scopolamine, an ester of scopine (Fig. 1a) with mandelic acid, is first hydrolyzed by alkali to scopine which is further derivatized with the heptafluorobutyric anhydride to the highly electrophilic heptafluorobutyl scopine, MW 351 (Fig.1b). This derivative elutes well on a commercial DB5 capillary GC column and can be detected by mass spectrometry, using positive chemical ionization or electron capture negative chemical ionization or electron impact ionization. The positive ion ionization leads to a protonated molecular ion at m/z 352; the electron capture negative ion leads to a M-HF fragment at m/z 331; electron impact ionization leads to a fragment at m/z 138, (Fig.1c) due to thermal decomposition. The ion yield of the first is low; thus, only the last two ionization methods are suitable for scopolamine quantitation.

In the electron impact ionization assay, the fragment at m/z 138 was monitored, using d₃-derivative (m/z 141) as an internal standard. In negative chemical ionization mode, the M-HF

ion at m/z 331 was measured, again using d_3 -deuterated scopolamine (m/z 334) as an internal standard.

The EI-SIMS profiles obtained from plasma and brain extracts obtained from rats 5 minutes after a dose of 2.5mg/kg scopolamine.HBr was injected i.p. is shown in Fig. 2. The internal standard concentration was about 50ng per GC injection. For lower doses, measurements at the high mass region by the negative chemical ionization mode which have a higher signal/background ratio, are likely to yield sensitivity greater than that of electron impact mode.

Fig. 1.

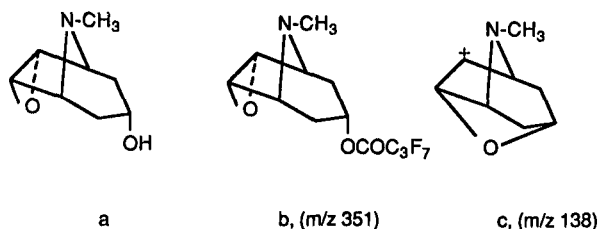
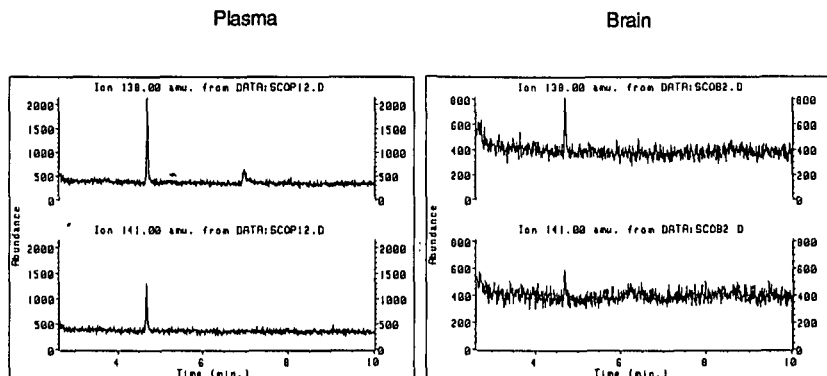


Fig. 2.



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STRUCTURE ELUCIDATION OF ISOMERIC HYDROXYALOINS AND
OXANTHRONE GLUCOSYLS BY HIGH RESOLUTION AND TANDEM MS

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Purpose. A method for the fast and reliable differentiation of isomeric anthrone and oxanthrone glucosyls is described.

Introduction

Samples: plant extracts of *Aloe capensis* and *Aloe barbadensis* (*Aloe vera*).

Activity: purgatives of the large intestine (1). Quantitative and qualitative analyses of active components are significant for the pharmaceutical industry (2,3).
Prior work: negative CI-MS/MS has been used to determine aloin and its derivatives in plant extract of *Aloe* species (4).

This work: NCI-MS/MS is used to differentiate between isomeric hydroxyaloins and oxanthrone glucosyls.

Experimental

Plant extracts: commercially available. Fractionated by HPLC: dissolved in CH_3OH , 1:1000; 20 μl injected on a RP-10 column, eluted with a $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ ratio of 9:5, 1 ml min^{-1} .

Mass Spectrometry: Finnigan MAT HSQ 30 and MAT 900Q. MS1 resolution: 1,000 (for MS/MS) and 10,000 (10 % valley) (for exact mass measurements). Internal standard: perfluorokerosin. MS2 (QQ) resolution: adjusted to unit (50 % valley). Collision gas: argon. Collision gas pressure: 0.5 Pa (for excitation of M^+); 7 Pa (for excitation of m/z 283). Collision energy: 45 eV.
Ionization: DCI, negative ions. Reagent gases: isobutane or ammonia. Electron energy: 70 eV. Emission current: 0.2 mA. Ion source pressure: 5 Pa.

Results

$\text{M}^+/(M-H)^+$: this ratio depends on reagent gas pressure. At a source pressure of 5-10 Pa: M^+ predominates. Above 10 Pa: $(M-H)^+$ predominates. See Figure 1.

MS/MS of M^+ and $(M-H)^+$: differences in spectra are seen with excitation by low-energy collisions, but not by high-energy collisions. See Figures 2.

Fragmentation: the glucosyl group, 163 u, is lost; see Figure 5. Formation of m/z 283: by loss of H_2O (18 u) and $\text{C}_5\text{H}_{11}\text{O}_6$ (151 u) from the anions of oxanthrone glucosyls; see Figure 6. Loss of m/z 151: aided by HO^- at position 10 of the aglycone? See Figure 7. Fragmentation of m/z 283: requires a 10X higher collision pressure than for fragmentation of M^+ .

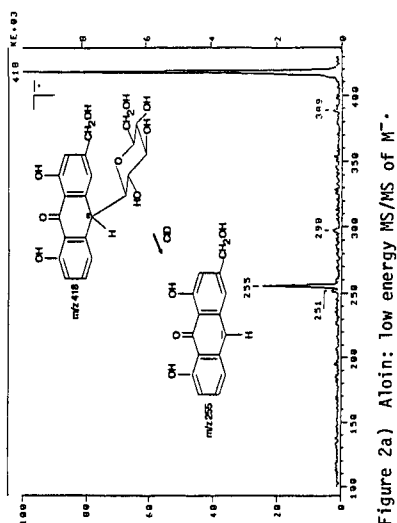
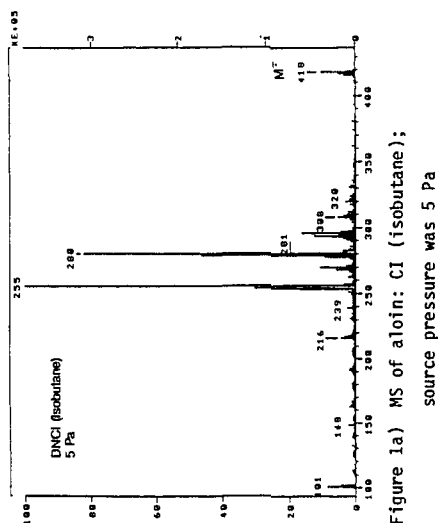
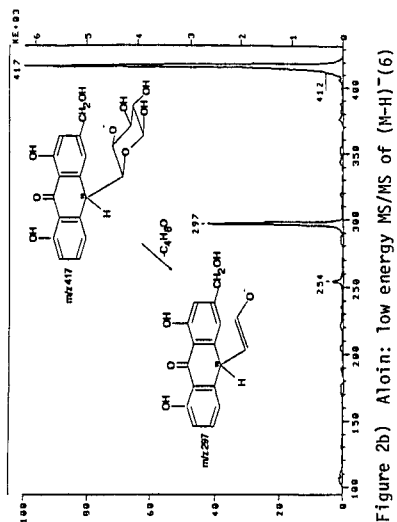
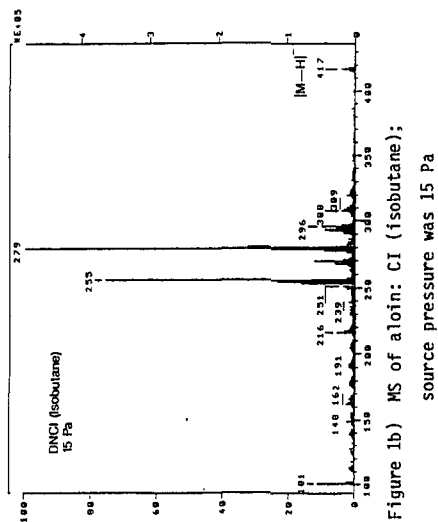
Discussions

These fragmentations were used for the fast differentiation of isomeric hydroxyaloins. Structures were verified by NMR and LC, using known compounds (5).

MS/MS is useful for analysis of single compounds in these complex biological matrices. NCI is a highly sensitive ionization technique for these compounds.

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SELECTIVE DETECTION OF SULFUR-CONTAINING COMPOUNDS BY REACTION INTERFACE/MASS SPECTROMETRY AND ITS APPLICATIONS IN METABOLISM STUDIES

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INTRODUCTION

Addition of a reactant gas to a low pressure microwave-induced plasma (MIP) creates a reaction interface in which complex molecules are converted into small stable neutrals. For a given reactant gas the molecules formed represent the elemental composition of original analyte. In this study HCl has been found most effective as a reactant gas for selective detection of sulfur-containing compounds using capillary gas chromatograph-reaction interface/mass spectrometry (GC-RIMS).

EXPERIMENTAL

The GC-RIMS has been described previously (1). The end of the a 30 meter DB-5, 0.25 μ m film thickness capillary column of an HP gas chromatograph was connected to a 4" long, 1/4" o.d. 1/32" i.d. ceramic tube by conventional vacuum components. The reactant gas was also added to this end of the ceramic tube. The other end of this tube was connected to an Extrel quadrupole mass spectrometer using deactivated, uncoated wide-bore fused silica tubing. The mass spectrometer was controlled by a Teknivent data acquisition interface, software, and an IBM AT computer. The microwave cavity was powered by 100 W, 2450 MHz power supply. In this study helium was used both as a carrier gas for the GC and the MIP.

RESULTS and DISCUSSION

Selection of the Reactant Gas: When oxygen was used as a reactant gas carbon forms CO and CO₂, nitrogen forms NO and NO₂, and hydrogen forms water. Results for sulfur were inconsistent, probably due to further reactions of newly-formed sulfur compounds with H₂O in the plasma and formation of adsorptive complexes. In order to solve this problem, a reaction gas with completely different chemistry was used, i.e. HCl. When HCl was used as a reactant gas, carbon in the presence of nitrogen predominantly produced HCN at m/z 27 along with other minor components of hydrocarbons, e.g., CH₄, C₂H₂, and chlorocarbons of the type C_nH_nCl_n. Nitrogen in the presence of carbon also predominantly produced HCN. Sulfur produced SCI at m/z 67, with other minor components including S₂Cl₂.

Selectivity: Detection at m/z 67 was completely selective for sulfur. The selectivity of this detection channel was demonstrated using thiopental and phenobarbital as examples of sulfur and nonsulfur-containing compounds. The GC-RIMS chromatograms are shown in Figure 1. m/z 27 represents a nonselective carbon channel while m/z 67 represents the selective sulfur channel.

Sensitivity: The GC-RIMS detection limit for thiopental was 20 picograms on-column at a S/N = 3 in the single ion detection mode with 600 ms integration time. The linear dynamic range of the calibration curve using phenobarbital as an internal standard is shown in Figure 2.

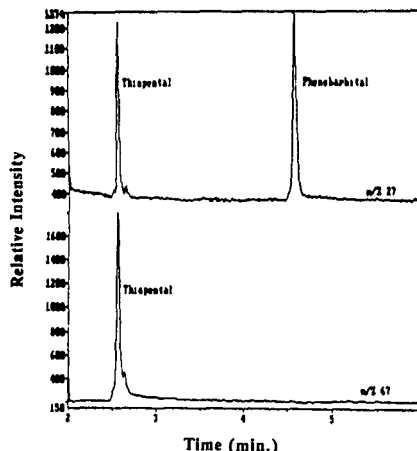


Figure 1. GC-RIMS selective detection of sulfur using HCl as a reactant gas. HCN at m/z 27 is a nonselective carbon channel. SCI at m/z 67 is a selective sulfur channel.

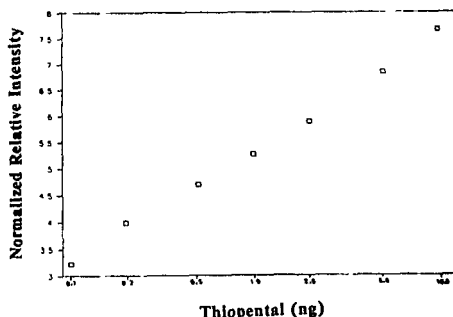


Figure 2. The linear dynamic range of the calibration curve for thiopental using phenobarbital as an internal standard.

Applications in Metabolism Studies: To study the application of this system, ^{13}C and ^{15}N -labeled phenytoin and its metabolites in urine were examined by GC-RIMS using $[2-^{13}\text{C}-, 1,3-^{15}\text{N}]$ -phenobarbital and thiopental as internal standards. To follow phenytoin metabolism, oxygen was first used as a reactant gas. Chromatograms showing nonselective ^{12}C and enriched ^{13}C and ^{15}N are shown in Figure 3. Selective detection of sulfur containing compounds in this urine was then followed using HCl as a reaction gas. The result is shown in Figure 4. Several of these peaks occur in urine samples from untreated animals. There are no known endogenous sulfur-containing compounds which would elute in this portion of a chromatogram. Further studies will be undertaken to obtain mass spectra of these unknown peaks to aid in their identification.

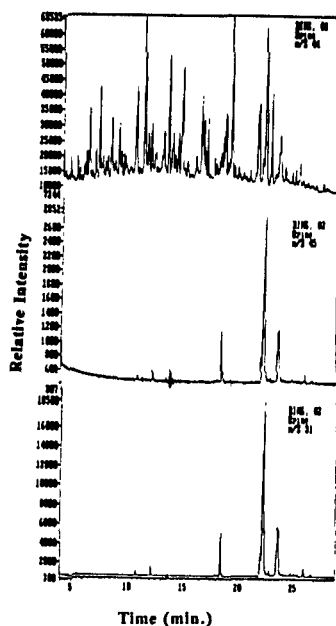


Figure 3. Application of RIMS to ^{13}C and ^{15}N -labeled phenytoin drug metabolism using oxygen as a reactant gas. From top to bottom; nonselective carbon channel selective ^{13}C and ^{15}N channels.

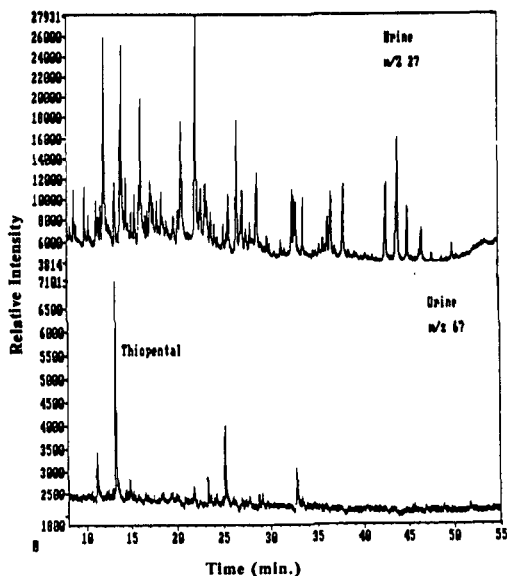


Figure 4. Selective detection of sulfur containing compounds in urine, using HCl as a reactant gas.

CONCLUSION

Reaction Interface mass spectrometry is a powerful technique which can be used both as an selective element and stable isotope detector. Its low detection limits, wide dynamic range, minimum instrumental modification and simplicity makes it an attractive tool in metabolism investigations. We have shown here that one can switch from study of stable isotopes such as ^{13}C and ^{15}N to selective element detection of sulfur and carbon by simply changing from one reactant gas (e.g. O_2) to another (e.g. HCl).

ACKNOWLEDGMENT

This work has been supported by the United States Public Health Service under Grant NIH-GM36143.

REFERENCES

1. D. H. Chace and F.P. Abramson, Anal. Chem., submitted for publication.

DETERMINATION OF ^{14}C -LABELED DRUGS AND THEIR METABOLITES BY CAPILLARY GAS CHROMATOGRAPHY - REACTION INTERFACE / MASS SPECTROMETRY (GC-RIMS)

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The coupling of a high resolution capillary gas chromatograph column to a proportional flow counter either degrades chromatographic resolution due to the volume of the radiodetector or has low sensitivity due to limited residence time required by narrow GC peaks. To circumvent these problems we have used a mass spectrometer as a selective ^{14}C detector.

A capillary gas chromatograph - reaction interface/ mass spectrometer (RIMS) technique has been shown to selectively detect ^{13}C -, ^{15}N -, and ^2H -labeled phenytoin and its metabolites in a sensitive, reproducible, and structure independent manner. Its design and operation has been described¹. A reaction interface is a microwave-powered device which decomposes a complex molecule in the presence of helium. The addition of a reactant gas forms stable products which reflect the elemental composition of the original analyte and which are detected by a conventional mass spectrometer.

A Varian Model 3300 gas chromatograph which was equipped with a Varian Model 1075 split/splitless injector, was used with a 30 meter DB-5, 0.25 mm i.d., 0.25 μm film thickness, capillary column. Ultra pure carrier grade helium was fitted with high purity stainless steel regulators and was operated with a helium flow of 1 or 2 mL per minute. The injector temperature was 265°C and the initial column temperature was 80°C. Following injection of 2 μL of sample, the chromatograph was programmed at various rates to 250°C. This work used a Dupont 21-492 mass spectrometer which was equipped with Trek Model 667 power supply. The Trek power supply was controlled by a Teknivent data acquisition interface, software, and an IBM XT Model 286 computer.

A male beagle dog used in the stable isotope experiments was placed in a metabolic cage 24 hours prior to administration of ^{14}C -labeled phenytoin during which time urine was collected, filtered, and frozen until analysis. Ninety μCi of 4- ^{14}C -labeled phenytoin (S.A. 55.8 mCi/mmol) or 0.41 mg, present in 1 mL of ethanol, was administered through a Y-tube into a saline solution which was flowing at a rate of 1 mL/min. The phenytoin solution was injected intravenously over a period of 1 minute. Urine was collected for 48 hours following the injection of labeled phenytoin, filtered, and frozen until analysis. Three hundred eighteen nCi of 2- ^{14}C -phenobarbital (Amersham, S.A. 55.8 mCi/mmol), was added as an internal standard to a 1% aliquot of the total volume of urine collected (10 mL). The samples were hydrolyzed, extracted using XAD-2 resin, and derivatized by extractive ethylation followed by trifluoroacetylation. One hundred microliters of toluene was added to the dry derivatized residue.

Detection of ^{14}C was achieved using hydrogen as the reactant gas and monitoring $^{14}\text{CH}_4$ at m/z 18.034 with a resolution of 1800 so that $^{14}\text{CH}_4$ was resolved from both H_2O , at m/z 18.011, and $^{15}\text{NH}_3$ at m/z 18.024. A non-selective chromatogram of carbon-containing compounds can be generated by monitoring CH_4 at m/z 16.031 at this resolution of 1800 which separates this mass from NH_2 at m/z 16.019, and O at m/z 15.995. Similarly, a chromatogram which detects nitrogen can be generated by monitoring NH_3 at m/z 17.027 or its isotopic variant, $^{15}\text{NH}_3$ at m/z 18.024.

Radioactivity in each standard was measured with a liquid scintillation counter. This calibrates the amount of ^{14}C labeled internal standard present in each chromatogram. These data were then manipulated to represent on-line flow ^{14}C monitoring. To preserve chromatographic integrity from GC peaks having 6 second half widths, a flow counter could retain a sample for no more than 1 second. Therefore, the total disintegrations per second in a peak should be divided by its half-width to express the peak height which would have been observed in a 100% efficient detector. For example, the ^{14}C -labeled peak in a chromatogram was equivalent to 4754 dpm or 4754 / 60 = 79 dps as measured by liquid scintillation counting. This value is then divided by the peak width at half height resulting in the calculation 79 dps / 6s = 13 dps per second. The right hand y-axis of the $^{14}\text{CH}_4$ chromatograms is labeled with this value. The quantity of radiolabel detected represents 30-50% of the amount of radiolabel added. This was a result of losses which occurred during the extraction, hydrolysis, and derivatization of each sample.

In separate experiments, between 4.8 nCi and 195 nCi of ^{14}C -labeled phenytoin was added to 10 mL of control urine. Each urine sample was hydrolyzed, extracted, and derivatized as described above. In a separate experiment, between 4.8 nCi and 48 nCi of ^{14}C -labeled phenytoin was derivatized directly. In each of these experiments, 159 nCi of ^{14}C -phenobarbital was added to each preparation as an internal standard. These samples were used to test the linearity for detection of ^{14}C . The ratio of areas under the peak for phenytoin and the internal standard in the chromatograms generated by monitoring m/z 18.034 was determined and plotted against the calculated ratio of nCi of phenytoin to nCi of internal standard. The data from each experiment was regressed and performed using a weighting of $1/y^2$. To evaluate the detection limit of ^{14}C -labeled phenytoin metabolites using RIMS, the smallest peak present in a ^{14}C - chromatogram was determined from the ratio of the area of this peak to the area of the internal standard which contains a known quantity of radiolabel.

Carbon-containing molecules are converted to $^{12}\text{CH}_4$ and detected at m/z 16.031. Figure 1 shows this non-selective chromatogram of urine from a dog administered ^{14}C -labeled phenytoin. This is similar to what would be obtained by a flame ionization gas chromatographic detector. A selected portion of a chromatogram of $^{14}\text{CH}_4$ at m/z 18.034 with a resolution of 1800 is shown in Figure 2 (bottom). Note that the majority of peaks present in the non-selective carbon chromatogram are not detected in the ^{14}C - chromatogram. However, the selectivity is not perfect. The peak at 18.4 min. in the ^{14}C chromatogram (Figure 2 bottom) is a result of NH_4^+ formed by ion molecule reactions from the NH_3 produced by large amounts of nitrogen-containing compounds. NH_4^+ cannot be easily resolved from $^{14}\text{CH}_4$. It was found that only nitrogen containing compounds in amounts greater than 250 ng on column are detected at m/z 18.034. Although phenytoin is a nitrogenous compound, the amount analyzed in each experiment was less than 10 ng on column. Therefore the nitrogen content of phenytoin did not contribute to the detection of ^{14}C -labeled phenytoin.

Nitrogen-containing peaks were detected in a chromatogram of NH_3 at m/z 17.027 as shown in Figure 2 (top). By comparison of the two chromatograms, peaks present in the $^{14}\text{CH}_4$ chromatogram which were likely produced from nitrogen can be determined; the remaining peaks are presumed enriched with ^{14}C . The peak shoulder at r.t. 24.4 minutes represents 34 pCi or 187 pg of a ^{14}C -labeled metabolite. The peaks at 22.7, 24.4, and 24.6 contain ^{14}C and are ^{14}C -labeled metabolites of phenytoin. These retention times coincide with phenytoin metabolites detected in previous studies using the stable isotopes, ^{13}C , ^{15}N , and ^2H .

The linearity of ^{14}C detection was determined by an analysis of ^{14}C -labeled phenytoin added to urine or when it was derivatized directly. The ratio of the ^{14}C signal for phenytoin to the internal standard, ^{14}C -labeled phenobarbital against the calculated ratio of nCi added was found to be linear and the two data sets were superimposable. The correlation coefficient of the pooled data set was 0.97 with a slope of 1.26. As little as 40 pCi (200 pg) on column of ^{14}C -labeled phenytoin was detected for phenytoin added to urine or derivatized directly.

Three metabolites of phenytoin were detected in a ^{14}C chromatogram compared to 9 using ^{13}C and ^2H and 20 for ^{15}N (Chace and Abramson, ASMS 1988). This relates to the dose of ^{14}C -labeled phenytoin which was 0.41 mg versus 50 mg for the stable isotope studies. RIMS provides detection of radiolabeled material at lower concentrations and higher resolution than other on-line radiocarbon detection schemes. Further improvement of selectivity will make the use of this method even better. However, the hazard of using radiolabels will always limit their application. We have demonstrated that detection of stable labels in a structure-independent manner is more selective.

The analysis of ^{14}C was not completely selective due to the presence of NH_4^+ from large quantities of nitrogen-containing compounds. If a ^{14}C -labeled compound coelutes with a nitrogenous compound present in large amounts on column, it will not be selectively detected. Although other radio-gas chromatographic methods are totally selective, resolution is inadequate. The use of an in-line cold trap for ammonia prior to detection by the mass spectrometer should improve selectivity substantially.

¹Chace, D.H. and Abramson, F.P., "Metabolism Studies using Stable Isotopes: Application of a Capillary Gas Chromatograph - Reaction Interface / Mass Spectrometer Technique (GC-RIMS) to the Selective Detection of ^{13}C , ^{15}N , AND ^2H , submitted for publication to Anal. Chem.

The authors acknowledge the financial support of this research by the US Public Health Service Grant NIH-GM36143.

Figure 1: Nonselective RIMS capillary gas chromatogram of a derivatized extract of urine from a dog receiving 90 μCi of $[4-^{14}\text{C}]$ -phenytoin obtained by monitoring $^{12}\text{CH}_4$ at m/z 16.031. The left side of the y-axis represents ion intensity. The x-axis represents time.

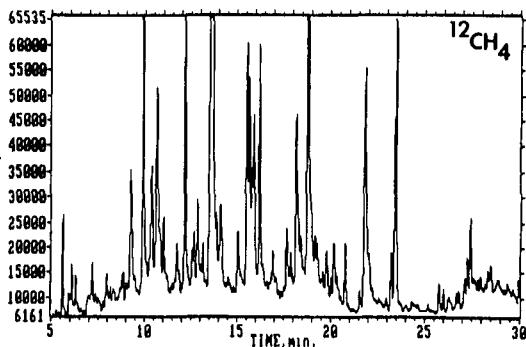
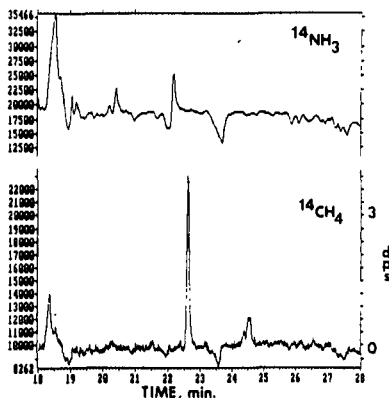


Figure 2: RIMS capillary gas chromatograms of a derivatized extract of urine from a dog receiving 90 μCi of $[4-^{14}\text{C}]$ -phenytoin representing detection of nitrogen as NH_3 at m/z 17.027 (top) and $^{14}\text{CH}_4$ at m/z 18.034 (bottom). The left side of the y-axis represents ion intensity and the right side represents dps calculated from the measured dpm (4754 dpm) of the internal standard. The x-axis represents time. The peak shoulder at r.t. 24.4 min. represents on column detection of 34 pCi which is equivalent to 187 pg of a ^{14}C -labeled phenytoin metabolite.



**MASS SPECTROMETRIC STUDIES ON AZIDOTHYMININE (AZT) USING FAB, THERMOSPRAY,
PLASMASPRAY AND MS-MS.**

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Azidothymidine (Zidovudine, 3'-azido-3'-deoxythymidine) has been shown to be efficacious in the treatment of AIDS. We studied the mass spectra using these ionization techniques to investigate which may be best for the development of a mass spectrometric analysis of AZT in serum.

EXPERIMENTAL. FAB: glycerol matrix, gun operated at 8 kV and 2 mA discharge current using xenon. Thermospray and plasmaspray: mobile phase 0.1 M ammonium acetate in methanol-water (1:1, v/v), flow rate 1.2 mL/min. No HPLC column used, samples directly injected via 20 μ L loop. MS-MS: collisional activation using argon at 2 uBar (5 eV energy). Mass range of 20-350 amu scanned in 1 s.

MASS SPECTRA. The FAB spectra (Fig. 1) consisted of the (M+1) ion, $m/z=268$ (60% intensity) and the base peak (after glycerol subtraction) at $m/z=128$, the sugar residue with loss of N from the azide group. The peak at $m/z=242$ (15% intensity) could not be interpreted as part of AZT. It also appeared in thermospray and plasmaspray using three batches of AZT. In the thermospray spectra (Fig. 2) the (M+1) ion was the base peak. Two fragments were found, $m/z=128$ and $m/z=115$, the latter representing further decomposition of the sugar residue (sugar residue-2N+H). Plasmaspray yielded the same fragment ions but, in general, at higher intensities which were strongly affected by the discharge current. At 300 μ A the (M+1) ion was the base peak with other fragments at 40-50% intensity (compare to thermospray). At 750 μ A the $m/z=128$ peak became the base peak and the (M+1) peak reduced to 40%; the ratio of $m/z=128$ to 115 increased 5-fold.

$m/z=242$. MS-MS was used to ascertain the relationship between the "unknown" at $m/z=242$ and AZT. The daughters of the (M+1) peak of AZT (Fig. 3) included one at $m/z=128$, which was also detected by the other ionization modes and another at $m/z=143$, designated as the (sugar residue+H) ion, detected only in MS-MS. The $m/z=242$ peak was not detected as a daughter of AZT. No parent of $m/z=242$ was found, except for the glycerol adduct peak. This suggests that the $m/z=242$ peak is the (M+1) or parent ion of the unknown. The only daughter ion of $m/z=242$ was at $m/z=117$ (Fig. 4). These data suggest that the structure of the unknown may be similar to that of AZT except the azide group is replaced by an amino group, 3'-amino-3'-deoxythymidine. Efforts to separate the compound from AZT by HPLC will establish whether it is an impurity, possibly related to the synthesis of AZT, or it originates during the ionization process; the latter would prompt further mass spectrometric studies.

AZT IN SERUM BY THERMOSPRAY. We demonstrated the feasibility of a novel approach in pilot experiments: serum ultrafiltrate (Centricon-30, Amicon Co.) was directly injected (50 μ L), without an HPLC column, and selected ion monitoring of the (M+1) ions to quantify AZT (Fig. 5). Injection of mobile phase did not effect the baseline of $m/z=268$. Blank serum (no AZT added) gave only small ripples. The int. std. commonly used in HPLC (3'-beta-azido stereoisomer of AZT) yields the same ions as AZT and could not be separated from AZT since no HPLC column was used. Detection of 30 ng/mL, which represent the low end of the AZT concentrations in patients on current experimental protocols, was readily achieved. Potentially available detection limit (5-10 ng/mL) appears adequate for anticipated pediatric applications. Advantages include simple and rapid sample preparation, high specificity, low detection limit and fast analysis (2-3 min/run). Work in progress include optimization of experimental conditions to eliminate annoying cloggings of the capillaries and a search for a suitable int. std. for improved quantification and reproducibility.

Supported by Grant 5U01-A127667 from NIAID and by the T.J. Martell Memorial Foundation for Leukemia and Cancer Research.

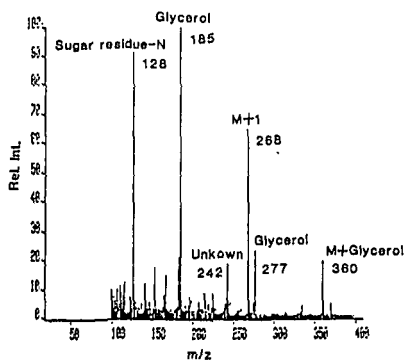


Fig. 1.

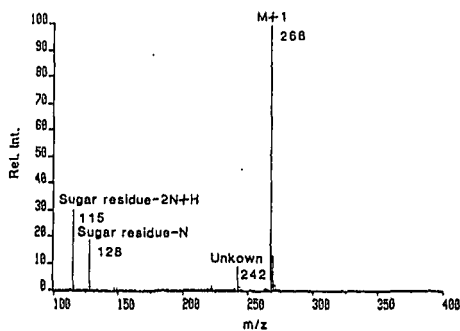


Fig. 2.

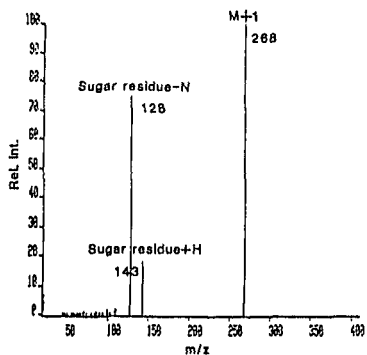


Fig. 3.

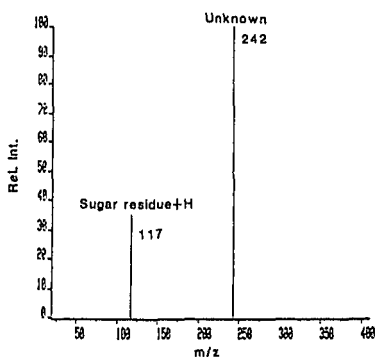


Fig. 4.

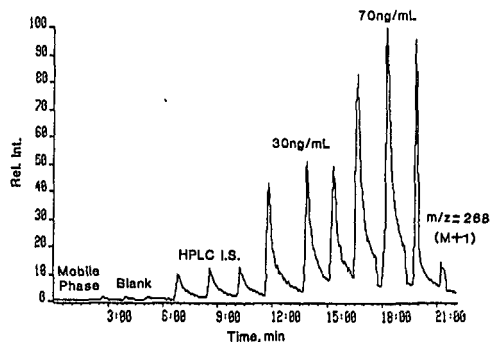


Fig. 5.

USE OF PYROLYSIS CI/MS OVER THREE DIFFERENT TEMPERATURE RANGES TO IDENTIFY BACTERIA

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J.M. Quarles, Jr., Department of Medical Microbiology and Immunology,
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The object of this research is to develop a rapid method for the identification of bacteria by MS using CI to reduce fragmentation. Time consuming steps such as solvent extraction or derivatization are to be avoided. The bacteria were pyrolyzed at lower temperatures than is the case with the well-known Curie-point method. The experimental procedure was the same as described previously [1] except that the discharge pulse rate was 2.5 kHz and three pyrolysis temperature ranges were used, 250-330, 330-390 and 390-440 C. Three temperature ranges were used for each sample to determine if the accuracy of the analysis could be improved by the additional data.

Five types of bacteria were studied, (1) Escherichia coli, (2) Staphylococcus aureus, (3) Staph epidermidis, (4) Listeria monocytogenes ATCC 9113 and (5) List monocytogenes ATCC 9115. Bacteria can vary from batch to batch, so different batches were used for the standard and "unknown" samples for each bacteria. The spectra were normalized at mass 18, and masses of 10-20% reproducible intensity were selected from the standard spectra for each bacteria. Then masses were selected at each temperature such that the reproducibility is good, but the intensity ratio between bacteria types is as large as possible. This produced the following sets of analysis masses:

Pyrolysis Temperature Range	Analysis Masses
Low	66,77,136
Medium	60,77,90,107
High	42,83,99,181

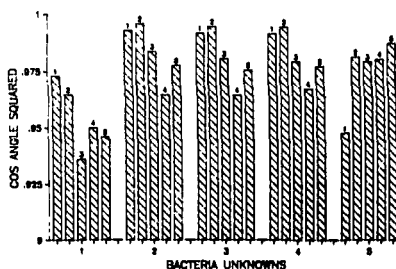
The unknowns and standards were represented by vectors using the intensities of the analysis masses. The $(\cos \phi)^2 \equiv T$ was calculated for each standard, where ϕ is the angle between the unknown vector and the standard vector. The results are shown in the three figures below, where the bacteria numbers have been defined above. One method for analysis is the 'T'-maximum method where the identification of a bacteria is considered to be correct if only the correct unknown has the highest 'T'-value when compared to the five standards. By this method E. coli and L. mono 9115 can be identified by low temperature pyrolysis and L. mono 9113 can be identified by high temperature pyrolysis. At the medium range S. aureus and S. epid would both be identified as the former.

Another way to identify the bacteria is from the five line patterns. It is seen from the figures below that the patterns can be used to identify all but S aureus and S epid, which can not be distinguished from each other. It is interesting that the patterns for L mono 9113 and L mono 9115 are so different, whereas those for S aureus and S epid are so similar. This could be a result of the fact that the selection of the analysis masses is somewhat arbitrary, so the selection may not have been optimized for the two Staph types.

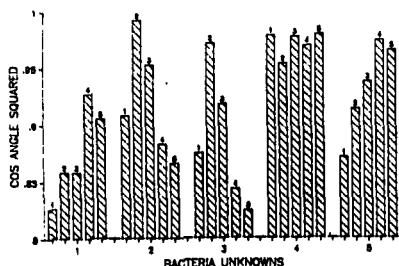
The use of three temperature ranges for the identification of the bacteria is an improvement over one temperature range. However, the method of selecting the analysis masses must be improved because the present sets do not distinguish S aureus from S epid.

Reference: 1. D.C. Conway, Humberto Lopez, and J.M. Quarles, Jr., 36th ASMS Conference on Mass Spectrometry and Allied Topics, pp. 1402-1403 (1988).

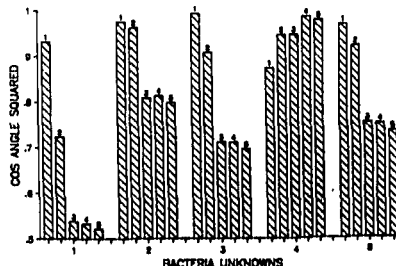
LOW TEMPERATURE PYROLYSIS MATCH



MEDIUM TEMPERATURE PYROLYSIS MATCH



HIGH TEMPERATURE PYROLYSIS MATCH



MOLECULAR WEIGHT DETERMINATION OF UNDERIVATIZED
GALACTURONIC ACID OLIGOMERS BY LIQUID SIMS

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Galacturonic acid oligomers (Gala) are derived from pectin, which are major components of plant cell walls. The generalized structure of a Gala oligomer is shown in Fig. 1. Galacturonic acid oligomers are believed to be involved in fruit maturation (1) auxin-induced stem elongation (4) and as potential elicitors for resistance to pathogens (3). In order to facilitate the study of these compounds, liquid SIMS was investigated as a possible tool for the rapid determination of molecular weight distribution of mixtures of Gala oligomers. Negative ion LSIMS spectra have been reported for underivatized Gala oligomers up to ten residues in length (4). These spectra are characterized by intense matrix peaks due to solvent suppression effects and a rapid decrease in peak intensity as a function of mass. We have developed a procedure for the molecular weight determination of mixtures of underivatized Gala oligomers up to 15 sugar residues in length using positive LSIMS.

Mixtures of Gala oligomers were produced by autoclave hydrolysis and characterized by anion exchange HPLC (5). A chromatogram showing the relative concentrations of Gala oligomers used for this work is shown in Fig. 2. Liquid SIMS was performed on a VG Analytical ZAB2-SE mass spectrometer equipped with a Cs⁺ primary ion gun operated at +30 kV. Samples were first dissolved in water, 10% trifluoroacetic acid (TFA) or 10% acetic acid (HAc) (10 ug/ul). Approximately one microliter of the oligosaccharide solution was then mixed with an equal amount of thioglycerol or NaCl/thioglycerol on the probe tip immediately prior to data acquisition.

First investigations involved the acquisition of positive and negative ion LSIMS spectra using a variety of matrices. Figure 3. shows a positive ion LSIMS spectrum of the Gala mixture in water and thioglycerol. Molecular ions of oligomers up to 13 sugar residues in length are detected. For most oligomers, the (M+H)⁺, (M+Na)⁺ and (M+K)⁺ forms of the molecular ions are detected (see Fig. 3B). The samples, solvents and matrix used in the acquisition of this spectrum were all deionized using ion exchange resin prior to use. It is apparent that for Gala oligomers the ionization efficiency for sodiated molecular ions is significantly higher than for their protonated counterparts. It is also important to note that the relative intensities of the molecular ion peaks do not correspond to the relative concentrations of the oligomers in the mixture (see Fig. 2). The appearance of various molecular ions can be attributed to the fact that the pH of the sample solution is well above the pKa of the carboxylic acid functionality of the Gala oligomers resulting in the partial deprotonation of the molecule. Studies on the effect of pH of the positive-ion LSIMS spectrum of these oligomers shows that at low pH, the (M+Na)⁺ and (M+K)⁺ ions disappear. Dell (4) has reported similar results for negative ion LSIMS spectra of underivatized Gala oligomers. In positive ion mode, however, little increase in the intensity of the (M+H)⁺ peaks is observed. We have found that the best results are obtained when the sample is dissolved in a low pH medium—i.e. 10% TFA— followed by incorporation into a thioglycerol matrix containing NaCl. When no NaCl is added to the matrix, the (M+H)⁺ ion predominates but has little intensity. At higher pH (i.e. 5% NaAc) no molecular ion peaks are observed. As NaCl is added to the thioglycerol, the (M+Na)⁺ increases in intensity and at high NaCl concentrations the (M+2Na-H)⁺ ion begins to appear. Figure 4 shows the molecular ion region of the positive LSIMS spectrum of pentadeca-galacturonide (15mer) in 5% HAc incorporated into thioglycerol containing 0.02M NaCl. We believe that with the high efficiency of ionization of sodiated Gala oligomers, further optimization of pH and NaCl concentration will result in molecular ion detection well beyond 3000 m/z.

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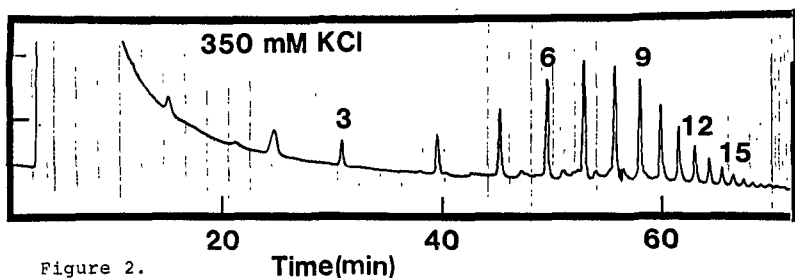


Figure 2.

POS-LSIMS GALA 350M IN WATER/THIO

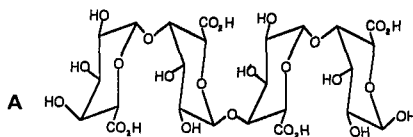
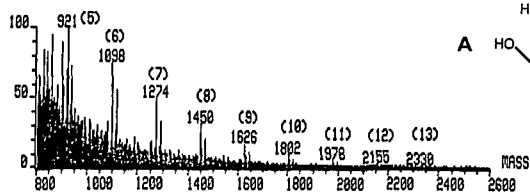


Figure 1.

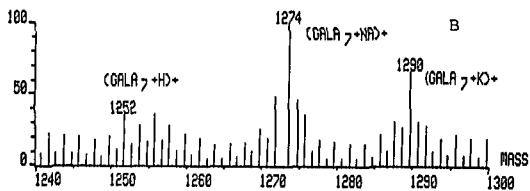


Figure 3.

POS-LSIMS PENTADECALACTUROSIDE IN 5% HAc/0.02M NaCl/THIO PT=

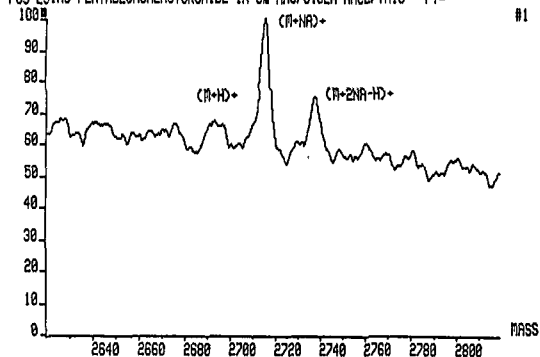


Figure 4

The authors wish to acknowledge the technical support of M. Perreria and the financial support of DOE (DE GFD5 ER13496), the OSU Agricultural Experiment Station and the NSF (Equipment grant BSS-870489).

CF-FABMS OF PROTEOLYTIC DIGESTS OVER TIME
FOR PRODUCING SEQUENCE ORDERED TRYPTIC MAPS

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CF-FABMS has been shown to be an effective tool for the analysis of the peptide fragments generated by proteolytic digestion (1). The use of CF-FAB for sample introduction into the mass spectrometer allows the facile monitoring of a proteolytic hydrolysis over time, enabling the analyst to correlate the release of many of the protease fragments from the original peptide structure (2). For a tryptic digest the mass spectra of reaction products from early time points in the hydrolyses reveal masses which are combinations of two or more tryptic fragments. These masses disappear from the spectra as the hydrolysis proceeds to completion but their presence early in the reaction allows the identification of adjacent tryptic fragments. For example, data taken from several time points in the tryptic hydrolysis of calcitonin allowed all four tryptic peptides of calcitonin to be located in the original structure relative to one another.

Carboxy-terminal sequence information can also be obtained by monitoring the reaction products of carboxypeptidase hydrolysis of whole peptides or tryptic fragments over time. The exopeptidase gives rise to fragments whose masses differ from one another by the exact mass of an amino acid residue. This mass difference allows identification of the amino acid present in the C-terminal position. Thus, the C-terminal fragment of calcitonin can be identified by determining the tryptic fragment whose C-terminal sequence matches that of the whole peptide. This information, combined with information from the tryptic digest of calcitonin, allows accurate mapping of all the tryptic fragments into their correct positions in the original peptide structure.

Tryptic digests of calcitonin were performed with trypsin-TPCK from Worthington at an enzyme to substrate molar ratio of 1:40. Carboxypeptidase Y from Sigma was used for C-terminal hydrolysis of calcitonin at an enzyme to substrated ratio of 1:27. Carboxypeptidase Y hydrolysis of calcitonin tryptic fragments was performed at an enzyme to substrated ratio starting at 1:250 and increasing up to 1:2. All proteolytic reactions were performed in 50 mM pyridine acetate (pH = 6.2). Aliquots of 5 μ L were removed at specified time intervals and were either quick frozen with dry ice or treated with 2N HCl to stop the enzymatic reaction.

All samples were analyzed by CF-FABMS performed on a Finnigan MAT 90 using the Finnigan MAT Bioprobe. A flow probe solvent of 5% glycerol/3% acetonitrile in water was employed at a flow rate of 7 μ L/min. Samples were injected via a Rheodyne valve Model 7410 fitted with a 1 μ L sample loop. The mass spectrometer was generally run with a resolving power of 1400 and an accelerating voltage of 4.8 kV.

Figure 1 shows the mass spectrum of the reaction products of the tryptic digest of 200 pmoles of calcitonin after 3 minutes. Spectra for aliquots taken 5 to 15 minutes into the reaction are identical to that seen in Figure 1, indicating that the masses 1122, 854, 777 and 716 represent the final products of the tryptic digest. These four masses, when combined, would yield a peptide of a molecular weight equal to that of calcitonin. In a 1 minute aliquot of the tryptic digest, several peaks were identified which each represent combinations of two tryptic fragments of calcitonin. The mass at 1613 is a combination of 854 and 777, while the mass at 1474 is a combination of 777 and 716. A peak was also found at m/z 1960 which is a combination of 1122 and 854. In addition, two peaks were identified which each represent a combination of three of the tryptic peptides of calcitonin. A peak at 2717 represents a combination of 1122, 854 and 777, while a peak at 2311 represents a combination of 854, 777 and 716. The

presence of these combinations indicates these tryptic fragments must be adjacent in the original calcitonin structure. This information allows the analyst to determine the order of the tryptic fragments in calcitonin as: (1122)(854)(777)(716) or (716)(777)(854)(1122). The identification of either 1122 or 716 as the C-terminal fragment enables one of these to be differentiated as the correct sequence.

The results of the digest of whole calcitonin by carboxypeptidase Y are shown in Figure 2. After 60 minutes a peak at m/z 3318.4 appears in place of the molecular species at m/z 3415. The difference between these masses is 97, and is equal to the mass of a proline residue. A digest of the tryptic fragments of calcitonin indicates the fragment with a mass of 716 also contains proline as its C-terminal amino acid, identifying it as the carboxy terminal tryptic fragment. This allows the correct tryptic map of calcitonin, (1122)(854)(777)(716), to be deduced.

The work presented here demonstrates the utility of incorporating the dimension of time into the analysis of protease digests. The mass specificity of mass spectrometry is critical in following the various fragments in multiple sample analyses. The dimension of time permits the analyst to observe combinations of tryptic fragments which allow them to be linked together in the original sequence. This results in the deduction of an ordered tryptic map which is useful for the quick and accurate characterization or verification of protein structure.

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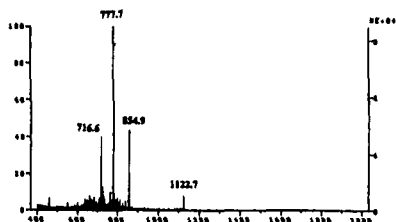


Figure 1. Three-minute tryptic digest of calcitonin.

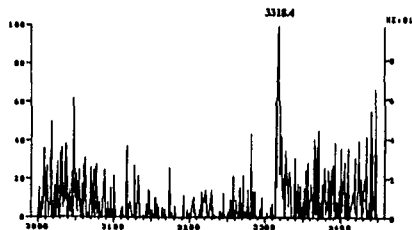


Figure 2. 60 minute carboxypeptidase Y digest of calcitonin.

TANDEM MASS SPECTROMETRY OF LIPOSIDOMYCIN ANTIBIOTICS

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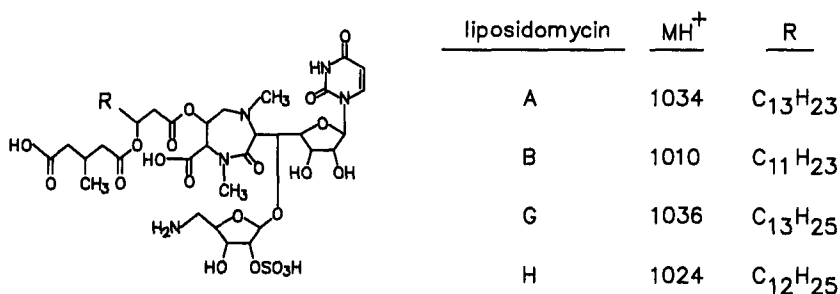
The liposidomycins are a family of novel nucleoside antibiotics which strongly inhibit bacterial cell wall synthesis¹. These lipid-containing nucleosides have several unusual structural components such as a seven membered heterocycle and a sulfate moiety. As part of the complete structure determination of the liposidomycins², MS/MS proved useful in establishing the interconnectivity of structural subunits by determining detailed decomposition pathways from a series of collision-induced dissociation (CID) reactions. Mass spectrometry (including high resolution and GC/MS of hydrolysis products) was used in concert with 2-D NMR to establish intermediate structural models and to confirm specific structural features. The present study also demonstrates the utility of tandem mass spectrometry as an efficient method for screening samples (or fractions collected by HPLC) to identify molecular ions, common structural features, and modifications of these antibiotics. Low-energy CID spectra were acquired with a VG 70-SEQ instrument (EBQQ geometry) using argon or krypton as collision gas with incident ion translational energies of 15 to 50 eV, and FAB ionization with glycerol matrix.

Liposidomycins A, B, G, and H were analyzed by MS/MS and CID spectra were obtained from molecular ions and several fragment ions appearing in primary mass spectra of these compounds. Decomposition pathways established from these CID reactions showed losses corresponding to structural components of sulfate, an amino sugar, uracil, ribose a seven membered heterocycle, and a complex fatty acid (Fig. 1). The four liposidomycin structures were found to differ only in the fatty acid side chain, R. For instance, a net structural difference of C₂ in liposidomycin A, compared with liposidomycin B, was determined to be located in the fatty acid based on shifts of 24 mass units in the CID spectra and on high resolution data.

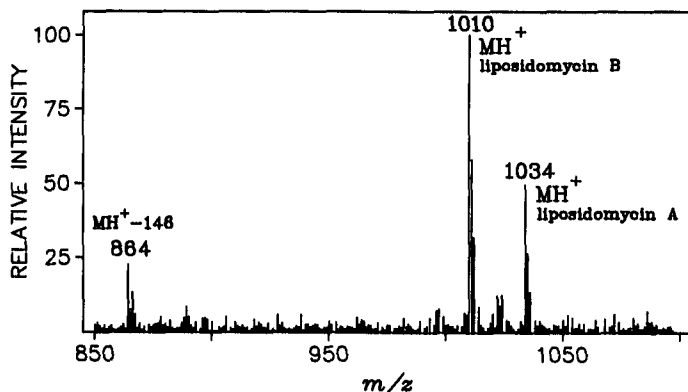
Reaction pathways were also instrumental in determining how all of the components are interconnected in the antibiotics. For example, the ribose component in liposidomycin B was determined to be bound between uracil and the seven membered heterocycle as indicated by ions at m/z 555 (fatty acid and seven membered heterocycle) and m/z 315 (seven membered heterocycle and ribose) in the CID spectrum of m/z 687. Ions at m/z 315 and m/z 183 (seven membered heterocycle) in the CID spectrum of m/z 427 support uracil as being bound directly to ribose. MS/MS analysis in the negative ion mode was essential for the placement of the sulfate moiety on the amino sugar and attachment of the amino sugar to the seven membered heterocycle.

The liposidomycins readily lose SO₃ from the sulfate moiety to form an intense MH⁺-80 ion. This unusual mass loss was utilized to screen several HPLC fractions collected from *Streptomyces* cell extracts by scanning for a neutral loss of 80 mass units. Molecular ions were identified for liposidomycins A, B, G, and H (e.g. Fig. 2) as well as several new modified liposidomycins which were otherwise difficult to

determine from their primary mass spectra. For screening biological samples, neutral loss experiments such as this can be used as a relatively efficient method for testing specific differences in structurally similar antibiotics or other natural products which are potentially biologically active.



(Fig. 1) Structures of liposidomycins A, B, G, and H.



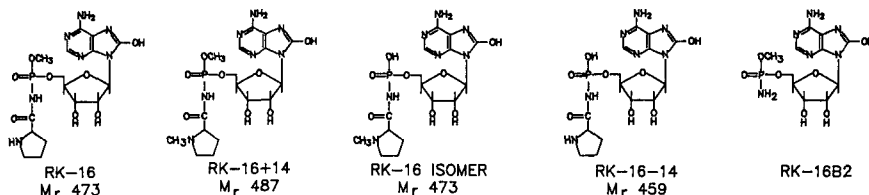
(Fig. 2) Neutral loss scan for loss of 80 amu (SO₃), liposidomycins A and B.
This work was supported by NIH grant GM 29812.

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STRUCTURE DETERMINATION OF A NOVEL ANTIFUNGAL NUCLEOTIDE ANTIBIOTIC

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RK-16 is an antifungal nucleotide antibiotic which occurs in *Streptomyces durhamensis*. RK-16 cannot be easily isolated, making structure determination difficult. FAB of the isolate shows only one molecular ion, RK-16 (MH^+ 474). No other molecular ions were observed contrary to expectations from HPLC data, indicating possible ion suppression by RK-16. It is believed that RK-16 readily isomerizes to form what has been designated "RK-16 Isomer". Microscale isopropylidene derivatization of RK-16 followed by FAB shows m/z 474 shifts to m/z 514 indicating the presence on one cis-diol. RK-16-14 and RK-16+14, not detected previously, are easily seen in the FAB spectrum due to enhanced surface activity conferred by the hydrophobic isopropylidene group. MS/MS of the isopropylidene products indicate the cis-diol is contained in the sugar. Deuterium exchange experiments using [3H]NBA (V.V. Mykytyn, A.M. Reddy and K.H. Schram, 36th ASMS Conference, 171 (1988)) indicate the number of active hydrogens in RK-16 is seven. MS/MS of MD⁺ demonstrated that two of the active hydrogens reside in the side-chain, two in the sugar, and three in the base.

Acid hydrolysis of the RK-16 isolate followed by isolation of the products and NMR studies show that RK-16 contains L-proline. Tandem mass spectrometry and NMR show RK-16 contains 8-oxoadenine. Base hydrolysis of RK-16 produces a product designated RK-16B2. Treatment of RK-16B2 with additional base at elevated temperature, produces a product which was compared to authentic 8-oxoadenosine by LC/MS. When hydrolyzed RK-16B2 is co-chromatographed with authentic 8-oxoadenosine, no peak broadening is observed thus confirming RK-16 contains 9-B-ribofuranosyl-8-oxoadenine.

MS/MS and LC/MS were used to overcome isolation problems by direct analysis of the nucleotide isolate. Detailed parent-daughter ion relationships were established by experiments involving mass selection and collision induced dissociation (CID) of MH^+ and its fragment ions, Figures 1 and 2. The ions m/z 115 and 129 in the CID spectrum of m/z 209 (figure 2) were the best indicators of the existence of an RK-16 isomer. Positive assignment of m/z 115 and 129 ions were accomplished only with the use of LC/MS which shows the ions come from the side-chains of RK-16, and RK-16 Isomer, respectively.

LC/MS of the isolate showed the presence of four major compounds: RK-16, RK-16 Isomer, RK-16-14 and RK-16+14. Traces of the side-chain ions m/z 115 (belonging to RK-16-14 and RK-16) and 129 (belonging to RK-16+14 and RK-16 isomer) show the difference between RK-16 and the RK-16 isomer to reside in the side-chain. Selected ion monitoring of the proline group m/z 70 and m/z 84 (methylated proline) demonstrated the RK-16 isomer and RK-16+14 contain a methylated proline whereas RK-16 and RK-16-14 do not.

Instrumentation:

Primary FAB spectra were acquired on a MAT 731 mass spectrometer using Xe gas. MS/MS data were acquired with a VG 70-SEQ (EBQQ geometry) instrument using FAB with Xe. Kr was the collision gas and the ion translational energy (lab frame) was 13-40 eV.

A Vestec 201 instrument with thermospray ionization was used for all of the LC/MS work, with a Supelcosil LC-18-S column. The LC system is that of Buck et al., Anal. Biochem. 129, 1 (1983) with a flow rate of 1.5 ml/min.

Derivatization:

1-5 ug of the nucleotide were placed in a capillary tube with 30 ul of 2,2-dimethoxypropane, to which 1 ul of concentrated HCl was added. The reactants were stirred for 5 min. and then dried using a Savant Speed Vac concentrator.

MS/MS of RK-16 ISOPROPYLDENE ISOMERS

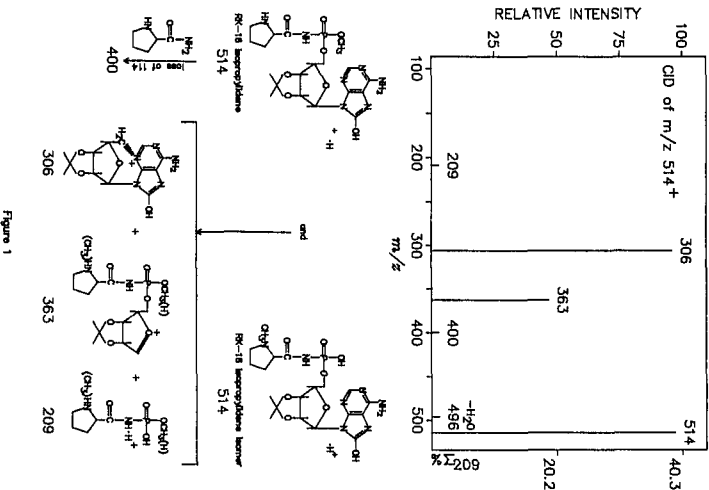


Figure 1

MS/MS of the SIDE-CHAIN of RK-16
ISOPROPYLDENE ISOMERS

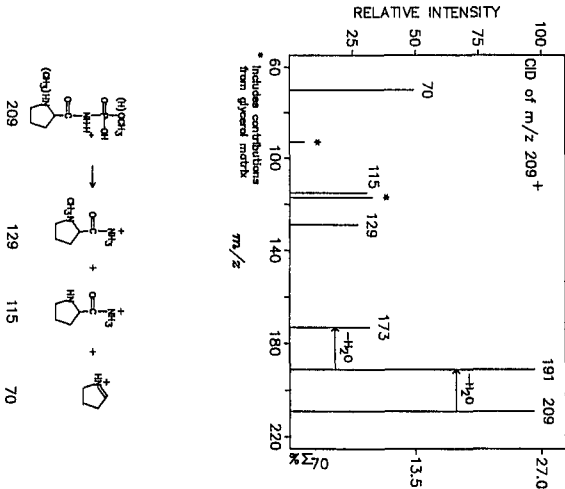


Figure 2

STUDIES OF OLIGONUCLEOTIDE IONIZATION BY CONTINUOUS-FLOW FAST ATOM BOMBARDMENT

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Continuous-flow fast atom bombardment (CF-FAB) enables the quantitative study of ion yields of oligonucleotides since the samples can be introduced directly into the source in a highly reproducible manner. In addition, the effects of hydrophobicity/hydrophilicity are reduced [1], thus reducing effects of relative surface activities.

It has been recognized for some time that the efficiency of ionization of oligonucleotides, by any ionization method, is lower than for peptides. This places greater limitations on nucleotide measurements by desorption mass spectrometry, both from the standpoint of sensitivity, and in terms of the maximum molecular size that can be studied. In order to gain a better understanding of the factors contributing to low ion yields with FAB ionization of oligonucleotides, a homologous series of oligonucleotides $[d(pA)_n, n = 1 \text{ to } 8]$ has been examined by CF-FAB and the ion yields compared to those of peptides of similar mass.

Ion yields of peptides depend strongly on both the molecular weight and the particular amino acid composition of the peptide considered. Naylor *et al.* found that peptides containing more hydrophobic residues generally are ionized more efficiently by FAB [2]. Table I shows that a typical dinucleotide ApC has a lower ion yield than both a very hydrophobic peptide, leu-enkephalin and a hydrophilic peptide, tuftsin. There is some improvement when negative ions are considered but there is still a 50-fold difference in ionization efficiency between leu-enkephalin and ApC.

TABLE I: PEPTIDES VS NUCLEOTIDES

	leucine enkephalin	tuftsin	ApC
Mr	555.3	500.3	571.2
Amt.	1 nmol	1 nmol	1 nmol
Hydrophobicity ^a	-596	+310	n.a.
RELATIVE PEAK AREAS ^b			
$[M+H]^+$	100	6	1
$[M-H]^-$	100	c	2

leucine enkephalin: Tyr-Gly-Gly-Phe-Leu

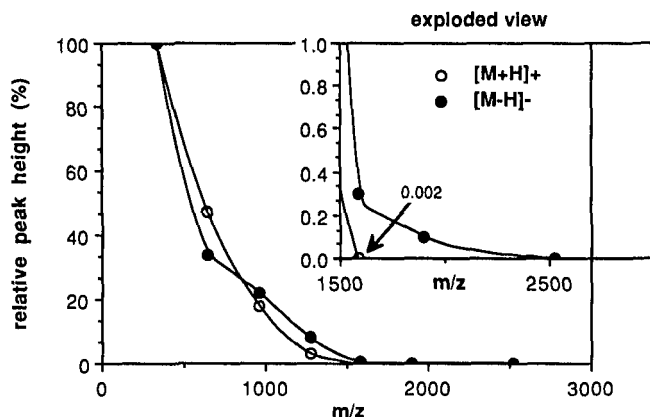
tuftsin: Thr-Lys-Pro-Arg

a. Calculated according to the indices for amino acids given in [3]. The more negative the index the more hydrophobic the peptide.

b. Normalized to leu-enkephalin, the ratio of responses $[M+H]^+ / [M-H]^-$ is not addressed.

c. Not measured.

FIGURE 1. Ion yields of both $[M+H]^+$ and $[M-H]^-$ ions of the oligonucleotides $d(pA)_n$, $n = 1$ to 8 (5 nmol). In each case the peak heights have been scaled to that of the mononucleotide pA. The yields of pA $[M+H]^+$ and $[M-H]^-$ ions are of the same order of magnitude.



We have found much less variation in ion yields of nucleotides of similar mass compared with peptides. This is an expected result in as much as the structural features of nucleotides are not as diverse as those of amino acids. There is some correlation of hydrophobicity for mononucleotides, with the greatest ion yields measured for the most hydrophobic of the common nucleotides, pA. Fig. 1 shows the dramatic decrease in ion yields as the number of nucleotide residues considered is raised. This clearly demonstrates the increasing difficulty with FAB ionization of large oligonucleotides.

The differences in ion yields of oligopeptides and oligonucleotides may be attributed to the substantial structural differences between the two classes of biopolymers. For oligonucleotides there are more solvating matrix molecules (up to 14 per residue for peptides compared with 20 per nucleotide unit), with protic solvents or glycerol matrix bound by unusually strong hydrogen bonds to the phosphate groups. Normal hydrogen bonds are of the order of 3-6 kcal/mol whereas bonds between water molecules and ionized phosphate groups have been estimated to have energies up to 10 kcal/mol [4]. Thus it is reasonable to assume that the energy required for desolvation during ionization of oligonucleotides will be substantially greater than for oligopeptides of similar size. The greater hydrophilicity of oligonucleotides also may reduce the concentration of molecules at the surface of the matrix, leading to reduced ion yields. In addition, the polyanionic phosphate backbone of oligonucleotides may be difficult to protonate during vaporization (solution $pK \sim 1$), although the thermochemical gas phase acidity of organic phosphodiester is presently unknown. Finally, steric constraints may limit the ability of oligonucleotides to self-solvate in the gas phase compared with peptides, thus contributing to lower ion yields for oligonucleotides.

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FAB-MS VS TIME-OF-FLIGHT SIMS: NEGATIVE ION STUDIES OF O-ALKYL DI- AND TRINUCLEOTIDES

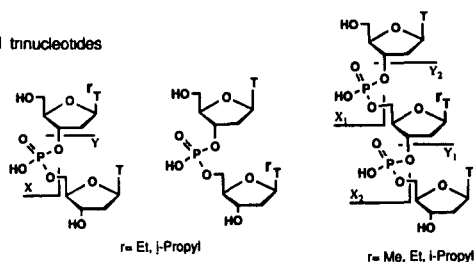
E. Lafortune*, W. Buchannon, G. W. Buchko, F. E. Hruska,
K. L. Sadana, K. G. Standing and J. B. Westmore,
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INTRODUCTION

O-alkylation of nucleic acids and their precursors currently generates much attention in view of its possible role in mutagenesis and carcinogenesis. It is known, for example, that the mutagenic and carcinogenic potential of alkylating agents correlates with their tendency to attack oxygen (1) and with the size of the alkyl group (2). To obtain information about the effects of alkylation on nucleic acid components in solution and in a solvent-free environment, we have analyzed a series of O4-alkylated di- and trinucleotides by fast atom bombardment (FAB) as well as by time-of-flight (TOF) secondary ion mass spectrometry (SIMS) (3,4).

These compounds include two pairs of isomeric dinucleotides, $d(r^4TpT)$ and $d(Tpr^4T)$, where $r = Et$ or $i-Pr$, as well as three trinucleotides, $d(Tpr^4TpT)$, with $r = Me, Et$ or $i-Pr$ (Fig. 1). We here outline some similarities and differences observed between negative ion mass spectra of these modified nucleotides, obtained by TOF-SIMS and FAB-MS.

Figure 1: O4-alkyl di- and trinucleotides



EXPERIMENTAL

1. Sample preparation:

For FAB-MS analysis, the titled compounds were dissolved in glycerol at a concentration of *ca* 10 mg/ml. A few μ L of each solution were then deposited on a stainless steel sample probe-tip. For TOF-SIMS analysis, the compounds were dissolved in a mixture of methanol and water (80:20) to give a final concentration of *ca* 1 mg/ml, and a few μ L of the solutions were deposited on aluminum foil treated to give a boehmite, $AlO(OH)$, surface (5).

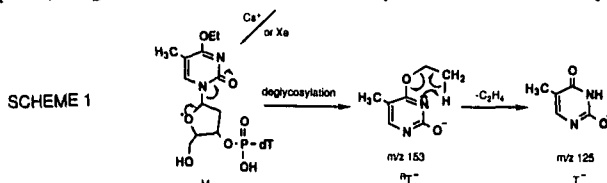
2. Mass spectrometry:

Negative FAB mass spectra were recorded with a VG 7070E-HF double focussing mass spectrometer. Samples were bombarded by 8 keV xenon atoms generated in a Saddle-field neutral beam gun, and the negative ions produced were accelerated to 6 keV. Negative TOF-SIMS mass spectra were recorded on the Manitoba TOF II mass spectrometer, which incorporates an ion mirror (3). Sample targets were bombarded by a pulsed beam of 28 keV Cs^+ primary ions and the negative secondary ions produced were accelerated to 10 keV.

RESULTS AND DISCUSSION

1. Dimers:

For the two pairs of positional isomers, several features that allow unequivocal indications of isomeric form were found to be common to both FAB and TOF-SIMS mass spectra. First, all compounds show intense quasimolecular ion signals; the relative intensity of these peaks is always greater when the alkyl group is on the 3'-end nucleobase. Moreover, the loss of a nucleobase (TH or ¹TH) from the [M-H]⁻ ion is always more prevalent from the 3'-end than from the 5'-end of the molecule. Additionally, the ratio 4-alkylthymine/thymine anions is always greater when the alkyl group is on the 3'-end thymine, an indication that dealkylation occurs more slowly and/or that deglycosylation (scheme 1) is taking place more rapidly for these compounds. On the other hand, notable differences between TOF-SIMS and FAB mass spectra include a greater of 5'-phosphate/3'-phosphate ratio of sequence ions (i.e. Y⁻/X⁻, see Fig. 1) in TOF-SIMS spectra, and greater loss of a nucleobase from sequence ions in FAB mass spectra.



2. Trimers:

We have noticed more differences than similarities between TOF-SIMS and FAB mass spectra of the trimers with the central nucleobase alkylated at the O4- position. Some similarities include (i) a $(X_1 + Y_1)/(X_2 + Y_2)$ ratio of sequence ions that is greater than one in all instances, meaning that the sugar-phosphate bonds on the central nucleoside are more readily cleaved than those on terminal nucleosides, and (ii), the ratio $^1\text{T}^-/\text{T}^-$, understandably smaller for trimers than for dimers, becomes even smaller with bulkier alkyl groups such as ethyl and i-propyl.

As to differences observed between TOF-SIMS and FAB spectra of these modified nucleotides, we have found that fragmentation of quasimolecular ions takes places to a much lesser extent under FAB conditions. A second major difference is the presence of sodiated quasimolecular and sequence ions under SIMS conditions. Interestingly, only those ions that contain the central alkylated nucleoside were found to be sodiated, leading to e.g. $[\text{M}-2\text{H}+\text{Na}]^-$, $[\text{X}_2 \text{ (or } \text{Y}_2)\text{-H}+\text{Na}]^-$, and not X_1^- or Y_1^- . These observations support our earlier findings that suggest an increased stability for thymidines alkylated at the O4-position (6).

This negative ion studies of O-alkyl di- and trinucleotides has provided many examples of the complementary nature of FAB-MS and TOF-SIMS. Each of these "soft" ionization techniques has not only allowed full characterization of these modified nucleotides, but has revealed clear indications of the site of alkylation in addition to its effect on fragmentation patterns.

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THE IDENTIFICATION OF 5,6-DIHYDROURIDINE IN NORMAL HUMAN URINE
BY COMBINED GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Partial purification and isolation of 5,6-dihydrouridine (D; Figure 1) from normal human urine by boronate gel affinity chromatography and reversed-phase HPLC preceded its characterization by combined GC/MS techniques. Structure proof is based upon a comparison of mass spectral and chromatographic features of the urinary component to those of an authentic reference sample. Additional data derived from high resolution mass measurements and deuterium isotope-labeling experiments provide confirmation of fragment ion structures.

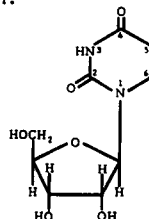


Figure 1.

A pooled normal urine (PNU) sample obtained from more than 300 normal male and female subjects at the PL Osaka Health Control Center was used in the analysis. The boronate gel affinity chromatography methods used to isolate the urinary nucleosides were a modification of a published procedure (1). A Shimadzu HPLC system equipped with uv detection (260 nm) was used in the isolation of the individual nucleoside fractions. Analytical and preparatory HPLC procedures used a 5 μ m Develosil ODS-5 reversed phase column and the following solvent gradient (v/v; time in parentheses): water (0 min); 13:87 methanol-water (25 min); 45:55 methanol-water (35 min); water (40 min). Each HPLC fraction was lyophilized and TMS derivatives prepared by heating in a 50 μ l solution of BSTFA/TMCS and pyridine (80:20 (v/v)) for one hour at 100 $^{\circ}$ C (2). GC/MS analyses were performed using a Varian Model 3400 gas chromatograph coupled to a Finnigan MAT90 double-focusing (BE) instrument. Sample introduction was via splitless injection onto a 30 m fused silica DB-5 capillary column heated from 150 $^{\circ}$ C to 300 $^{\circ}$ C at 6 $^{\circ}$ C min $^{-1}$. Standard low resolution, EI mass spectrometer conditions were used for detection.

For several of the HPLC-separated fractions, preliminary identification of the major constituent(s) was made by comparing the HPLC retention times and uv absorption spectra of the urinary components to reference samples. For example, the fraction eluting at 6.63 min (denoted PNU/1) exhibited an absorbance maximum at 257 nm, which corresponded to that of the modified nucleoside m 1 A (HPLC retention time 6.13 min). However, a GC/MS examination of the TMS derivative of PNU/1 revealed the presence of several possible nucleosides in addition to m 1 A. One apparent nucleoside, having a GC retention time of 21.73 minutes, was given the designation PNU/1/A for identification purposes.

The 70 eV EI mass spectrum of PNU/1/A is shown in Figure 2. Structural assignments and mechanistic origins for most of the molecular- (M), sugar- (S) and base-related (B) ions have been described (3). Molecular weight information was obtained by examining the [M] $^{+}$ and [M-15] $^{+}$ regions, the latter ion formed via the loss of a methyl radical. Although a molecular ion was not observed, the molecular weight of the sample was assigned as 534 Da on the basis of a [M-CH $_3$] $^{+}$ ion at m/z 519 (2.7% RI). Other molecular weight-related ions detected were: [M-CH $_2$ OTMS] $^{+}$ (m/z 431, 0.6%), [M-CH $_3$ -TMSOH] $^{+}$ (m/z 429, 0.2%) and [M-(TMSOH) $_2$] $^{+}$ (m/z 354, 0.7%). Ions normally associated with a ribose-(TMS) $_3$ sugar were observed, namely [S-H] $^{+}$ (m/z 348, 0.6%), [S-TMSOH] $^{+}$ (m/z 259, 1.6%), [S-H-CH $_2$ OTMS] $^{+}$ (m/z 245, 2.5%), [S-O-TMSOH] $^{+}$ (m/z 243, 2.9%), [C $_4$ H $_4$ O $_2$ (TMS) $_2$] $^{+}$ (m/z 230, 4.7%) and [C $_3$ H $_5$ O $_2$ (TMS) $_2$] $^{+}$ (m/z 217, 100%). Subtraction of the mass of the ribose

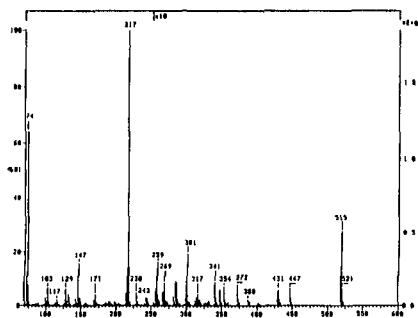


Figure 2

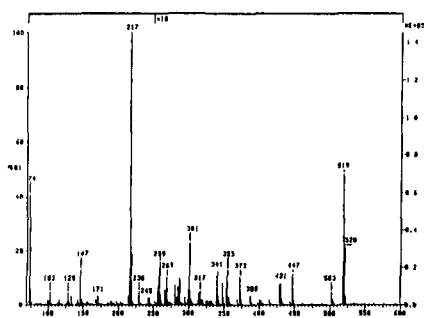


Figure 3

(349 Da) from the suspected molecular weight of the unknown (534 Da) gave an aglycone mass of 185 Da, suggesting a maximum incorporation of one TMS group in the base. Analysis of the TMS-²H₉ derivative of PNU/1/A confirmed the presence of three TMS groups in the sugar and one in the base. The assignment of 185 Da for the weight of the aglycone was substantiated by other ions commonly associated with a pyrimidine base: [B+C₂H₂O(TMS)₂]⁺ (m/z 373, 0.8%), [B+C₂H₃O₂TMS]⁺ (m/z 317, 0.4%), [B+C₂H₃OTMS]⁺ (m/z 301, 1.9%), [B+CHOTMS]⁺ (m/z 287, 0.9%), [B+C₂H₂OTMS-CH₃]⁺ (m/z 285, 0.9%), [B+H+TMS]⁺ (m/z 259, 1.6%), [B+TMS-CH₃]⁺ (m/z 243, 2.9%), [B+H+CHO]⁺ (m/z 215, 4.6%), [B+CH]⁺ (m/z 198, 1.1%) and [B+H-CH₃]⁺ (m/z 171, 4.1%). Subtraction of 72 Da, the weight of one TMS group less a hydrogen, from 185 Da gave 113 Da as the weight of the free base, which was indicative of 5,6-dihydrouracil. Comparison of the mass spectrum of PNU/1/A with a library spectrum of 5,6-dihydrouridine-(TMS)₄ resulted in a close match. Final confirmation of identity was obtained by comparing the GC and mass spectral behavior of PNU/1/A with that of an authentic reference sample of 5,6-dihydrouridine-(TMS)₄. The GC retention time for the urinary component was 21.73 minutes while the reference compound had a retention time of 21.32 minutes (1.9% error). The low resolution EI mass spectrum of D-(TMS)₄ (Figure 3) exhibited fragmentation and ion intensities essentially identical to its urinary counterpart. Therefore, on the basis of strong similarities in GC retention times and mass spectra between the reference compound and PNU/1/A, the identity of the unknown urinary component was established as 5,6-dihydrouridine.

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GC/MS ANALYSIS OF SPE ISOLATED URINARY NUCLEOSIDES. Victoria V. Mykytyn and Karl H. Schram. University of Arizona, College of Pharmacy, Tucson, AZ, 85721.

Modified nucleosides released during the process of tRNA turnover are not reutilized, but rather excreted intact in urine. Although normal subjects excrete low and constant levels of modified nucleosides, there appears to be an increase in excretion levels of certain modified nucleosides in a wide variety of cancer patients.¹ Previous methods used for the isolation of urinary nucleosides have been time-consuming and, in general, identification and quantitation of these compounds has been based on the use of non-specific detectors.² In addition, isolation procedures that expose base sensitive nucleosides to an alkaline environment may lead to structural modification. For example, Wilson and McCloskey have reported that 1-methyladenosine converts to N⁶-methyladenosine at pH 11.³ Likewise, 3-methyluridine has been shown to be unstable in the presence of Dowex-1 (borate) at pH 9.0.⁴ In order to avoid conditions which may result in the alteration of structure in base sensitive nucleosides, a solid phase extraction (SPE) procedure⁵ has been developed for the simple and rapid isolation of 1-methyladenosine (m¹A), 1-methylinosine (m¹I) and 1-methylguanosine (m¹G) from human urine under neutral conditions.

Human urine (pH 7.0) was centrifuged at 12,000 X g for 10 minutes at 4°C. A 3.0 ml aliquot containing 10 ug of 1,N⁶-ethenoadenosine as the internal standard was then transferred to a 6 ml, 500 mg C-18 SPE column. Under vacuum, the nucleosides retained in the column were washed with 500 ul of distilled water. GC/MS analysis showed that none of the compounds of interest were eluted in the water wash. The nucleosides were then eluted with 3 X 500 ul of methanol. m¹A, m¹I and 1,N⁶-ethenoadenosine had a recovery of 60% while m¹G showed a 100% recovery (n=3). The methanol was then evaporated under a stream of dry nitrogen. The sample residues were derivatized with 10 ul of pyridine + 40 ul of BSTFA containing 1% TCMS at 100°C for 1 hour.

Samples were analyzed by GC/MS using a Finnigan MAT 90 mass spectrometer coupled to a Varian 3400 GC. For capillary GC, a 30m DB-5 column was used. Injections were made in the splitless mode using helium as the carrier at a head pressure of 10 psi. The injection port temperature was 300°C. The column temperature was programmed from 150 to 300°C at a rate of 6°C/min. The transfer line to the mass spectrometer was maintained at 300°C. Electron ionization was employed at a source temperature of 250°C. The scan range was 450-600 daltons at a scan speed of 1 sec/dec. The ion chromatograms of the most intense ion from each compound were plotted. These were m/z 498 for m¹I, m/z 507 for 1,N⁶-ethenoadenosine, m/z 585 for m¹G and both m/z 554 and 482 for m¹A which represent the TMS₄ and the TMS₃ derivatives, respectively.

Quantitation was achieved using the peak area ratios of each compound to the internal standard followed by linear regression analysis. Calibration curves were very reproducible with correlation coefficients of 0.999 for m¹I, 0.992 for m¹G and 0.980 for m¹A (n=9). (See Fig. 1) The lower limit of detection is approximately 6 ng injected.

Table 1 lists the mean concentration of each nucleoside isolated from the urine of 6 normal subjects. The concentrations of these same compounds in a pooled urine sample of 300 normal subjects are also shown. The values from the pooled urine sample represent the average values from 7 replicate aliquots. Table 1 compares the values from the current study to those published by Chheda, et al.. (Units converted for direct numerical comparison.)

The C-18 SPE procedure described is simple, rapid and avoids conditions which may alter the structure of pH sensitive nucleosides. Specifically, m¹A, m¹I, m¹G and 1, N⁶-ethenoadenosine can be isolated from human urine with good recovery and reproducibility. Precise quantitation of m¹A, m¹I and m¹G by GC/MS is easily performed.

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Figure 1. Standard curves for urinary nucleosides.

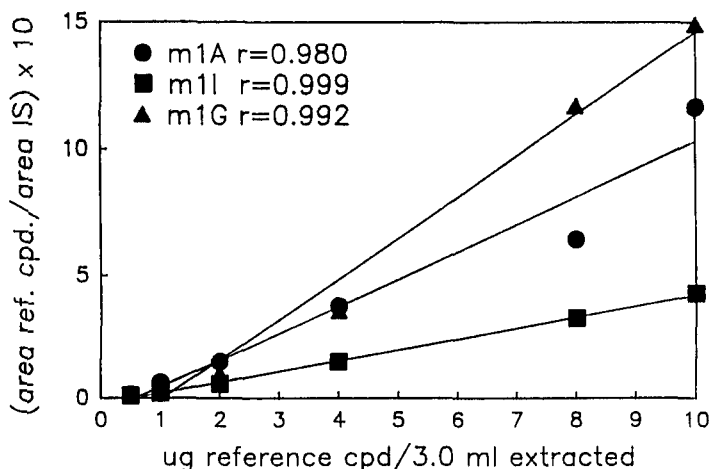


Table 1.

Urinary Nucleosides Derived from tRNA

Compound	ug/3.0 mls, Published ^a	ug/3.0 mls ^b	ug/3.0 mls, PNU ^c
N ¹ -Methyladenosine	14.26 - 14.33	18.87	15.52 (n=6)
N ¹ -Methylinosine	2.65 - 2.72	10.60	7.95 (n=8)
N ¹ -Methylguanosine	3.00 - 3.51	4.50	4.51 (n=6)

^a Derived values from Chheda, G.B., et al. in "Chromatographic and Other Analytical Methods in Nucleic Acid Research", Vol. II, C.W. Cherké and K.C. Kuo, Eds., Elsevier Science Publishers, Amsterdam, The Netherlands (1989) in press.

^b Values represent mean of 6 normal subjects.

^c PNU= Pooled Normal Urine: represents pooled urine from 300 normal subjects. n value is number of replicates analyzed from pool. Pooled sample obtained from: Nakano, K., Yasaka, T., PL Comprehensive Research Institute, Osaka, Japan.

Structural Characterization of Alkyl and Hydroxyalkyl DNA Bases
as Pentafluorobenzyl Derivatives

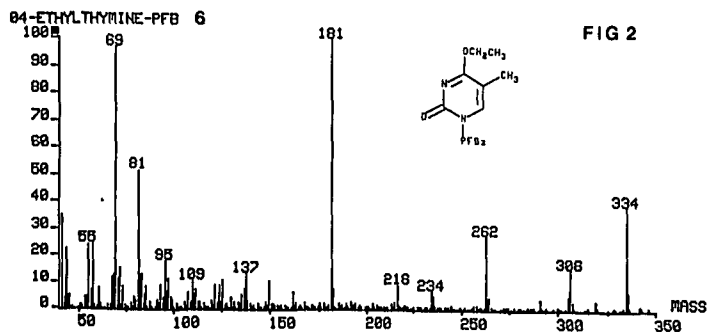
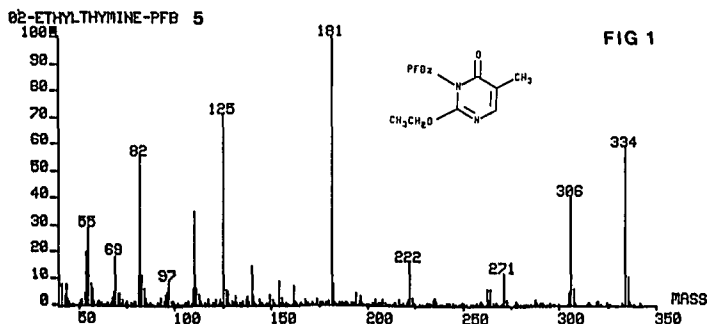
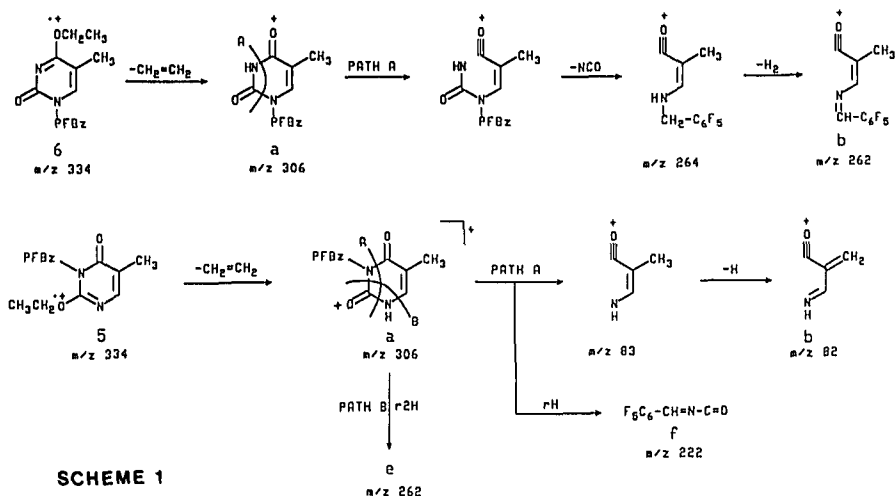
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The use of electrophore labeling to increase the sensitivity of analytes to electron capture MS is well established. In the field of nucleic acid chemistry detection limits as low as 1 femtogram have been reported for perfluoroalkyl and perfluoroacyl derivatives of nucleobases under ECNI-MS conditions. Pentafluorobenzyl derivatives (PFBz) of DNA bases are easily prepared under mild conditions and thus are suitable as a general electrophoric derivative. In this study, a number of alkyl and hydroxyalkyl modified DNA bases have been prepared as PFBz derivatives and their EI spectra investigated for structural utility. Fragmentation pathways were investigated using deuterium labeling, high resolution accurate mass measurements, and metastable analysis. The chromatographic properties and sensitivity of these derivatives under GC-ECNI-MS conditions was also investigated.

While in general the electron impact fragmentation patterns of the PFBz derivatives follow the modes described by previous researchers for the common nucleobases, the PFBz group exerts some influence on the fragmentation, especially in the case of bis PFBz derivatives. Furthermore, modification of the bases via substitution at various heteroatoms induces some alternative cleavages. Common to all of the derivatives is the loss of the PFBz group with the charge being shared by either fragment. The PFBz ion at m/z 181, which is always one of the most intense peaks in the spectrum, subsequently undergoes loss of HF to produce a low abundance ion at m/z 161. Low abundance ions arising from the loss of F or HF from the molecular ion are also common. Molecular ion peaks for the PFBz derivatives are typically well defined. Fragment ions yield information about both the final derivative and the starting nucleobase. In general the positive charge tends to remain with the base fragments, and rearrangement ions are of low intensity. In fact even for those ions formed by a rearrangement process, the resulting charged species still contains the nucleobase. This contrasts with the behavior of the commonly employed TMS derivatives which are highly prone to rearrangements. In the latter, the charge is frequently localized on the TMS function, so that the spectra are generally rich in TMS fragment ions.

The complete mass spectra of the isomeric compounds O^2 - and O^4 -ethylthymine as PFBz derivatives are shown in Figures 1 and 2. While the spectra of the underivatized compounds differ by only one ion, several distinct differences in the spectra of the derivatives permit the complete characterization of these compounds. Both derivatives lose $CH_2=CH_2$ from the molecular ion to yield an ion a, as shown in Scheme 1. A retro Diels Alder cleavage proceeding through the preferred N3-C6 and C2-N1 bonds (path A) results in two distinctly different ions. Subsequent ejection of two hydrogens yields the highly conjugated and stable ions b. In the O^4 isomer this ion contains the PFBz group and thus appears at high mass (m/z 262), while its counterpart, O^2 does not have the PFBz and consequently appears at a low mass (m/z 82). That some loss of HNC O also occurs in the O^4 compound is evidenced by low intensity peaks at m/z 262 (ion e) and 264 which presumably result from the alternative pathway B (cleavage through the N3-C $_4$ and N1-C2 bonds). Pathway A, which produces ion b in the spectrum of O^2 also results in formation of a complementary ion $C_6F_5CH_2NCO$, which upon further loss of a hydrogen yields ion f at m/z 222.

These derivatives exhibit good chromatographic properties and can be detected at the low femtogram level using ECNI-GC-MS and single ion monitoring conditions.



FAB MASS SPECTRA OF SOME NATURALLY OCCURRING MODIFIED NUCLEOTIDES AND THEIR ANALOGS. S.P. Dutta, J. Wesolowski and G.B. Chheda, Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York 14263

Fast Atom Bombardment (FAB) mass spectrometry has been proven to be of major importance in the analysis of polar and thermally labile biological molecules such as nucleotides, peptides and oligosaccharides. The ease of sample preparation coupled with the production of normally intense quasimolecular ion $(M+H)^+$ or $(M-H)^-$ and other structurally significant fragment ions make FAB a method of general utility in the analysis of biomolecules. In our laboratory we have been interested in the characterization and quantitation of the naturally occurring modified and chemically altered nucleotides of nucleic acids. With a view to developing a mass spectrometric detection and assay method for the modified nucleotides present in nucleic acid hydrolysates, we have examined the FAB mass spectra of some of the modified nucleotides present in tRNA.

The modified nucleotides were prepared synthetically in our laboratories or obtained from commercial sources. Approximately 1-2 μ g of the sample nucleotide was dissolved in 0.5 μ l of a 1:1 mixture of glycerol (containing 10% water) and thioglycerol and placed on the FAB target. Mass spectrometric analyses were carried out on a Finnigan MAT 90 mass spectrometer equipped with an Ion Tech 11NF saddle field gun. Xenon (99.995%) was used as the primary beam gas and the FAB Gun was operated at 8 kV potential and 2-4 mA emission current. Low resolution spectra (Res. 2000-2500) were recorded at ambient temperature with a scan rate of 20-30 s/decade.

The spectra of these nucleotides exhibited strong quasimolecular ions $(M+H)$ or $(M-H)$ in addition to other characteristic fragment ions. The positive ion FAB spectra (Table 1) showed strong peaks for $(M+H)^+$ and for $(B+H)^+$, S^+ , unmodified compound+H (5'-AMP or 3',5'-cAMP), adenine+H and in many cases a dimeric peak $(2M+2H)^+$. The negative ion spectra (Table 2) showed a very strong $(M-H)^-$ peak (which in most cases was the base peak in the spectrum after subtraction of the matrix peaks). In addition to that other peaks in the negative ion spectrum were due to B-H, unmodified compound-H (5'-AMP, 3',5'-cAMP), adenine-H and in some cases a dimer peak at $2M^-$. The fragmentation pattern were in general agreement with that shown earlier^{1,2}. In general the FAB spectra in the negative ion mode were much cleaner and showed less fragmentation than the spectra in the positive ion mode. The negative ion spectra did not show any adduct ions such as $(M-H+Na)^-$ or $(M-H+K)^-$. The FAB spectra of the NH_4^+ salts of these 5'-nucleotides or 3',5'-cyclic nucleotides generally lost NH_3 and ionized as the free acid.

This paper describes the FAB mass spectra of some of the modified nucleotides present in tRNA, some other analogous compounds and the corresponding 3',5'-cyclic nucleotides.

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Table 1 Comparative intensities of the (M-H)⁺ and other fragment ions (m/z) in the negative ion FAB mass spectrum of 5'-nucleotides and 3',5'-cyclic nucleotides.

Compound	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)
1 5'-AMP	347.8 (82)	136 (100)					136 (100)
2 5'-GMP	467.2 (61)	171.9 (100)					171.9 (100)
3. M ⁺ -Benzyladenosine-5'-monophosphate	428.2 (31)	469.4 (9)	275.6 (23)	212.7 (10)	348.1 (21)		375.9 (100)
4. M ⁺ -Benzyladenosine-3',5'-cyclic monophosphate	420.1 (51)		225.8 (51)	194.7 (51)			335.9 (100)
5. M ⁺ -Methyladenosine-5'-monophosphate	362.0 (67)		149.9 (100)	212.8 (41)	345.2 (21)		
6. M ⁺ -Methyladenosine-3',5'-cyclic monophosphate	360.0 (100)		148.9 (100)	211.8 (41)			
7. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 5'-monophosphate (1:1)	423.5 (100)	515.6 (22)	201.0 (20)	212.8 (25)	348.1 (25)		
8. M ⁺ [(3,5-bisphosphoryl)-adenosine-5'-monophosphate (PMP)]	515.5 (100)	520.4 (6)	205.5 (6)		347.8 (6)		627.3
9. M ⁺ [(ε ²)-isopentenyladenosine-3',5'-cyclic monophosphate (c-UMP)]	348.2 (14)	420.2 (5)	205.5 (62)		329.8 (4)		796.3 (0.5)

Table 2 Relative intensities of the (M-H)⁺ and other fragment ions (m/z) in the negative ion FAB mass spectrum of 5'-nucleotides and 3',5'-cyclic nucleotides.

Compound	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)
1 5'-AMP	346.0 (100)	133.9 (60)					694.2 (11.5)
2 5'-GMP	465.9 (100)	156.1 (40)					725.8 (4)
3. M ⁺ -Benzyladenosine-5'-monophosphate	455.9 (100)	224.3 (14)	545.8 (136)		133.9 (15)		
4. M ⁺ -Benzyladenosine-3',5'-cyclic monophosphate	418.1 (100)	223.9 (11)	328.2 (5)				
5. M ⁺ -Methyladenosine-5'-monophosphate	359.9 (100)	147.9 (67)	245.9 (40)				
6. M ⁺ -Methyladenosine-3',5'-cyclic monophosphate	360.0 (100)	147.9 (54)	246.1 (35)		133.9 (37)		
7. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 5'-monophosphate (1:1)	491.2 (69)	278.9 (84)	345.5 (40)	7 (2.5)			
8. M ⁺ [(ε ²)-isopentenyladenosine-3',5'-cyclic monophosphate (c-UMP)]	414.0 (100)	201.8 (10)	246.1 (9)		134.0 (8)		829.6 (4)

Compound	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)
10. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 5'-monophosphate	433.3 (54)	455.5 (2.5)	225.9 (100)	212.8 (5)	348.2 (3)		865.9 (7)
11. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 3',5'-cyclic monophosphate	449.4 (5)	255.0 (5)	194.7 (5)	330.1 (4)			136.0 (100)
12. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 3',5'-cyclic monophosphate	415.1 (22)	437.2 (1)	225.8 (23)	330.1 (21)			136.0 (100)
13. M ⁺ -furfuryladenosine-3',5'-cyclic phosphate	410.2 (8)	421.3 (33)		194.8 (13)			

*p stands for the unmodified compound, i.e., 5'-AMP or 3',5'-c-UMP

Table 2 cont'd

Compound	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)	(M-H) ⁺ (R1)
9. M ⁺ [(ε ²)-isopentenyladenosine-3',5'-cyclic monophosphate (c-UMP)]	335.9 (82)	201.8 (42)	228.1 (13)	134.0 (14)			793.8 (48)
10. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 3',5'-cyclic phosphate	437.4 (100)	257.7 (5)	194.8 (18)	330.1 (48)			
11. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 3',5'-cyclic monophosphate	431.2 (100)	218.8 (5)	194.8 (21)	330.1 (22)			864.0 (10)
12. M ⁺ [(9-β-D-Ribofuranosyl)-purine-6-yl]-L-threonine 3',5'-cyclic phosphate	413.0 (100)	218.8 (10)	194.8 (16)	330.1 (48)			
13. M ⁺ -furfuryladenosine-3',5'-cyclic monophosphate	408.0 (100)	213.8 (35)	194.8 (6)	330.1 (6)			817.6 (2)

*p stands for unmodified compound

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COMPARISON OF THE MAJOR LIPIDS OF SCENT GLAND SECRETIONS FROM THE
WESTERN DIAMONDBACK RATTLESNAKE (*CROTALUS ATROX*)
AND DUMERIL'S GROUND BOA (*ACRANTOPHIS DUMERILI*)
BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY:
EVIDENCE FOR TAXONOMIC AND EVOLUTIONARY VARIATION.

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When disturbed by predators, many snakes secrete foul-smelling substances from two glands located at the base of the tail. Little is known about the biological significance or the chemical composition of these ubiquitous snake secretions. Several studies have described the chemical classes of compounds present in them. Electrophoretic analysis indicates taxonomic differences in scent gland proteins¹. The thin layer chromatograms of boid, colubrid and viperid scent gland secretions displayed bands consistent with sterols, sterol esters, and free fatty acids². In separate studies, gas chromatography³ and mass spectrometry⁴ have identified free fatty acids and cholesterol. Finally, we have identified cholesterol and a number of fatty acids and fatty amides in the scent gland secretions of Dumeril's ground boa (*Acrantophis dumerili*) by gas chromatography-mass spectrometry (GC-MS)^{5,6}. Extending our studies on the composition of these secretions, we report here a comparative study of the scent gland secretions from the western diamondback rattlesnake (*Crotalus atrox*) and Dumeril's ground boa.

After extensive experimentation with the boa secretion, the following standard method was adopted. Chloroform extracts of secretions were fractionated by preparative thin-layer chromatography (TLC). A representative TLC, with the standard fractions indicated, is shown in Figure 1. The TLC fractions were scraped, eluted with ether, and analyzed with a Hewlett-Packard 5995C quadrupole GC-MS. Following initial analysis, the fatty acids (fraction 3) and sterols (fraction 2) were derivatized and reanalyzed. The fatty acids were converted to methyl esters with ethereal diazomethane. The sterol fraction was trimethylsilylated. Three capillary columns were evaluated; a DB-1, a DB-5, and a DB-1701 (all 30 m x 0.32 mm i.d., 0.25 μ film thickness, J&W Scientific). Best overall resolution of components was obtained with the DB-1701. Chromatographic conditions were, in general: 30:1 split injection, 100°-300°C @ 10°C/minute, and hold. Mass spectrometric conditions were: 70 eV, scan m/z 20-620 for 0.71 sec cycle time, ion source temperature 200°C, transfer lines 300°C.

Free fatty acids and cholesterol constitute the major components of the chloroform-soluble fraction of the scent gland secretions from both species. There was no definitive evidence for sexual variation in the composition of the secretions. The fatty acids identified in *A. dumerili* included the even carbon number saturated acids from C_{16} to C_{26} , the C_{17} acid and a $C_{18:1}$ acid. Lactic, phenylacetic and 3-phenylpropanoic acids were also identified as minor constituents after derivatization. Hexadecanoic (palmitic) and octadecanoic (stearic) acids were the major fatty acids found. The fatty acid composition of the *C. atrox* secretions ranged from C_{16} to C_{25} , with C_{20} and C_{22} being the major acids.

Cholesterol was the most abundant lipid found in the secretions of both species. This sterol accounted for appx. 30% or more of the total ion current in injections of unfractionated $CHCl_3$ extracts. A cholestadiene isomer was also identified in the secretion of *A. dumerili*. This steroid was a minor component, but the exact structure is yet to be determined. Trace amounts of some other steroids were detected, as well as thermal degradation products of cholesterol occurring in the GC injection port. Both species contained several fatty amides. The C_{16} , C_{18} , C_{20} and C_{22} amides were found in *A. dumerili*, while the C_{18} , C_{20} , C_{22} and C_{24} amides were found in the *C. atrox* secretion. Shifts in the abundance of the fatty amides from C_{16} and C_{18} in *A. dumerili* to C_{20} and C_{22} in *C. atrox* reflect the shift in the abundances of the corresponding fatty acids.

While the lipid composition profiles of the secretions from these two species are qualitatively similar in that the major constituents are fatty acids and cholesterol, there is a quantitative difference in the distribution of the fatty acids found and in fatty acid/cholesterol ratio. In *A. dumerili*, the fatty acid distribution is broad -- from C_{16} to C_{26} , with C_{16} and C_{18} dominating. In *C. atrox*, the fatty acids range from C_{16} to C_{25} , but the quantitative distribution is narrower, with C_{20} and C_{22} dominating. Compare Figures 2 and 3. This finding is consistent

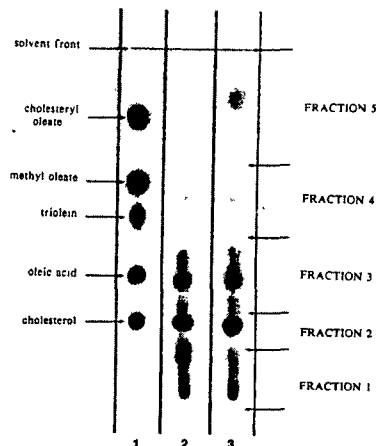


Figure 1: TLC of total lipid extract of scent gland secretion from *A. Dumerilii*. Lane 1: standards. Lane 2: pooled male secretion. Lane 3: pooled female secretion.

with the results reported⁴ for two species from the family Viperidae and one from the family Crotalidae, in which the vipers showed C₁₆ and C₁₈, whereas the crotaline (similar to our findings with *C. atrox*) showed only C₂₀ and C₂₂. Our results, taken with these other reports,^{3,4} suggest that fatty acid distributions in these secretions may reflect taxonomic differences. Data on a wider variety of species is needed, however, before it will become clear whether this suggestion will hold true, and to what extent it will support current snake systematics.

Of more immediate interest is the function of these secretions. The majority of evidence suggests involvement in predator-prey interactions, although most of this evidence is anecdotal. The foul odors associated with these secretions suggest a defensive role. Some evidence for this role exists from studies involving the Texas blind snake (*Leptotyphlops dulcis*)⁸, and interactions between the kingsnake (*Lampropeltis getulus*) and carnivores⁹. Our efforts at describing the chemical constituents of these snake secretions is preparatory to studies aimed at identifying the specific chemical(s) involved in such interspecies interactions. While it is clear that the major components are fatty acids and cholesterol, it is also probable that these are not the odoriferous "defensive" components, but are probably the "perfumer's base" extenders used to decrease the volatility of the active trace ingredients and prolong the efficacy of the secretion. This is in contrast to the observation that a 1:1 mixture of oleic and linoleic acids possessed an odor "nearly the same" as the secretion of *Leptotyphlops dulcis*.³ Close coordination of studies on the chemical composition of fractions and the behaviors which those fractions elicit in test subjects will be required to identify active ingredients.

Figure 2: TIC of *A. dumerili* secretion total lipid extract. DB-1701 column. 1 hexadecanoic acid (17%); 2 heptadecanoic acid (<1%); 3 octadecanoic acid (3%); 4 octadecanoic acid (25%); 5 hexadecanamide (<1%); 6, 7, 10, 11, 12 unknowns; 8 eicosanoic acid (1%); 9 octadecanamide (<1%); 13 eicosanamide (<1%); 14 cholestadiene isomer (<1%); 15, 18, 19 cholesterol degradation products; 16 docosanamide (<1%); 17 cholesterol (31%).

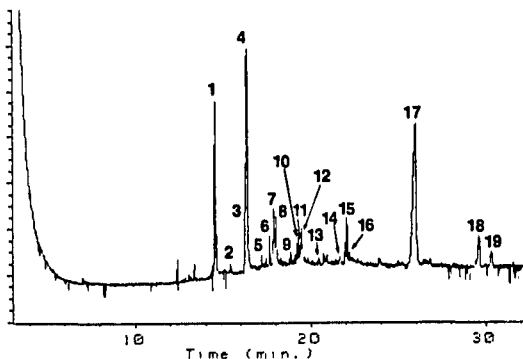
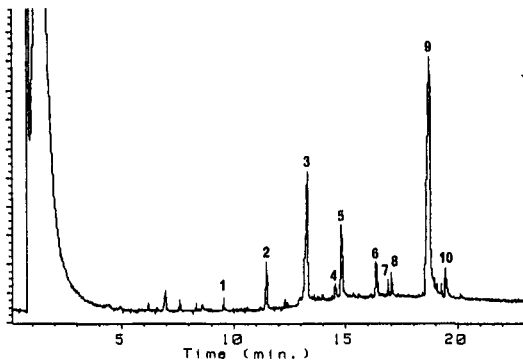


Figure 3: TIC of *C. atrox* secretion acid fraction. DB-1701 column. 1 hexadecanoic acid (<1%); 2 octadecanoic acid (5%); 3 eicosanoic acid (19%); 4 unknown (2%); 5 docosanoic acid (7%); 6 docosanamide (4%); 7 C_{24:1} acid (1%); 8 tetracosanoic acid (2%); 9 cholesterol (58%); 10 C₂₅ acid (1%).



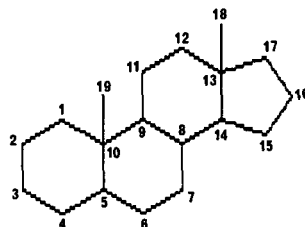
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ELECTRON IMPACT MASS SPECTRA OF STEROIDS: ANDROSTANES AND DERIVATIVES

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As a continuation of the Thermodynamics Research Center Spectral Data Program -- Mass Spectra (formerly the American Petroleum Institute Research Project 44 -- Mass Spectral Data), we report the electron impact mass spectra of a group of 78 derivatives of androstane.

Methods: The androstane parent structure ($C_{19}H_{32}$, m.w. 260) is shown. All compounds were obtained from Steraloids, Inc., (Wilton, New Hampshire) and used as received. The composition of the collection is indicated in Table I. Many compounds have multiple types of functional groups as well as multiple functional groups. The categories in Table I represent types of compounds in the collection. The sum is therefore larger than the total number of compounds examined. All data were collected on a VG Analytical 70-S mass spectrometer and VG 11/250J datasystem. All the compounds were crystalline solids, which were introduced into the mass spectrometer on the direct insertion probe. The probe was heated slowly in order to distill the compound being analyzed to evaluate sample purity and to avoid thermal degradation.



Spectra have been searched against the National Bureau of Standards library available on the datasystem (42000 spectra; purity fit search, fit factor 700 or greater). The results are given in Table II. 46% of the spectra of these compounds yielded no matches of any quality. Most of the parent unesterified steroids all show high intensity molecular ions. Ironically, esterification with any of several acyl groups substantially decreases or, in some cases, eliminates the molecular ion, producing indistinguishable mass spectra. Example spectra for 5α -androst-3 β -ol-17-one ($C_{21}H_{30}O_3$, m.w. 288) and its acetate (m.w. 330) and benzoate (m.w. 392) esters are shown. While M^{+} is base peak for the parent compound, M^{+} for both esters is absent. Base peaks of the ester spectra correspond to the androstadien-17-one resulting from elimination of the carboxylic acid by rearrangement.

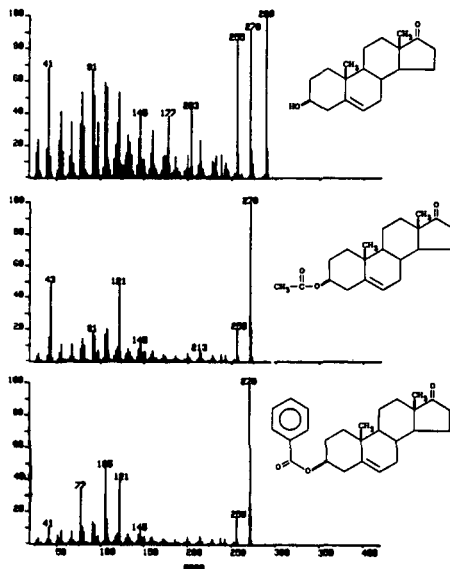
Full spectral data of all the compounds described here, as well as for a large number of other compounds and compound types, are available from the Thermodynamics Research Center. A list of compounds, M^{+} , and 6 largest peaks is given. Funds for purchase of instrumentation were provided in part by NSF Grant CHE-8705697.

TABLE I:
Collection Composition

Compd type	Number ^a
alkanes	1
alkenes	27
alkynes	1
dienes	14
alcohols	20
diols	6
ketones	50
diones	5
triones	2
esters	36
diesters	6
ethers	1
diethers	1
halo cmpds	7
oxa cmpds	1
oximes	1
a. See methods	

TABLE II
NBS Library Search Results

Fits	18	23%
Ambiguous fits	24	31%
No Fits	36	46%
Total	78	



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CAS Ref #	mol wt.	X1	m/z	X1	m/z	X1	m/z	X1	m/z	X1	m/z	X1	FORMULA	SUBSTITUTION IN POSITION												
														1	2	3	4	5	6	9	11	17				
438-22-2	260	85	245	100	95	71	203	60	81	60	67	52	55	47	C ₁₉ H ₃₂											
963-75-7	272	85	218	100	190	35	91	35	79	34	41	33	93	30	C ₁₉ H ₃₀	=	0		ah				CO			
963-74-6	274	100	109	49	67	48	230	40	41	40	55	38	81	32	C ₁₉ H ₃₀				ah				CO			
2639-53-4	274	76	220	100	79	49	91	48	93	47	41	42	176	41	C ₁₉ H ₃₀	=			ah				BOH			
897-06-3	284	13	122	100	121	23	91	20	159	17	123	13	41	13	C ₁₉ H ₃₀	=		CO					CO			
63-05-8	286	100	244	55	124	45	41	37	148	23	287	23	79	21	C ₁₉ H ₃₀			CO					CO			
846-48-0	286	100	122	100	121	23	91	19	123	13	41	13	147	10	C ₁₉ H ₃₀	=		CO					BOH			
2484-30-2	286	100	136	87	133	37	91	32	107	29	151	29	79	29	C ₁₉ H ₃₀			CO					CO			
846-46-8	288	100	41	398	244	38	217	38	67	32	55	29	124	28	C ₁₉ H ₃₀					ah			CO			
58-22-0	288	82	124	100	245	87	147	30	41	29	203	26	91	22	C ₁₉ H ₃₀			CO					BOH			
53-43-0	288	100	270	92	255	85	41	68	91	66	105	59	107	56	C ₁₉ H ₃₀			BOH					CO			
1229-12-5	288	100	41	56	67	44	55	38	81	32	79	32	244	31	C ₁₉ H ₃₀			CO					CO			
53-41-8	290	100	108	53	107	52	41	48	67	42	55	37	93	35	C ₁₉ H ₃₀			acOH					CO			
481-29-8	290	100	41	37	107	33	246	30	67	30	108	32	55	28	C ₁₉ H ₃₀			BOH					CO			
521-18-6	290	100	231	93	41	28	232	21	81	21	67	21	55	21	C ₁₉ H ₃₀			CO					BOH			
521-17-5	290	100	272	77	107	73	105	68	41	68	91	67	79	59	C ₁₉ H ₃₀			BOH					BOH			
571-22-2	290	100	41	67	220	60	81	57	55	57	67	51	247	46	C ₁₉ H ₃₀			CO					BOH			
1156-92-9	290	67	220	100	41	52	91	47	107	66	93	44	81	43	C ₁₉ H ₃₀			BOH					BOH			
1852-53-5	292	100	215	80	233	72	41	72	107	69	55	65	81	61	C ₁₉ H ₃₀			BOH					BOH			
571-20-0	292	100	233	58	215	54	107	54	41	50	81	49	55	49	C ₁₉ H ₃₀			BOH					CO			
7738-93-4	298	100	160	77	121	60	91	58	41	49	131	40	159	38	C ₁₉ H ₃₀	=		CO					CO			
382-45-6	300	65	122	100	121	37	41	23	91	20	123	20	301	13	C ₁₉ H ₃₀			CO					CO			
72-63-9	300	8	122	100	121	37	41	23	91	28	161	28	105	19	C ₁₉ H ₃₀	=		CO					CO			
1039-17-4	300	29	242	100	227	77	267	54	43	54	285	29	105	24	C ₂₀ H ₃₂			CO					CO			
58-18-4	302	100	43	45	124	40	41	27	303	21	245	20	229	20	C ₂₀ H ₃₂			CO					CO			
521-11-9	304	40	43	100	41	68	271	65	247	64	81	60	55	60	C ₂₀ H ₃₂			CO					CO			
521-10-8	304	59	253	100	43	82	213	70	27	47	105	43	91	42	C ₂₀ H ₃₂			BOH					CO			
2436-48-8	305	61	69	100	112	67	289	50	246	50	41	45	230	39	C ₂₀ H ₃₂			NOH					BOH			
53-39-4	306	16	71	100	43	89	41	42	29	40	249	38	81	38	C ₂₀ H ₃₂			CO					CO			
641-83-8	306	57	100	107	77	81	64	291	57	165	56	41	53		C ₂₀ H ₃₂	0		BOH					CO			
434-03-7	312	100	124	52	245	45	297	35	229	35	79	35	91	33	C ₂₀ H ₃₂			CO					CO			
972-46-3	314	1	286	100	124	65	244	60	41	41	79	38	91	37	C ₂₁ H ₃₄			OEt,					CO			
2324-10-9	316	60	43	100	202	98	262	75	93	45	79	41	91	40	C ₂₁ H ₃₄			CO					CO			
1807-02-9	318	100	43	95	300	82	124	64	137	55	242	51	41	46	C ₂₀ H ₃₂			CO					CO			
2363-59-9	328	100	122	100	43	44	121	25	147	22	91	15	123	14	C ₂₁ H ₃₄			CO					CO			
2352-19-4	328	29	43	100	133	93	136	66	268	33	286	33	91	20	C ₂₁ H ₃₄			CO					CO			
853-23-6	330	0	270	100	43	49	121	48	107	21	255	21	105	17	C ₂₁ H ₃₄			Acyl ¹					CO			
1045-69-8	330	42	43	100	124	92	147	70	288	38	228	33	91	22	C ₂₁ H ₃₄			CO					CO			
1164-95-0	332	8	272	100	43	50	108	43	257	32	218	30	107	28	C ₂₁ H ₃₄			acetyl ¹					CO			
1239-31-2	332	30	272	100	43	85	108	51	107	50	67	50	218	57	C ₂₁ H ₃₄			Acyl ¹					CO			
1164-91-6	332	23	272	100	43	91	257	58	149	45	231	29	94	29	C ₂₁ H ₃₄			Acyl ¹					CO			
1639-43-6	332	0	272	100	43	43	121	33	107	24	273	21	257	20	C ₂₁ H ₃₄			Acyl ¹					CO			
1164-92-7	332	19	43	100	101	52	272	50	94	29	81	29	55	28	C ₂₁ H ₃₄			CO					CO			
977-32-2	342	16	122	100	57	72	29	50	147	39	121	39	91	21	C ₂₂ H ₃₆			CO					CO			
25862-97-9	342	20	57	100	133	64	136	50	29	42	286	40	268	22	C ₂₂ H ₃₆			CO					CO			
57-85-2	344	38	57	100	124	82	147	65	29	56	228	31	302	24	C ₂₂ H ₃₆			CO					CO			
855-22-1	346	10	105	100	77	19	133	18	268	8	106	8	136	8	C ₂₂ H ₃₆			CO					CO			
5953-68-4	346	4	272	100	108	39	218	32	27	37	21	29	31		C ₂₂ H ₃₆			acetyl ²					CO			
15253-55-1	346	12	272	100	57	51	218	50	29	45	108	37	107	32	C ₂₂ H ₃₆			Acyl ²					CO			
18170-14-1	346	12	57	100	272	63	29	55	94	25	81	23	55	22	C ₂₂ H ₃₆			CO					CO			
4834-82-6	352	20	44	100	41	74	272	66	67	43	108	61	79	61	C ₂₂ H ₃₆			acetyl ⁸					CO			
15233-27-9	352	50	108	100	308	99	67	93	41	89	107	83	79	81	C ₂₀ H ₃₂			Acyl ⁸					CO			
65928-84-9	352	70	41	100	231	92	55	90	81	88	280	84	67	73	C ₂₀ H ₃₂			CO					CO			
28507-01-9	366	80	41	100	67	82	368	79	55	73	79	72	81	71	C ₂₀ H ₃₂			acBr					CO			
29041-91-6	368	51	41	100	81	93	55	87	67	80	79	76	68	75	C ₁₉ H ₃₀			acBr					CO			
2099-26-5	374	0	314	100	43	84	121	33	146	27	133	26	107	22	C ₂₃ H ₃₈			Acyl ¹					CO			
5424-40-8	376	0	43	100	316	73	256	30	107	28	93	25	241	23	C ₂₃ H ₃₈			Acyl ¹					CO			
2061-86-1	388	100	43	0	268	74	328	49	253	42	91	32	147	32	C ₂₄ H ₃₈			Acyl ¹					CO			
9041-66-8	390	10	105	100	122	85	77	38	147	22	121	13	91	11	C ₂₄ H ₃₈			CO					CO			
115387-45-6	390	14	105	100	77	19	133	18	268	8	106	8	136	8	C ₂₆ H ₄₀			CO					CO			
2080-86-6	392	0	270	100	105	47	121	40	77	33	271	22	255	17	C ₂₆ H ₄₀			Acyl ³					CO			
2088-71-3	392	27	105	100	77	46	147	24	124	23	228	11	270	10	C ₂₆ H ₄₀			CO					CO			
5953-69-5	394	4	272	100	105	44	77	30	218	29	107	29	257	24	C ₂₆ H ₄₀			acetyl ³					CO			
6696-39-5	394	3	272	100	218	3																				

FATTY ACID AND HYDROCARBON CONTENT OF SALMONELLA TYPHIMURIUM BY GC/MS.
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Introduction. The occurrence of some unusual fatty acids in the lipids of microbial strains¹ has stimulated efforts to correlate these unusual molecules with virulence and disease.² The presence of both 2-OH-14:0 and 3-OH-14:0 have been associated with lipid A in Salmonella typhimurium, and appear to be incorporated into lipopolysaccharide (LPS).³ LPS is known to be associated with many aspects of the pathogenesis of Salmonellosis.⁴ Endotoxins from gram-negative bacteria stimulate interleukin 1 (IL1), and are made up of both LPS and lipid A. The lipid A component of LPS is active in inducing IL1, and hydroxylated fatty acids specifically are important for the IL1 inducing properties of lipid A.⁵

Objective. The objective of this study was to look for interesting and unusual molecules, that potentially may play a role in the virulence of S. typhimurium, in the following compound classes: 1) fatty acids or their derivatives, 2) hydrocarbons, and 3) other lipophilic components.

Instrumentation. The mass spectrometer (MS) used for these EI GC/MS measurements was a double-focussing VG 70-250 EHF spectrometer. The MS source block was directly coupled to an HP 5890 GC. The source block, interface, and GC injector temperature was 190, 260, and 250°C, respectively. A Supelco SPB-1 fused silica capillary column with a 0.25 µm coating was used. Accurate mass measurements were conducted at 2,000 resolution over a narrow mass range, encompassing two known PFK peaks at 10s/decade. Samples were injected in the splitless mode with helium as carrier gas (15 psi head pressure). The injector was purged 30 sec after injection. A double temperature ramp GC program was used: 40°C for 3 min; ramped at 10°C/min to 140°C; held at 140°C for 1 min; ramped at 5°C/min to 260°C; and held at 260°C for 20 min.

Results and Discussion. The EI GC/MS of the methylated total fatty acids are shown in Fig. 1. This chromatogram shows the two hydroxylated fatty acids, 2-hydroxy-tetradecanoic acid methyl ester (2-OH-14:0) and 3-hydroxy-tetradecanoic acid methyl ester (3-OH-14:0) with its accurate mass determined to be m/z 258.2196 (calculated for C₁₅H₃₀O₃, 258.2195). Two cyclic fatty acid methyl esters also were observed, 17-carbon cyclopropane (C17Δ) observed at m/z 282.2562 (calculated for C₁₈H₃₄O₂, 282.2559) and a 19-carbon cyclopropane (C19Δ) observed at m/z 310.2864 (calculated for C₂₀H₃₈O₂, 310.2872).

The hydrocarbons observed in small amounts during the fatty acid run are listed with their accurate mass values in Table 1. Hydrocarbons were observed from C₁₇H₃₆ to C₂₉H₆₀ in the C_nH_{2n+2} series.

Dehydroabietic acid methyl ester (DHAAME) was found with the fatty acids in the diglyceride fraction. DHAAME was observed at m/z 314.2246 (calculated for C₂₁H₃₀O₂, 314.2246). The mass spectrum of DHAAME is shown with its structure in Fig. 2. DHAA is generally considered to be a resin acid from pine wood,⁶ but it is diversely found from honey mushroom⁷ to Black Sea Bryozoa,⁸ which are minute, colonial animals. DHAA has low toxicity in rats,⁹ but, its toxicity to fish greatly changes when the pH is slightly changed.¹⁰

Conclusion. The fatty acid profiles of S. typhimurium were in agreement with those earlier reported.¹¹ The hydrocarbons are in low concentrations and do not appear unusual. Since both 2-OH-14:0 and 3-OH-14:0 have been associated with lipid A, which has been associated with virulence, the presence of these hydroxy fatty acids suggests their association with virulence by S. typhimurium. The unusual cyclic fatty acids, C17Δ and C19Δ, may play a role in virulent character. It is unusual to find a resin acid like dehydroabietic acid (DHAA) in a bacterium. Its role is not understood. But, it has low toxicity in the rat and its toxicity in fish drastically changes by slightly changing the pH. Therefore, DHAA potentially may be associated with virulence by S. typhimurium.

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Table 1. Hydrocarbons found in Salmonella typhimurium.

Observed mass	Formula	Calculated mass	Deviation ^a
240.2804	C ₁₇ H ₃₆	240.2817	1.3
254.2976	C ₁₈ H ₃₈	254.2974	0.2
268.3120	C ₁₉ H ₄₀	268.3130	1.0
282.3309	C ₂₀ H ₄₂	282.3287	2.2
296.3402	C ₂₁ H ₄₄	296.3443	4.1
310.3602	C ₂₂ H ₄₆	310.3600	0.2
324.3774	C ₂₃ H ₄₈	324.3756	1.8
338.3886	C ₂₄ H ₅₀	338.3912	2.6
352.4059	C ₂₅ H ₅₂	352.4069	1.0
366.4212	C ₂₆ H ₅₄	366.4226	1.4
380.4397	C ₂₇ H ₅₆	380.4382	1.5
394.4556	C ₂₈ H ₅₈	394.4539	1.7
408.4655	C ₂₉ H ₆₀	408.4695	4.0

^aDeviation is in millimass units (mmu).

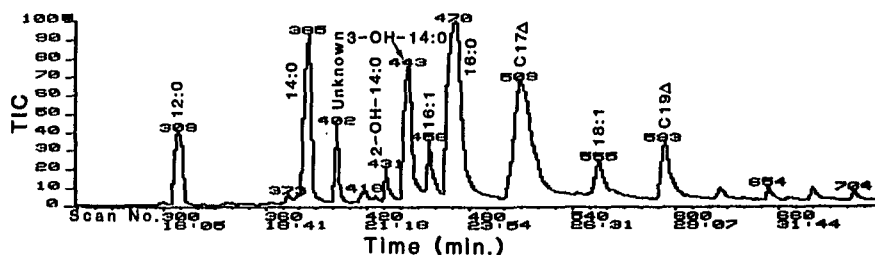


Figure 1. Total ion current (TIC) profile of fatty acids in S. typhimurium.

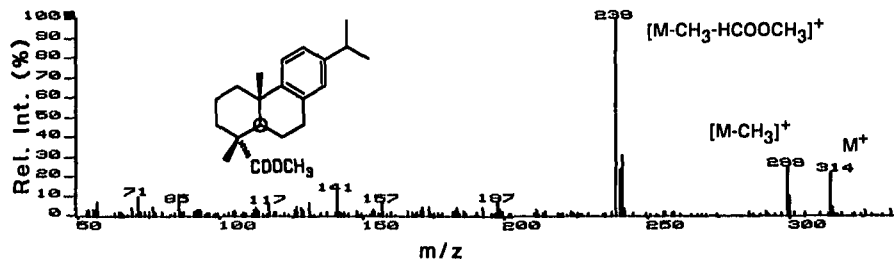


Figure 2. The mass spectrum of dehydroabiestic acid methyl ester.

DETERMINATION OF ANOXIA-INDUCED PHOSPHOLIPASE A2 (PLA2)
ACTIVATION IN FRESHLY ISOLATED RABBIT PROXIMAL TUBULES
USING NICI .

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The mechanisms of ischemia induced phospholipid breakdown in kidney cells have not been completely elucidated. In the arterial occlusion model of renal ischemia several investigators have found marked increases in free fatty acids (FFA) and diacylglycerol within the first 15 minutes . These effects were accompanied by changes in lysophospholipids suggesting the action of enzyme of the phospholipase A(s) class . We have evaluated the role of anoxic injury on phospholipase(s) activation by determining changes in arachidonic acid release measured by NICI GCMS after manipulation with PLA2 inhibitors as well as protein synthesis inhibitors in rabbit proximal tubules subjected to anoxia.

METHODS : Renal cortical tubules were isolated from rabbits according to published methods.

After isolation, tubules were preequilibrated for 20 min at 37°C in a 95% O_2 /5% CO_2 atmosphere; then anoxia was maintained for 40 min under a N_2 atmosphere at 37°C and then reoxygenated for additional 40 min.

AA was extracted after addition of acidified methanol and 10 ng of octadeuterated (D8) AA as internal standard. AA is isolated on C18 columns and converted to pentafluorobenzyl esters. AA mass is determined by GCMS NICI mode monitoring m/z 303 for protium and m/z 311 for deuterium .

In alternate experiments in tubules labeled with ^3H AA, AA and diacylglycerol (DAG) were separated by TLC.

PLA2 was assayed in proximal tubules mechanically disrupted and subjected to serial high speed centrifugations to separate microsomal and cytosolic fractions. PLA2 activity was measured as the amount of AA released after incubations of the fractions with cold PC , PE and 14C 2 arachidonyl PC, PE .

RESULTS : Anoxia induced a time dependent stimulation of AA release from basal levels of 4.33 ± 71 ng/mg prot. (normoxia) to 139.03 ± 35.33 ng (after 40 min.) . AA release went up to 165.33 ± 36.41 ng/mg during 40 min of reoxygenation. Pretreatment of proximal tubules prior to anoxia with actinomycin D significantly inhibited AA release during reoxygenation from 202.66 ± 15.70 ng/mg to 108 ± 25.77 ng/mg prot.. A similar effect although less pronounced was observed with cycloheximide . Quinacrine and dibucaine did not have a significant effect on AA release during anoxia or reoxygenation . . TLC analysis of fatty acids in tubules previously labeled with ^3H AA confirmed the changes observed with AA mass determinations as well as a significant formation of 1,2 DAG in anoxic conditions. We conclude : 1) Anoxia stimulates AA release in proximal tubules through activation of phospholipases presumably PLA2 . 2) Activation of the enzyme (s) responsible for AA release require protein synthesis. Further studies are required to elucidate substrate specificity and other factors associated with this activation.

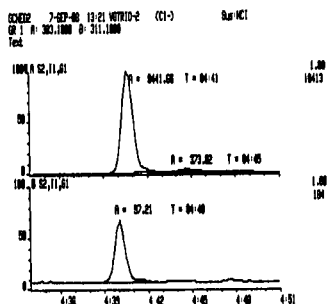


FIGURE 1. Selective ion monitoring of arachidonic acid (m/z 303 upper panel) and D8 octadeuterated AA (m/z 311 lowerpanel) both derivatized as pentafluorobenzyl esters.

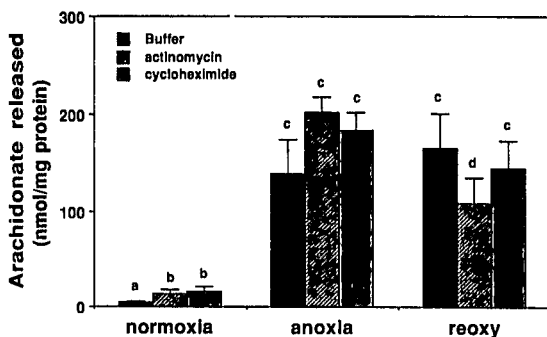


FIGURE 2. Effect of protein synthesis inhibitors on AA release of proximal tubules subjected to normoxic, anoxic and reoxygenation conditions.

DIRECT ANALYSIS OF MODIFIED FATTY ACIDS THROUGH THEIR OXAZOLINE DERIVATIVES
BY COMBINED IN-BEAM ELECTRON-IMPACT (IBEI)-B/E LINKED SCANNING TECHNIQUE

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Location of double bond and other substituents in long-chain fatty acids (FA) has been a topic of continuing interest in organic and biomedical analysis. Of the numerous methods so far advanced, the approach that first suggested by Vetter in 1971 [1] on the basis of "remote functional group modification" is particularly attractive. In this way, it is possible to handle complex mixtures of unknown acids by derivatization of the COOH group ("remote site") followed by MS or GC-MS of the reaction products.

Most often FA were converted to N-acyl pyrrolidides [2], picolinyl esters [3], or heterocycles (eg., benxoxazoles, BOX [4]; or 4,4-dimethyloxazolines, DMOX [5]) that would afford more specific spectra regarding the chain structure. The main advantage is peak identification without the use of standards that are not available for the "unknowns". The significantly improved diagnostic power of these derivatives aroused immediate interest to test them in direct analysis without prior chromatographic separation. Thus, Deterding [6] reported, collision activation (CA) of the $(M+H)^+$ in fast atom bombardment (FAB) spectra of the unsaturated FA (UFA) derivatized as picolinyl esters was able to reveal typical fragmentation, from which the site of double bonds was readily apparent. Usually, the assignment is possible for cases of di- and trienoic acids, but becomes more difficult for samples with higher degrees of unsaturation.

We wish to report here a very simple and efficient technique for direct analysis by using B/E-linked scan coupled with in-beam electron-impact (IBEI) mass spectrometry of DMOX derivatives (cf. ref. 7 and 8).

DERIVATIZATION

DMOX were prepared by condensation of FA with 2-amino-2-methylpropanol (AMP) according to the procedure reported earlier [5].

MASS SPECTROMETRY

Instrument: MAT 711 coupled with SS 166 data system. Operating parameters: accelerating voltage 8 kV, electron energy 70 eV, emission current 0.8 mA, source temperature 20°C, resolution 1000.

IBEI: the sample (ca. 1 μ g) in $CHCl_3$ was loaded on the stainless steel target of the FAB direct inlet probe and then inserted into the ion source 3-4 mm away from the electron beam.

B/E-linked scan: performed by using a Finnigan-MAT Metascan unit, linear scanning speed 128 sec/decade, the spectra were registered on a UV-recorder. The measured daughter ion m_2 was calculated according to the equation $m_2 = (m \times m_1)^{1/2}$, where m_1 = mass of the precursor, m = apparent mass of the marker.

RESULTS AND DISCUSSION

Good reproducibility in mass measurement (deviation <0.2 amu) can be obtained based on a calculation from the results of repeated scanning starting from m/z 335 [DMOX of 18:1(9)].

The daughter ion spectra originating from the molecular ions of nine UFA, two acetylenic, and five hydroxy acids have been obtained. The site of unsaturation (and substitution) can be deduced according to the empirical rule outlined before [5,8].

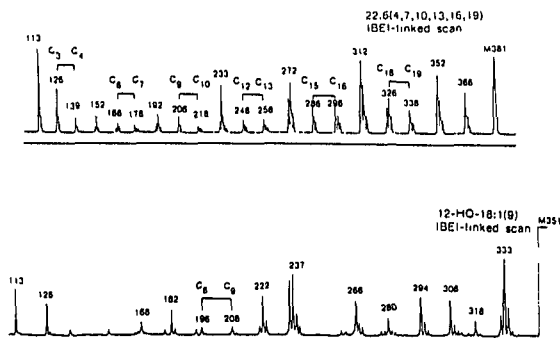
One important implication of the use of this technique is related to the analysis of mixtures. Usually, when going from EI to IBEI mode, a pronounced increase in intensities occurs for the molecular ion peaks (200-1000 % enhancement), while the increase for other peaks are relatively less significant (10-30% enhancement). The predominating feature of the results allows easy distinction of the odd-mass molecular ions from the even-mass fragment ions in the spectra resulting from mixture samples.

CONCLUSION

The method described above provides an alternative means for direct location of unsaturated bonds (for polyenes with up to 6 double bonds) and hydroxyl groups in long-chain FA. It will be beneficial for the analysis of samples which are not amenable to GC-MS due to poor volatility or scanty MS information attainable by other means. Artifact peaks can arise from unrelated species that fall within the mass window of $\pm 2.5\%$ with respect to the target precursors. Nevertheless, this drawback can be overcome by using tandem mass spectrometry.

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CHARACTERIZATION OF FATTY ACYL HYDROXYLATION OF TUMOR CELL GANGLIOSIDES BY FAB-CAD-MS/MS

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Many tumor cells are known to express glycosphingolipids structurally distinct from those of cells from analogous normal tissue. Often these differences are located in the oligosaccharide portion of the molecule (1). However, structural variation has also been found in the ceramide (lipid) portion of the both gangliosides (2,3) and neutral glycosphingolipids (4,5) of some tumor lines. Ceramide composition has usually been determined by methanolysis and subsequent analysis by GC-MS of the derivatized sphingoid base and fatty acid.

Although negative ion FAB-MS analysis provides oligosaccharide sequence information, direct characterization of the ceramide structure by examination of the prominent $[\text{CerO}]^-$ fragment (representing the total ceramide composition) in the spectra of gangliosides is not possible because of potential structural isomerism (e.g., d18:1 sphingoid base, C18:0 fatty acid vs d20:1, C16:0 or d18:1, C18:0 vs d18:0, C18:1).

The introduction of FAB-CAD-MS/MS techniques in both positive and negative ion modes (6) has permitted the composition of the ceramide residue to be determined in underivatized glycosphingolipids. We have recently reported the use of this analytical approach in combination with HPLC purification of individual ganglioside molecular species to characterize the ceramide composition of neuroblastoma (NB) GM2 and compare it to that of normal human brain (HB) GM2 (7).

The separation of individual molecular species prior to analysis by FAB-CAD-MS/MS allowed the ceramide structure of minor GM2 components to be determined, even though they do not produce sufficient parent ion intensity in a mixture of ceramide species.

The FAB-MS spectra of oligosaccharide homogeneous normal human brain (HB) and neuroblastoma (NB) GM2 differ dramatically in the molecular ion region, indicating the ceramide molecular species pattern of GM2 from each tissue is distinct. This conclusion is confirmed by the reversed phase HPLC separation of these two samples into their individual GM2 ceramide molecular species. The NB GM2 has many more ceramide species than the HB GM2.

When the fragment ion in the negative FAB spectrum representing the ceramide moiety, $[\text{CerO}]^-$, is used as the parent ion in a CAD-MS/MS experiment, two daughter ions, S and T (8), indicative of the fatty acyl group are observed. Since the weight of the total ceramide is known, the sphingoid base present can be derived by difference.

The negative FAB-CAD-MS/MS spectra of HPLC separated molecular species are greatly improved over those obtained from the same compound in a mixture of other GM2 congeners. Two examples of FAB-CAD-MS/MS analysis of separated NB GM2 molecular species are shown in Figure 1. These two congeners, not present in HB GM2 both contain hydroxy fatty acyl residues. Examination of other NB GM2 ceramide species demonstrated that fatty acyl hydroxylation is characteristic of neuroblastoma GM2.

An alternative FAB-CAD MS/MS approach in the positive ion mode, using an analogous ceramide fragment provides complimentary information on the sphingoid base though, again, the other part of the ceramide can be easily deduced. The lack of sensitivity in the positive FAB mode for underivatized gangliosides, however, makes the negative ion mode experiment preferable.

The combination of HPLC purification of molecular species and FAB-CAD-MS/MS is a powerful technique for determining ceramide structure in underivatized gangliosides. This approach has been used to characterize the GM2 composition of normal human brain and neuroblastoma tumors. The dramatic differences observed were due to the presence of additional hydroxy fatty acyl and longer chain fatty acyl residues in the ceramide of neuroblastoma tissue.

We have now extended this analytical approach to other neuroblastoma gangliosides. The ceramide composition of NB GM1 was found to vary from that of HB GM1 in a similar manner. NB GM1 molecular species at m/z 1580, 1586, and 1642 were absent from HB GM1. These represent ceramide species containing sphingoid base d18:1 and the hydroxy fatty acids h18:0, h20:0, and h24:1, respectively. Preliminary analysis of NB GD3 and HB GD3 showed a similar pattern. Thus, it appears that fatty acid hydroxylation is characteristic of neuroblastoma tumor gangliosides.

HPLC: Extracted gangliosides (7) were separated and recovered by normal phase HPLC on a 25 cm Hilar RT Lichrosorb Si100 NH₂ column. The solvent gradient of Gazzotti (8) was used. Gangliosides were detected at 215 nm. The resulting oligosaccharide homogeneous gangliosides were then separated into their individual ceramide molecular species by reversed phase HPLC on a Hilar RT Lichrosorb Si100 RP-8 column. An acetonitrile-5mM phosphate buffer solvent gradient (7) was employed. Eluted gangliosides were then desalted on a Sephadex G-50 gel filtration column prior to mass spectral analysis.

Mass Spectrometry: Approximately one μl of a methanolic ganglioside solution (1nmol/ μl) was mixed with 2 μl of triethanolamine matrix on the FAB probe tip. Ions were produced by bombardment with a 6kV beam of Xe atoms in a JEOL HX-110 double focusing mass spectrometer operating in negative ion mode. The accelerating voltage was 10kV.

and the resolution was set at 3000. For CAD-MS/MS, helium was used as the collision gas in a cell located in the first field-free region. The helium pressure was adjusted to reduce the abundance of the parent ion, $[\text{CerO}]^-$, by 75%. A JEOL DA-5000 data system generated linked scans at constant B/E.

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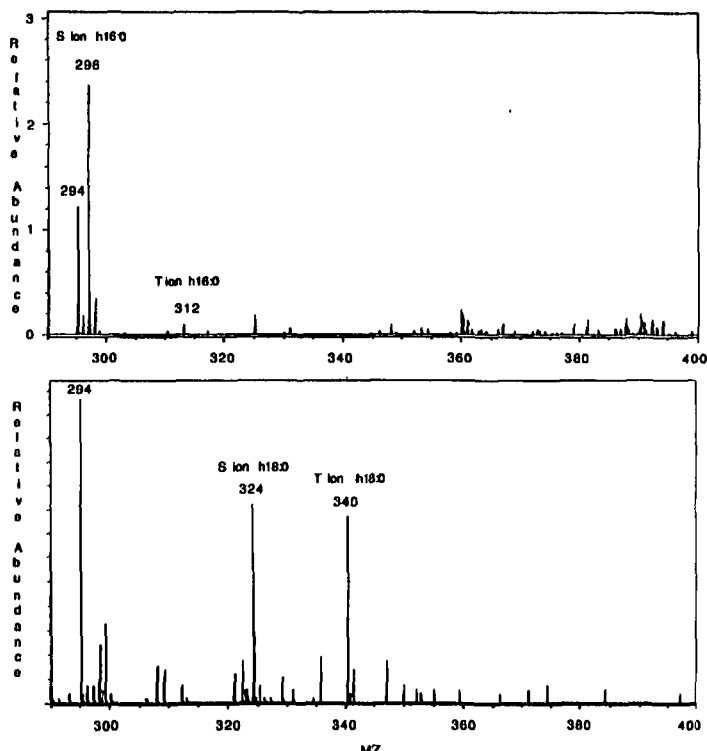


Figure 1. The negative FAB-CAD-MS/MS of two different purified NB ceramide molecular species with $[\text{M-H}]^-$ ions at m/z 1398 and 1370. The presence of diagnostic S and T daughter ions permit their ceramide fatty acyl residues to be unambiguously identified as hydroxystearate (h16:0) and hydroxypalmitate (h18:0), respectively.

**EC-NI CAD MS/MS ANALYSIS OF THE FRAGMENTATION OF ABSICISIC ACID:
THE ROLE OF OXYGEN-INDUCED ACTIVATION**

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Electron Capture Negative Ionization (EC-NI) mass spectrometry is a highly sensitive method for detection of the methylated derivative of abscisic acid (ABA-Me), an important plant growth regulator. Use of methane or ammonia as the buffering reagent gas promotes the formation of M^- as the most intense ion in the mass spectrum. In EC-NI mass spectrometry, the presence of O_2 in the ion source can significantly alter the mass spectrum. Both gas-phase reactions and wall-catalyzed reactions involving oxygen are known to induce formation of additional ions.¹ In the spectrum of ABA-Me, oxygen enhances the formation of structurally-informative fragment ions of mass 136, 141, and 152. In a previous study² of ABA-Me by EC-NI, the oxygen-enhanced ions of mass 141 and 152 were observed without the deliberate introduction of oxygen into the ion source. However, small quantities of oxygen were no doubt present in the reagent gas or GC carrier gas.

Figures 1a and 1b are the mass spectrum with and without oxygen present in the source, respectively. The oxygen-induced fragmentation reaction is being studied systematically with a Finnigan triple quadrupole mass spectrometer (TSQ-70). Conditions for EC-NI are established in the ion source and the molecular anion of ABA-Me (m/z 278) is transmitted via Q_1 into the collision cell (Q_2) for energy-controlled reactions with $^{16}O_2$, $^{18}O_2$, or Ar. Molecular oxygen is required for formation of abundant ions of mass 141 and 152, and a minor ion of mass 136 (see Figure 2a,b). The abundance of these ions decreases as the K.E. of the M^- is increased from 0 to 10 eV/Lab; beyond 10 eV, CAD fragments dominate. In contrast, when M^- interacts with Ar in Q_2 at low K.E. (0-40 eV Lab), no peaks are observed at m/z 136, 141, or 152.

The oxygen-activated ions are formed through a pathway in which a $(M+O_2)^-$ adduct (m/z 310) is an intermediate. This short-lived adduct decomposes to produce the ions of mass 136, 141, and 152. The adduct ion can be stabilized in a high pressure CI ion source so that it can be chosen as the parent ion in a MS/MS experiment. The CAD spectrum of m/z 310 (figure 3) shows that the ions of mass 136, 141, and 152 arise from the $(M+O_2)^-$ adduct ion.

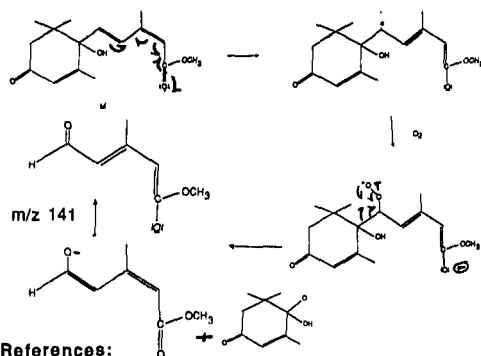
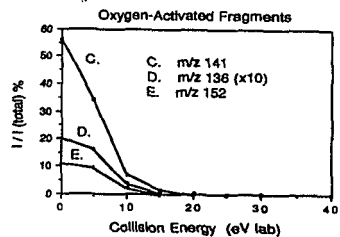
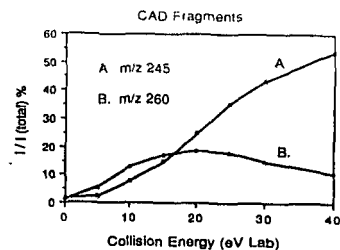
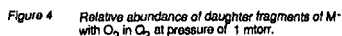
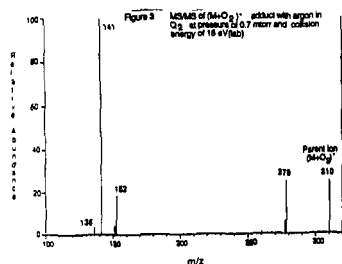
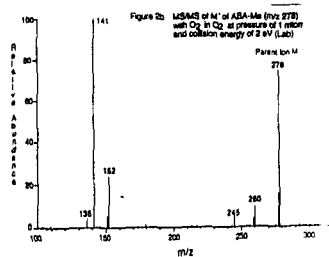
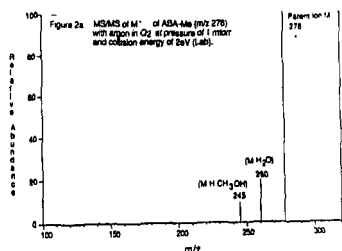
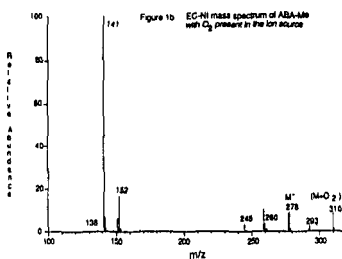
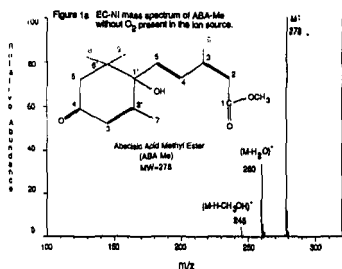
Two other prominent ions in the spectrum at mass 260 and 245 are not oxygen-activated fragments, but CAD fragments. ^{18}O - and 2H -labelled analogues of ABA-Me have enabled the determination of fragmentation mechanisms for these two significant dissociations. $(M-18)^-$ results from the loss of the tertiary hydroxyl group and a hydrogen from the side chain, presumably at the 4 or 5 position. $(M-33)^-$ arises from sequential loss of H^- from the 5' position and CH_3OH from the tertiary hydroxyl group and one of the gem-dimethyl groups from the ring as proposed by Netting et al.² MS/MS reveals that m/z 245 may also result from loss of CH_3^- from the ion at m/z 260. Figure 4 summarizes the relationship between the fragment ions formed and the collision energy. As the kinetic energy of the parent ion is increased, the formation of CAD fragments (m/z 260 and m/z 245) increases, whereas the oxygen-activated daughter ions decrease.

When $^{18}O_2$ was introduced into the Q_2 cell and M^- of ABA-Me was chosen as the parent ion, the fragment at m/z 141 shifted to m/z 143. There was no shift in mass for the other oxygen-activated fragment ions. The ion of mass 141 not only contains the two carboxyl oxygens, as shown by isotope labelling studies, but a third oxygen whose origin is molecular oxygen. The following mechanism involving a $(M+O_2)^-$ intermediate accounts for the formation of this oxygen-activated ion.

Oxygen contamination may significantly alter the mass spectrum in EC-NI. Most often addition products are observed due to reactions with oxygen impurities present in the reagent gas. The EC-NI spectrum of ABA-Me produces structurally-informative fragment ions which are a result of oxygen-induced fragmentation. The use of triple quadrupole mass spectrometry allows selective reactions to be examined in an environment free of interfering reactions. This method has been used to elucidate reactions between M^- of ABA-Me and molecular oxygen, and these fragments have proved useful in ^{18}O isotope labeling studies of ABA biosynthesis.^{3,4}

Experimental:

All spectra were recorded on a Finnigan TSQ 70 triple quadrupole mass spectrometer equipped with a Varian 3400 Gas Chromatograph. The column used was a DB-5 capillary column (30m x 0.247mm; J&W Scientific, Inc.). Source pressure was maintained at ca. 0.8-0.9 mtorr methane or ammonia, and source temperature was held at 150 C. Unless otherwise indicated, pressure of O_2 in Q_2 was 0.9 mtorr, and collision energy was 0-5 eV(Lab frame).



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TANDEM MASS SPECTROMETRY OF UNDERIVATIZED PROSTAGLANDINS

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The metabolic oxidation of arachidonic acid leads to formation of a wide variety of biologically active molecules including prostaglandins and leukotrienes (1). Historically, mass spectrometry has played a central role in the structure elucidation of these hydroxy, unsaturated carboxylic acids. However, most experiments have involved extensive derivatization and/or chemical degradation of these molecules (2). Thus, there has been a continuing need for the development of methods to probe the chemical structure of oxygenated, unsaturated fatty acids to facilitate such characterizations. We report here studies performed on underivatized prostaglandins using fast atom bombardment mass spectrometry, using 3-nitrobenzyl alcohol saturated with BaCl₂ as matrix. Ions were desorbed from this matrix initially as (M-H+Ba)⁺. Previous work has involved the study of the decomposition of prostaglandins cationized as lithium salts (3)

The fragmentation of the bariated molecular ion upon high energy collision activation (CA) was observed to be quite different from that of the protonated form, in that charged localization due to the barium ion permitted charge-remote fragmentation to occur (4). In the case of the prostaglandins, localization of the cationic site at the carboxylic acid moiety was facilitated by the use of the divalent barium ion in the desorption matrix. Major ions were observed for the loss of water from each of the hydroxyl groups present in the prostaglandin structure. These are shown in Figures 1 and 2 with the loss of a single moiety of water (PGA₂ and PGB₂) and for a loss of two molecules of water in Figures 3 and 4 (PGD₂ and PGE₂). Two major ions were observed at m/z 209 and 263 for these prostaglandins which arose from a charge-remote fragmentation process indicating the double bond position in these prostanoid structures at C5-C6. The structures of these ions are shown below. Another structurally informative ion was observed at m/z 343 which could be rationalized as arising from the loss of the alkyl chain at carbon 12. The formation of these latter ions involve a proton loss from carbon 8, a proton that is not available in the PGB₂ structure because of the double bond in the cyclopentenone ring.

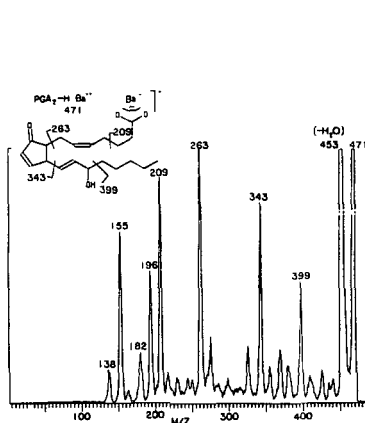


Figure 1 Collision-induced dissociation of bariated PGA₂ (m/z 471)

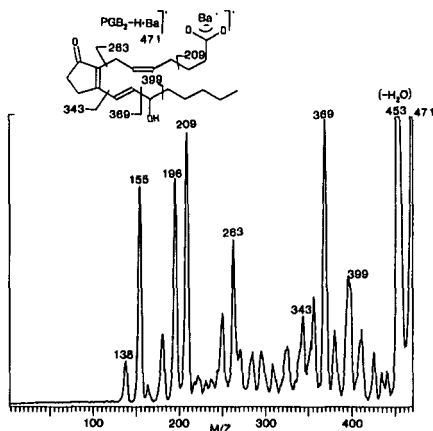


Figure 2 Collision-induced dissociation of bariated PGB₂ (m/z 471)

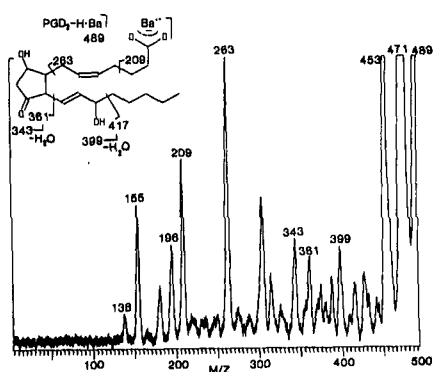
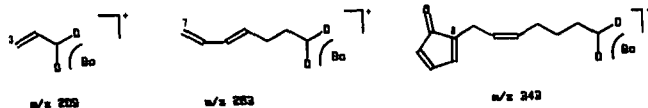


Figure 3 Collision-induced dissociation of bariated PGD_2 (m/z 489)

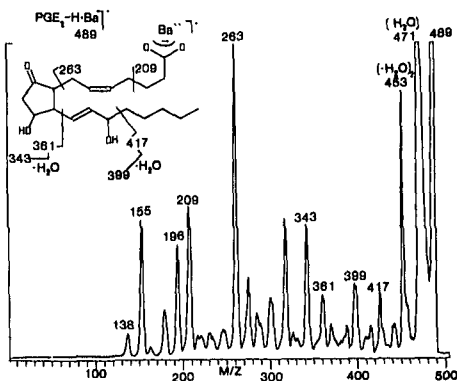


Figure 4 Collision-induced dissociation of bariated PGE_2 (m/z 489)

The loss of water from the various hydroxyl moieties in the prostaglandins has a negative effect on the CA spectra observed for all eicosanoids most likely because isomerization can occur along the C20 carbon chain. Also likely is an interaction of the barium ion with the double bonds or other functional groups present in these complex molecules, resulting in alternative fragmentation pathways that are not well understood at the present time. Studies of the collision-induced dissociation of the $(M-H-H_2O+Ba)^+$ may be of more value to probe the complex structure of these polyhydroxy, unsaturated fatty acids.

Acknowledgements

This work was supported, in part, by grants from the National Institutes of Health (HL25785 and RR01152).

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NEGATIVE ION FAB/MS OF PLATELET ACTIVATING FACTOR

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Fast atom bombardment mass spectrometry is a powerful method of measuring platelet activating factor (PAF, 1-O-alkyl-2-O-acetyl-glycero-3-phosphocholine). This technique is limited, however, by the necessity of separating PAF from other molecules which are chemically quite similar and may be isobaric. Hexadecyl PAF is the major species found in most biological mixtures and this PAF species is isobaric with 1-O-octadecanoyl-lyso-glycero-3-phosphocholine which can be expected to be in these mixtures at concentrations equal to or exceeding those of PAF. MS/MS procedures were investigated as a means to measure PAF in biological mixtures without extensive chromatographic separations.

METHODS: Dynamic (continuous flow) and static FAB/MS was carried out using either a VG 7070E in a normal mode or using the collision cell in the first field free region and performing B/E linked scans to study product ions or B²/E scans to study precursor ions. Dynamic FAB was performed with a microsyringe pump (Isco Inc., Model uLC-500) using a glycerol-based matrix (methanol/water/glycerol, 80/20/2) at 5 μ l/min through a 1 m X 50 μ m untreated fused silica tube. This mass spectrometer was equipped with a saddle-field fast atom bombardment gun and a commercial FAB ion source. FAB desorbed ions were produced with 8 KeV xenon atoms with a gun current of 2 mA. Collision-induced dissociation took place with helium as collision gas in the first field free region collision cell with a 10-50% suppression of the selected ion beam. B/E data from the VG 7070E were obtained using a Teknivent data acquisition system and software. Calibration of these scans was performed at each parent mass by linking the accelerating voltage with the B/E scan then linear scanning all three parameters (B/E/V) simultaneously. This arrangement permitted transmission of all source ions at their proper m/z ratios. The mass range was calibrated with glycerol cluster ions. Accelerating voltage was then unlinked and the B/E scan obtained for product ions.

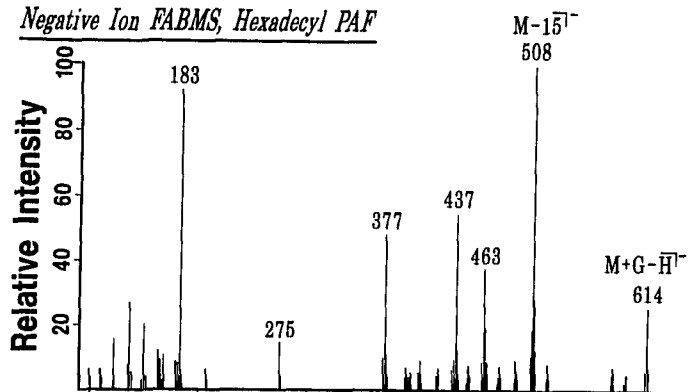
RESULTS: Positive ion FAB/MS of phosphocholines are characterized by strong (M+H)⁺ ions and another intense ion at m/z 184 derived from the phosphocholine head group. Collisional induced dissociation of the isobaric protonated molecular ions of PAF and stearoyl-lyso-GPC followed by linked scanning (B/E) of the ESA and magnetic fields of a VG 7070E resulted in product ion spectra which were virtually indistinguishable between the two species. The major product ion from both compounds was m/z 184.

Negative ion FAB mass spectra of phosphocholines have been recently described to contain intense ions corresponding of M-15, M-60, M-86 as losses from the choline polar head group (Jensen et al., Lipids 21:580-588, 1986). Product ion spectra from the M-15 ion of these phospholipids contain ions diagnostic of acyl chain identity and position.

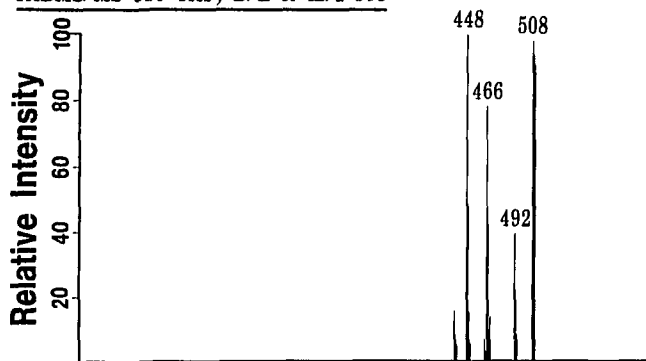
Negative ion FAB/MS of PAF was quite similar to that described for other phosphocholines, showing fragment ions of M-15, M-60 and M-86. However, the product ion spectrum from the M-15 ion revealed ions specifically related to the acetyl group, namely M-42 and M-60. These two ions were shown by isotopic labelling to correspond to the loss of ketene and acetic acid, respectively. The product ion spectrum from the M-15 ion of the isobaric lyso-GPC contained an intense ion at m/z 283, the stearoyl anion. Thus, product ions from the M-15 ion of these similar compounds gave spectra which clearly distinguished between the two structures.

During the course of these studies, a unique and interesting matrix adduct ion was observed in the negative ion FAB/MS of phosphocholines. The ion corresponds to attachment of one glycerol molecule minus a proton to the phosphocholine (M+G-H)⁻. For PAF this ion at m/z 614 was shown through CID and B/E linked scanning methods to be the immediate precursor for the ions previously described as M-15, M-60 and M-86. It may be this unusual chemistry of cluster adduct formation which allows the formation of intense negative ion from zwitterionic phosphocholines which contain a positively charged quaternary amino group.

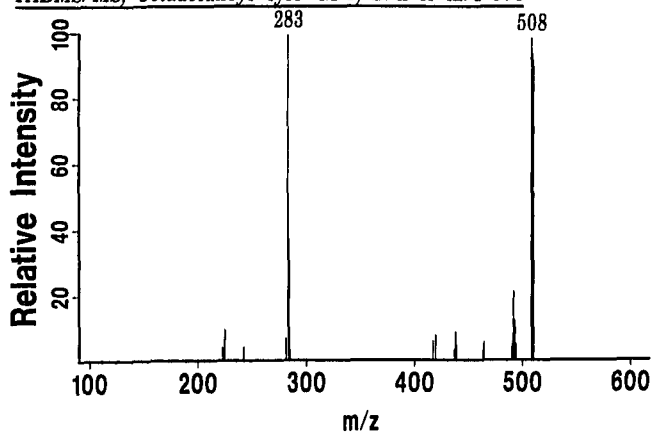
Negative Ion FABMS, Hexadecyl PAF



FABMS/MS C16-PAF; B/E of m/z 508



FABMS/MS, Octadecanoyl-lyso-GPC, B/E of m/z 508



DETERMINATION OF ENDOTHELIAL CELL DERIVED PLATELET ACTIVATING FACTOR BY FAB/TANDEM MASS SPECTROMETRY

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Mass spectrometric determination of lipid-derived mediators has been indispensable for structural elucidation and trace level analysis of these biologically significant molecules. The low concentrations of these agents, together with the complexity of the biological milieu, create stringent analytical demands of sensitivity and selectivity. We recently described (1) the development of a procedure for the detection of platelet activating factor (PAF; 1-O-alkyl-2-acetyl-sn-glycerol phosphoryl choline) using FAB/tandem MS with parent ion scanning on a BEQ instrument (VG ZAB SEQ). The method was rigorously validated and applied to the determination of PAF production by stimulated human neutrophils. A comparison of quantitative data obtained by the FAB/tandem MS method with values obtained by GC-MS of the dephosphorylated and derivatized analyte indicated excellent agreement.

We have recently extended the application of this procedure to the study of stimulated endothelial cells. The possible production of PAF by endothelial cells is of particular interest in defining mechanisms for their interaction with neutrophils. Cultured human umbilical vein endothelial cells (HUVEC) have been shown to produce PAF following stimulation with thrombin (2 U/ml) or calcium ionophore (10 μ M) (Figure 1). Cell extracts were fractionated by silica HPLC to separate PAF from other phosphatidyl choline species. Quantification using [²H₃-acetyl]PAF as internal standard and parent ion scanning indicated levels of hexadecyl-PAF to be (average values in ng PAF per mg protein): 5.7 for thrombin, 11.8 for ionophore and 1.7 for control (no stimulation). The demonstration of PAF production by stimulated endothelial cells qualitatively confirms earlier findings by Prescott et al. (2) who used bioassay techniques; our initial findings, however, suggest that the levels of PAF production may be lower than previously reported.

In preliminary experiments we have evaluated the use of continuous flow sample introduction and detection by selected reaction monitoring as an alternative approach to the analysis of PAF. Figure 2 shows repeat analyses of authentic hexadecyl-PAF (12 pg) with detection of the fragmentation m/z 524 $\rightarrow$ 184. A low picogram detection limit is indicated. Figure 2 also illustrates the satisfactory detection by selected reaction monitoring of PAF in extracts of stimulated endothelial cells.

This work was supported by NIH (GM 34120 and AI 26916)

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FIGURE 1 PAF PRODUCTION BY STIMULATED HUMAN ENDOTHELIAL CELLS

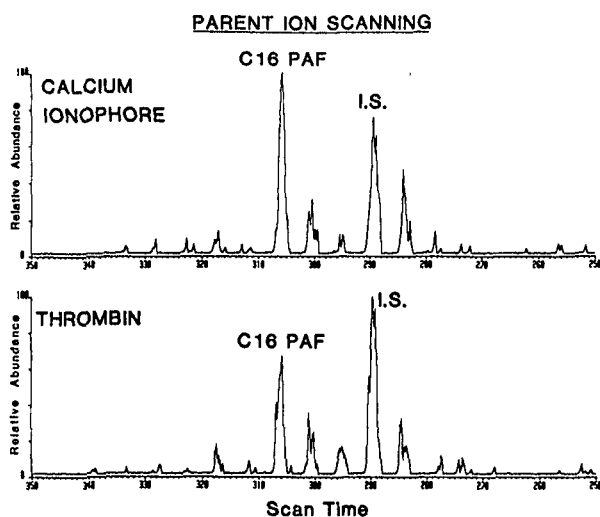
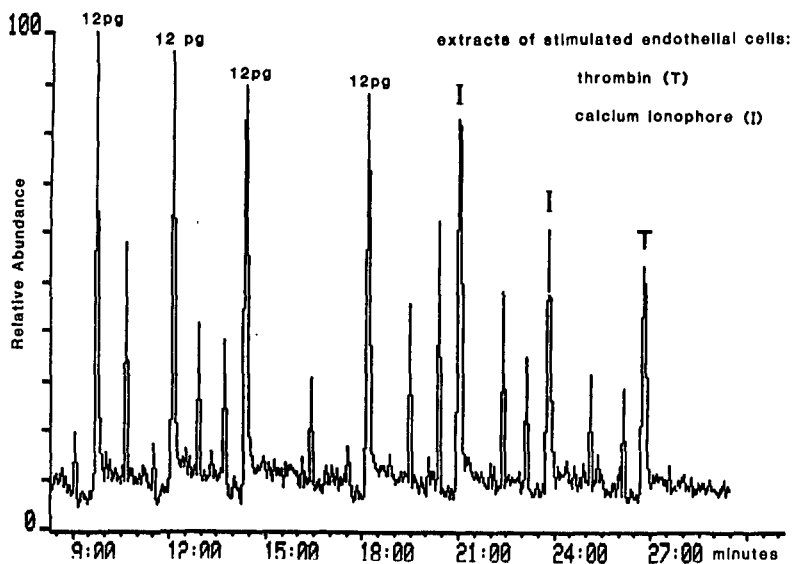


FIGURE 2

CONTINUOUS FLOW FAB SELECTED REACTION MONITORING (524→184)
PAF STANDARD AND BIOLOGICAL EXTRACTS



**ANALYSIS OF LEUKOTRIENES USING CONTINUOUS FLOW FAST ATOM
BOMBARDMENT/TANDEM MASS SPECTROMETRY.**

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Improved understanding of the role of 5-lipoxygenase products as mediators or products of cell injury and/or inflammation requires techniques for their accurate and precise quantification. We describe initial stages of our development of reference quantitative techniques based on tandem mass spectrometry. Hitherto development of analytical methods for the cysteinyl leukotrienes (LT's) has been limited by the expense and limited availability of authentic standards together with their instability.

LTC₄, LTE₄, their respective methyl esters and deuterium labelled analogues have been synthesized essentially according to published procedures¹⁻³. The collisionally activated decompositions of the $[M+H]^+$ and $[M-H]^-$ ions formed by fast atom bombardment have been studied by tandem mass spectrometry (ms/ms) using a hybrid sector quadrupole instrument (VG ZAB SEQ). Characteristic daughter ion spectra were observed for each ion studied, fragmentation pathways have been proposed and are supported by deuterium labelling. For example, the major daughter ion derived from the $[M+H]^+$ ion of ²H₂-LTC₄ (figure 1) is the formation of protonated glutathione (308), other losses include water (610), loss of [glutamine-OH] (499), loss of glutathione (321) and formation of C₁₄H₁₉²H₂ (191) (figure 2).

Initial studies using continuous flow fast atom bombardment/tandem mass spectrometry indicate low picogram quantities of cysteinyl LT's are detectable. Sample introduction conditions were: running solvent H₂O:CH₃CN:glycerol (70:20:10) at a flow rate of 3ul/min. Figure 3 shows the selected ion monitoring (SIM) chromatogram of m/z=628, which corresponds to the $[M+H]^+$ ion formed from ²H₂-LTC₄; three injections were made, each peak corresponds to 8 picograms of analyte.

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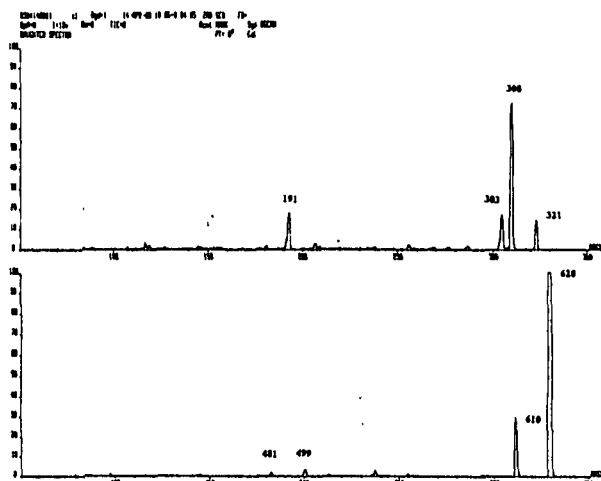


Figure 1. $[M+H]^+$ Daughter ion spectra of 2H_2 -LTC₄ (VG ZAB SEQ).

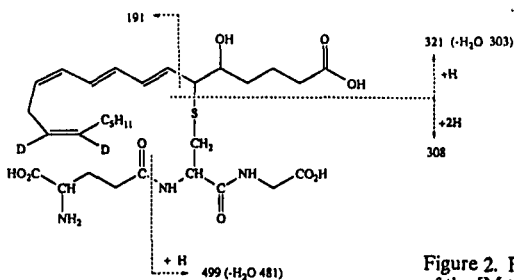


Figure 2. Fragmentation pattern of the $[M+H]^+$ ion of 2H_2 -LTC₄.



Figure 3. SIM chromatogram of $m/z=628$; 3 x 8pg introduced via continuous flow FAB.

LIPOXYGENATION OF POLYUNSATURATES BY HUMAN AND RAT PLATELETS:
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Polyunsaturated fatty acids can be oxygenated by lipoxygenases to form hydroperoxy and hydroxy derivatives. Many of these metabolites are known to possess significant biological activity, however, the type and the extent of these effects depend upon their molecular structure. In addition to arachidonic acid (20:4w6), w-3 polyunsaturated fatty acids have been shown to be oxygenated in various tissues including platelets, neutrophils and brain. However, our studies indicate that hydroxy compounds formed by rat brain homogenates are a racemic mixture, suggesting that they are formed mainly by autooxidation. In platelets, 12-lipoxygenase produces 14- and 11-hydroxy docosahexaenoic acid (HDHE) and 12-hydroxy eicosapentaenoic acid (HEPE) from docosahexaenoic acid (22:6w3) and eicosapentaenoic acid (20:5w3), respectively. The biological significance of these metabolites is not clear at this time, however, 14- and 11-HDHE are capable of inducing smooth muscle contraction and also antagonizing both the smooth muscle contractile and platelet aggregatory effect of thromboxane. 12-HEPE has also been shown to inhibit platelet aggregation induced by thromboxane.

We considered, therefore, that changes in the levels of these metabolites after dietary supplementation with fish oil, rich in 20:5w3 and 22:6w3, may result in a shift in the pattern of eicosanoid production and, consequently, in their biological actions. In our study, platelets obtained from rats fed either a fish oil or corn oil diet for 21 days were stimulated with ionophore, A23187. The various lipoxygenase products formed were characterized using reversed phase HPLC, thermospray LC/MS, chiral phase HPLC and GC/MS analysis; the levels of these lipoxygenase products were also compared. Four major peaks were observed in the reversed phase UV and thermospray ion chromatograms. These products were monohydroxy forms of 20:5w3, 18:2w6, 20:4w6 and 22:6w3, since their thermospray mass spectra contained $M \cdot NH_4^+ - H_2O$ and $MH^+ - H_2O$ at the corresponding m/z values. Monohydroxy derivatives of 20:4w6 and 22:6w3 coeluted in a chromatographic system using an Axxichrom-ODS column and a mobile phase of 0.1M ammonium acetate:methanol ramping from 40:60 to 10:90 in 60 min. These products were collected, methylated and separated on a chiral phase HPLC system using a Dinitrobenzoylphenylglycin (ionic) column and hexane:2-propanol:acetonitrile 497.5:2.5:0.5 as mobile phase. Analysis of the GC/MS/EI spectra indicated that the monohydroxy compounds formed were 12-HEPE, 13-HODD, 12-HETE and 14-HDHE. As an example, a thermospray spectrum and an EI spectrum of 12-HEPE are shown in figure 1. A decrease in 12-HETE and an increase in 12-HEPE were the most prominent changes observed in the stimulated rat platelets after a fish oil diet although there was also a significant increase in HDHE production (Table 1). These changes in the formation of lipoxygenase products seemed to correlate with the changes in the fatty acid distribution of the platelets (Table 2). Chiral phase HPLC analysis indicated that the 12-HEPE produced was in the S configuration, as we have previously reported for 12-HETE, 14-HDHE and 11-HDHE formed by human platelets.

Preliminary data indicate that 12(S)-HEPE also causes a contraction of rat aorta and antagonizes the contractile effects of a thromboxane-mimic. The observed decrease in the 20:4w6 substrate pool in combination with the increased level of w-3 fatty acids which can be metabolized to biologically active compounds may in part explain some of the effects of a diet high in w-3 fatty acids on platelet and vascular function.

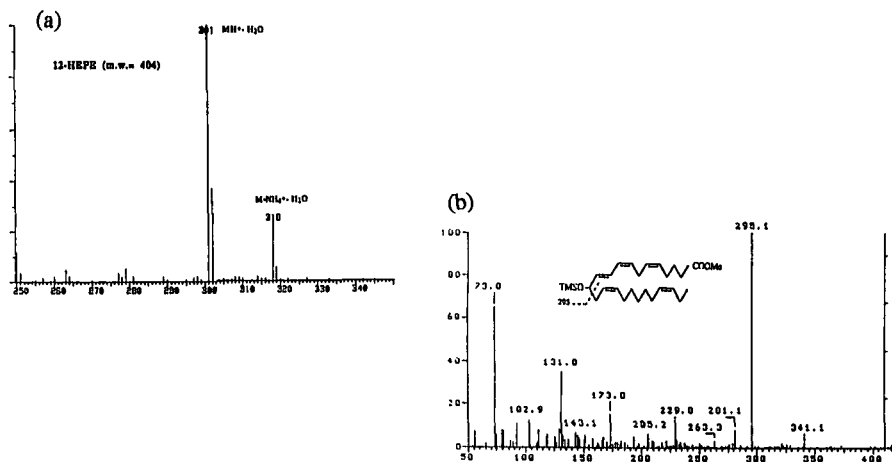


Figure 1. Thermospray (a) and GC/MS/EI (b) mass spectra of 12-HEPE obtained from stimulated rat platelets.

Table 1. Dietary Effect^a on Production of Hydroxy Polyunsaturated Fatty Acids in Rat Platelets^b.

Diet	nmoles/20min/mL			
	12(S)-HEPE	13(S)-HODD	12(S)-HETE	14(S)-HDHE
Corn Oil	0.31±0.11	1.55±0.81	5.56±1.50	0.12±0.01
Fish Oil	2.04±0.24	0.46±0.15	1.45±0.16	0.22±0.01

a. Male Sparague-Dawley rats (n=5 for each group) were fed either 10 wt% of corn oil or 1 wt% of corn oil + 9 wt% of fish oil for 21 days.

b. 6 x 10⁶ platelets/mL were stimulated with 20 μM calcium ionophore, A23187.

Table 2. The Fatty Acid Distribution of Rat Platelets in Animals Fed Either a Fish Oil or Corn Oil-Based Diet.

Diet	20:5w3	18:2w6	20:4w6	22:6w3
Corn Oil	nd	20.5±2.0	12.2±2.1	0.3±0.0
Fish Oil	8.4±0.7	5.3±0.5	7.1±1.5	2.4±0.1

nd = not detected

NOVEL METABOLITES OF LTB₄ FROM GUINEA PIG EOSINOPHILS

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Leukotriene B₄ (LTB₄) is a powerful chemoattractant for guinea pig eosinophils both *in vitro* (1) and *in vivo* (2). We have previously demonstrated (3) that guinea pig eosinophils, when activated either by phagocytotic stimuli or calcium ionophore, expressed an active arachidonic acid 5-lipoxygenase activity that led to the synthesis of LTB₄ and 5-hydroxyeicosatetraenoic acid (5-HETE). Furthermore, the time course of the LTB₄ synthesis indicated the presence of LTB₄ metabolic activity in these cells. Therefore, we examined the ability of guinea pig eosinophils to metabolize LTB₄.

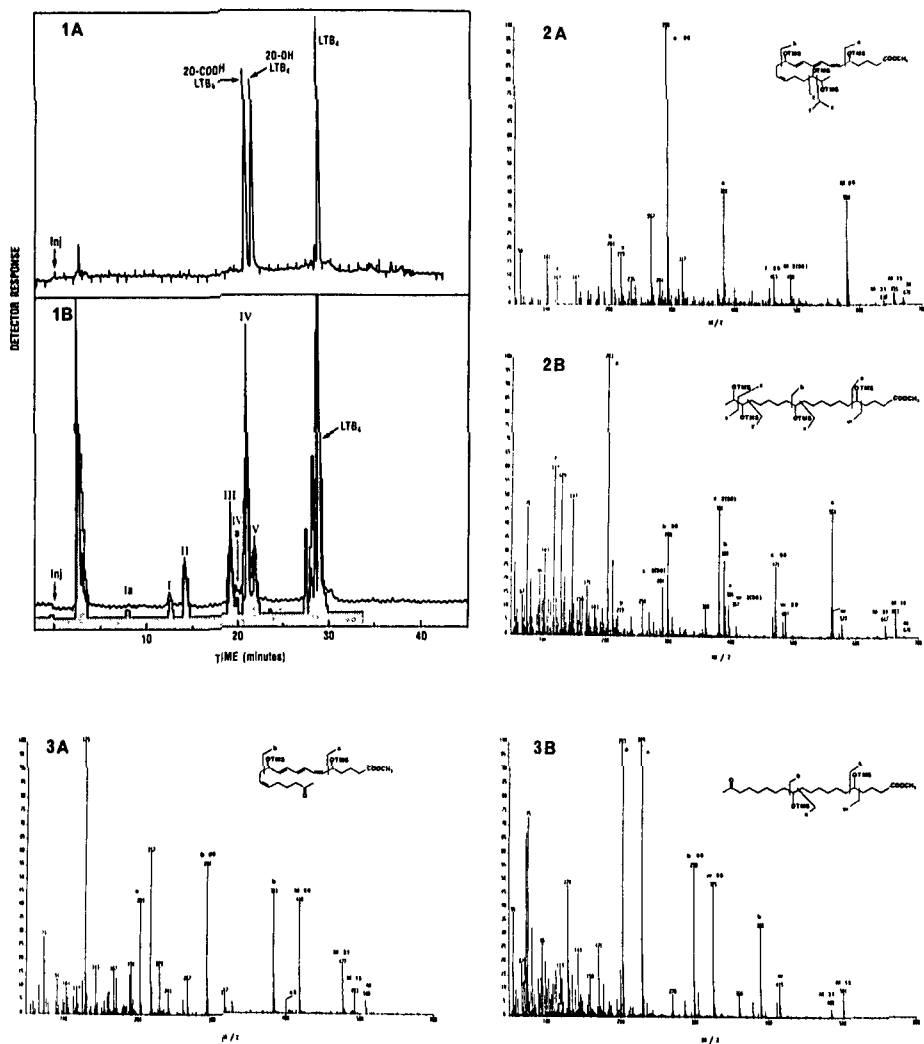
Incubation of LTB₄ with guinea pig eosinophils generated five prominent metabolites (I-V) and two minor metabolites with UV absorbance at 270 nm that can be readily separated by rp HPLC (Figure 1). All of these metabolites incorporated radioactivity from [³H]-LTB₄. All seven metabolites retained the UV triplet of 258, 270, 285 nm indicating that all seven metabolites retain the conjugated triene system of LTB₄. When the retention times were compared with authentic compounds, peak V matched with 20-hydroxy LTB₄, and peak IV matched with 20-carboxy LTB₄.

Metabolites I-V were analyzed by GC/MS following formation of the methyl ester, trimethylsilyl ether derivatives on a VG 70SE mass spectrometer equipped with a HP 5890 gas chromatograph. A 15m DB-1 fused silica capillary column was used. The mass spectra of metabolites V and IV were identical to previously published spectra of 20-OH LTB₄ and 19-OH LTB₄ respectively (5).

The mass spectrum of metabolite III (Figure 2A) exhibits a weak molecular ion at m/z 670 and other important ions at m/z 655 (M-15), loss of CH₃, m/z 639 (M-31), loss of -OCH₃; m/z 580 (M-90), loss of Me₃SiOH; m/z 490 (M-2(90)), m/z 463 (M-207) loss of Me₃SiOH and CH₂-CH₂-OSiMe₃, m/z 383 (b), m/z 293 (b-90); m/z 219 (Me₃SiO + = CH-CH(OSiMe₃)-CH₃, y); m/z 203 (a), and m/z 117 (z). The alpha cleavage ions at m/z 117 and 219 indicate that the two additional -OH groups are located at C18 and C19. This assignment is confirmed by the mass spectrum of hydrogenated metabolite III (Figure 2B). The molecular ion at m/z 678 and ions at m/z 663 (M-15) and m/z 647 (M-31) are 8 mass units greater than before indicating the presence of 4 double bonds. The ions at m/z 203 (a); m/z 117 (z) and m/z 219 (y) are at the same mass as the native metabolite. Fragment ions at m/z 389 (b), m/z 299 (b-90) are shifted up 6 daltons with respect to the corresponding ions in the native metabolite consistent with hydrogenation of three double bonds. Three new alpha cleavage ions are seen in the spectrum of the hydrogenated compound at m/z 577 (M-CH₂-(CH₂)₂-COOCH₃, w), m/z 391 (M-CH₂-(CH₂)₅-CH(OSiMe₃)-(CH₂)₃-COOCH₃, x) and m/z 561 (M-CH(OSiMe₃)-CH₃, c). These fragments lose Me₃SiOH to give rise to ions at m/z 487 (w-90); m/z 471 (c-90); m/z 397 (w-2(90)); m/z 381 (c-2(90)) and m/z 291 (c-2(90)). The spectrum of hydrogenated metabolite III confirms the position of the hydroxyl groups to C5, C12, C18 and C19. This metabolite is identified as 18,19 dihydroxy LTB₄.

The mass spectrum of methyl ester, trimethylsilyl ether derivative of metabolite II is shown in figure 3A. This spectrum shows a molecular ion at m/z 508 and ions at m/z 493 (M-15), loss of CH₃; m/z 477 (M-31), loss of OCH₃; m/z 418 (M-90), loss of Me₃SiOH; m/z 383 (b); m/z 293 (b-90); m/z 203 (a) and m/z 129 (base peak), Me₃SiO + = CH-CH=CH₂. The ions at m/z 383 (b), 293 (b-90), and 203 (a) are characteristic of the C1-12 portion of the leukotriene B₄ molecule and are consistent with the UV data which indicates that the triene chromophore of LTB₄ is intact. The mass spectrum of metabolite II after hydrogenation is shown in figure 3B. The molecular ion is not seen but abundant ions at m/z 501 (M-15) and 485 (M-31) indicate that the mass of metabolite II has been increased 8 daltons by hydrogenation, indicating the presence of 4 double bonds. The b ion is not shifted while the a and a-90 ions are shifted up 6 daltons consistent with hydrogenation of the three double bonds of the C1-12 portion of LTB₄ (6,8,10). Additional ions at m/z 415 (M-101, w), loss of CH₂-(CH₂)₂-COOCH₃ and m/z 229 (M-287, x) are seen. Based this information, metabolite II is proposed to be 19-oxy LTB₄.

Four of the five major eosinophil metabolites have been identified as 20-OH LTB₄, 19-OH LTB₄, 18,19-diol LTB₄ and 19-oxy LTB₄. Guinea pig eosinophils possess an active omega oxidation system capable of metabolizing LTB₄.



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CAD STUDIES OF ALKYL- AND HYDROXY-SUBSTITUTED AROMATIC IONS FOR THE
STRUCTURAL ELUCIDATION OF HYDROXYLATED METABOLITES OF AROMATIC COM-
POUNDS

David C. Young and Paul Vouras

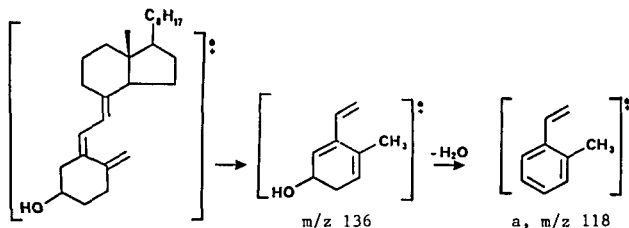
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The use of collisionally activated decomposition (CAD) to determine the structure of ions, based on comparison with the CAD spectra of known ions, has been well established. Judicious selection of compounds used in generating precursor ions, taking basic principles of ion chemistry into account, allows one to generate standard CAD spectra which can be used with reasonable confidence to assign the structure of ions arising in the mass spectra of unknown molecules. We report here on the use of this principle towards determination of the A-ring hydroxylation of Vitamin D.

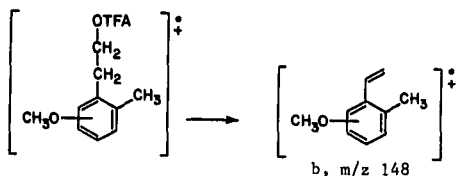
Although not intrinsically an aromatic compound, Vitamin D gives rise in its mass spectrum to an aromatic methylstyryl cation of m/z 118 following the loss of H_2O from m/z 136 (Scheme 1) [1]. A-ring hydroxylated metabolites of Vitamin D would thus incorporate the extra hydroxy group on the ion of m/z 118 shifting it to m/z 134. The position of substitution of the extra OH-group on a metabolite could thus be ascertained by comparing the collision spectrum of its m/z 134 fragment to those of the four possible hydroxymethylstyryl cations generated from synthesized authentic compounds. The hypothesis was tested using readily available 1,25-dihydroxyvitamin D_3 .



Scheme 1

Different approaches to the generation of these ions were pursued. It was soon realized that preparation of styryl compounds bearing the structure represented by ion a was complicated by their facile polymerization. We thus decided to explore the production of such ions in situ via the McLafferty rearrangement of trifluoroacetyl (TFA)

derivatives of the corresponding hydroxyphenyl-ethanols. We found, however, that the four isomeric hydroxy-2-methylstyryl cations of interest gave identical CAD spectra. In order to circumvent this problem, methylation of the aromatic hydroxy group was considered. CAD spectra were thus acquired for the four isomers of the methoxystyryl cations of m/z 148 which were generated from the TFA derivatives of the appropriate isomeric methoxy-2-methylphenyl ethanols (Scheme 2). Consistent with this approach, 1,25-dihydroxyvitamin D_3 was also permethylated and its mass spectrum gave rise to the analogous methoxystyryl ion of m/z 148. Significant differences in the CAD spectra of the four isomers were observed. This behavior is in contrast to that of the corresponding hydroxy analogs and is, in part, due to resonance processes through the aromatic ring to form benzoquinone type cations in the *ortho* and *para* isomers following the loss of a methyl radical. Moreover, as expected, only the CAD spectrum of the 3-methoxy-2-methylstyryl ion matched that of the m/z 148 ion from 1,25-dihydroxy vitamin D_3 . The CAD spectra of the different isomeric ions are partially summarized on Table I. This method provides a viable approach for the characterization of A-ring metabolites of vitamin D as well as for related aromatic systems in general.



Scheme 2

Table I

Partial CAD mass spectra of the m/z 148 ion generated from methoxystyryl ions of type *b* and 1,25-dihydroxy vitamin D_3 .

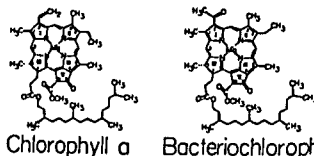
m/z	3-MeO	4-MeO	5-MeO	6-MeO	1,25-di-OMe-Vit- D_3
133	46	86	92	100	56
117	100	58	100	7	100
115	60	65	86	11	59
105	40	100	66	61	38
103	36	37	54	6	31
91	42	52	80	10	50
77	27	46	54	7	25

1. Zaretskii, Z.V.I.; Kingston, E.E.; Beynon, J.H.; Lauber, R.; Schlunegger, U.P., *Org. Mass Spectrom.*, 1985, **20**, 336.

Structural Determination of Modified Chlorophylls By Using Tandem Mass Spectrometry

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Improved methods for structural determinations are needed to further elucidate chlorophyll biosynthesis and photosynthetic mechanisms. We present here the first application of MS/MS to investigate a series of structurally modified chlorophylls. To evaluate MS/MS, we chose bacteriochlorophyll *a*, chlorophyll *a* and *b*, chlorophyll *a* and *b* allomers (10-*HO*-, 10-*HO*-lactone-derivatives) and the pheophytins as model systems.

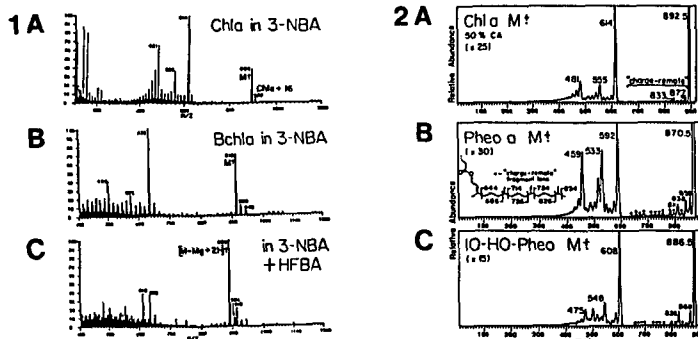


In addition, we examine the application of MS/MS to study the intrinsic reactivities of chlorophylls with alkali metal ions, and chlorophyll dimers. The study of dimers is especially interesting because of their role as the "special pair" in the photosynthesis reaction center.

FAB of *chl a* in a matrix of 3-NBA produces an abundant molecular ion cluster and fragment ions consistent with previously reported spectra. (Figure 1a) (1) The predominant molecular species is the radical cation. The loss of the phytyl chain gives the most abundant fragment ion (*m/z* 614).

The FAB matrix offers both advantages and problems for analysis of chlorophylls. The FAB matrix acts to modify the strong aggregation of *chl a*; thus *chl a* is desorbed with less internal energy and the "explosive" fragmentation observed in 252Cf-PD(1)(2) and LD(3) techniques is not observed by FAB. Reactions of chlorophyll with the FAB matrix, however, are problematic for accurately determining their purity. Oxidation products of *chl a* and *Bchl a* (10-*HO*- and 10-*HO*-lactone-derivatives) and chlorophyll-matrix adducts are observed in the FAB spectra (Figures 1a and b). Their relative abundances increase with continued bombardment of the sample.

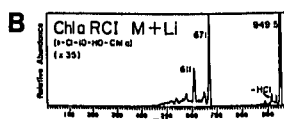
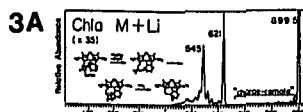
Demetallation of chlorophyll upon addition of hexafluorobutyric acid (HFBA) to the FAB probe yields the corresponding pheophytin (Figure 1c). This is analytically useful as the pheophytins yield more abundant structural information upon CA (e.g. charge-site-remote fragmentations).



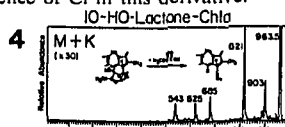
The CAD of the $M^{+\bullet}$ of the chlorophylls studied are similar to that of *chl a* (Figure 2a). Product ions in the range *m/z* 400-620 are similar to source-produced fragment ions in the full scan spectrum. The MS/MS experiment, however, is less ambiguous because only the decomposition of a single species is measured. Detection limits for acquiring good quality MS/MS spectra is ~10 pmoles (10 ng). The energy loss accompanying CAD with He limits the accuracy of mass assignments in the MS/CA/MS spectra. Mass assignments were confirmed by comparison with MS/MS metastable and CA B/E linked scan experiments. The energy loss accompanying CA with He associated with the ion of *m/z* 614 was 23.4 ± 0.1 eV, which is in good agreement with the value reported by others.(4)

- (1) Chait, B.T.; Field, F.H. *J. Am. Chem. Soc.* **1982**, *104*, 5519-5521. Chait, B.T.; Field, F.H. *J. Am. Chem. Soc.* **1984**, *106*, 1931-1938.
- (2) Hunt, J.E.; Macfarlane, R.D.; Katz, J.J.; Dougherty, R.C. *J. Am. Chem. Soc.* **1981**, *103*, 6775-6778.
- (3) Brown, R.S.; Wilkins, C.L. *J. Am. Chem. Soc.* **1986**, *108*, 2447-2448.
- (4) Bricker, D.L.; Russell, D.H. *J. Am. Chem. Soc.* **1986**, *108*, 6174-6179. Gueveremont, R.; Boyd, R.K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *84*, 47-67.

CA of the pheophytins produce more abundant structural information than the chlorophylls. A series of regularly spaced peaks are assigned to charge-remote losses of the elements of C_nH_{2n+2} from the phytol chain. Branch points are identified by interruptions in the series of alkane losses. The ion of m/z 838 is produced by loss of methanol and formation for the stable ketene ion.(5) The loss of methanol is not seen upon CA of the chlorophyll derivatives in which the C-10 hydrogen has been replaced by -OH. The ion of m/z 811 arises by loss $\cdot COOCH_3$ and is an abundant ion for all the chlorophylls studied.

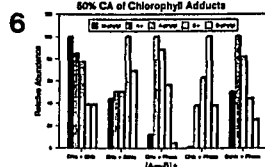
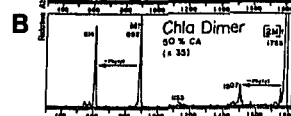
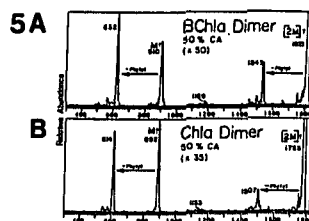


CA of chlorophylls cationized with alkali metal ions is complementary to the M^+ ion. CA of Chla $[M + Li]^+$ produces an ion of m/z 545 resulting from loss of the phytol chain and subsequent losses of methanol and CO_2 (Figure 3a). Metal ion attachment and enol formation at C-9 are consistent with chla solution chemistry. The above fragmentation is not produced in the CA of the $[M + Li]^+$ ion of chla RCI(6) because the C-10 hydroxyl prevents the loss of methanol(Figure 3b). Loss of HCl confirms the presence of Cl in this derivative.



An interesting ion produced by CA of the $[M + K]^+$ species of HO-lactone-chla, is the ion of m/z 821(Figure 4). The metal ion is being lost possibly by a mechanism in which the K^+ is chelated by the oxalic acid methyl ester. Subsequent loss of 278 amu (phytyl) from this ion produces the ion of m/z 543.

In addition to producing abundant M^+ ions, FAB of chlorophyll produces a less abundant dimer $[2M]^+$ (Figure 5a and b). CAD of chlorophyll dimers has not been reported.



Ions corresponding to M^+ and $[M - phytol]^+$ are the most abundant ions in the CA mass spectra. More revealing are the higher mass ions due to losses of CH_3OH , $\cdot COOCH_3$, and phytol from the dimer. The ions of m/z 1189 and 1153 from Bchla and chla respectively are proposed to result from successive losses of the two phytol groups and CH_3OH and CO_2 in a mechanism similar to that of the CAD of the $[M + Li]^+$ adducts.

It has been shown that coordination of the C-9 carbonyl of one chla to the Mg of the another chla is responsible for dimer formation in solution.(7) The higher mass ions demonstrate that the $C=O \cdots Mg$ interaction between the two chla molecules is weaker than but comparable to the critical energy for cleaving off the phytol chain. If our mechanism is correct, the ions of m/z 1189 and 1153 lend support to the $C=O \cdots Mg$ interaction.

CAD of mixed chlorophyll adducts allows us to qualitatively order the ionization potentials (IP) of the various chlorophylls by measuring the relative abundances of the M^+ species(Figure 6). These results suggest, in order of increasing IP: Bchla < Chla < Pheoa < Chlb.

- (5) Jackson, A.H.; Kenner, G.W.; Smith, K.M.; Aplin, R.T.; Bubzikiewicz, H.; Djerassi, C. *Tetrahedron* 1965, 21, 2913-2924.
- (6) Senge, M.; Dörnemann, D.; Senger, H. *FEBS Lett.* 1988, 234, 215-217. Senge, M.; Senger, H.; *Photochem. Photobiol.* 1988, 48, 711-717.
- (7) Katz, J.J.; Shipman, L.L.; Cotton, T.M.; Janson, T.R. in *The Porphyrins*; Dolphin, D. Ed.; Academic Press: New York, 1978; vol.5, Part C, pp. 401-458.

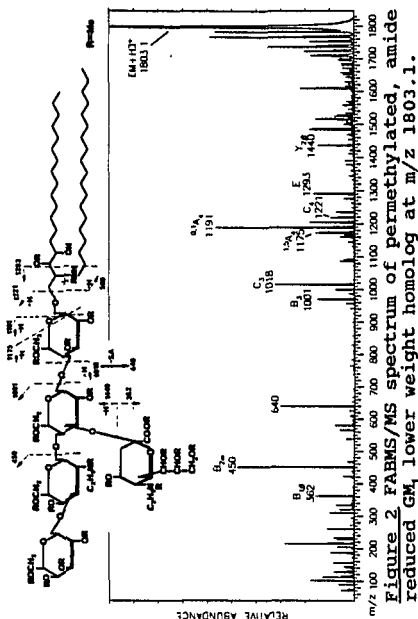
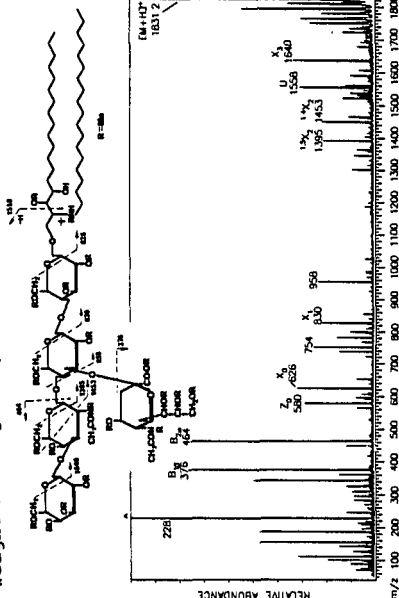
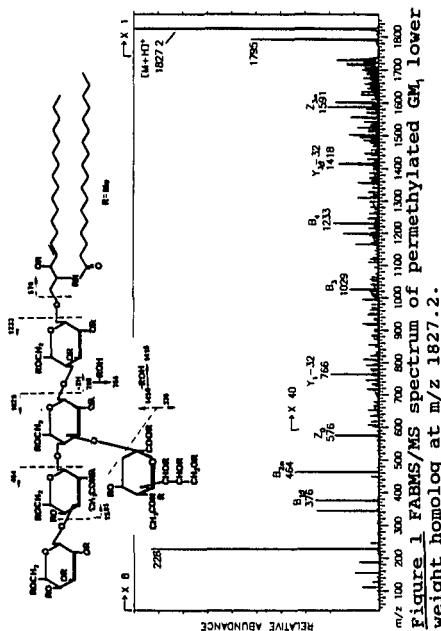
DERIVATIZATION OF GANGLIOSIDES AND THEIR ANALYSIS BY HIGH PERFORMANCE TANDEM MASS SPECTROMETRY J. E. Vath, B. Doman and C. E. Costello, Dept. of Chemistry, Mass. Institute of Technology, Cambridge, MA 02139

Derivatization of gangliosides shows promise for improved analysis by MS/MS, by increasing the parent ion abundance and creating groups which direct fragmentation toward structurally significant daughter ions. Permethylated-reduction has been very successful for EIMS characterization of glycolipids and permethylation has also been shown to increase FAB/MS signal. We have explored the use of similar derivatives for FAB/MS analysis of these compounds, with the expectation that the secondary amines (generated from the amides by reduction) would serve as sites for charge localization and therefore strongly influence the CID fragmentation pattern.

Permethylating using CH_3I and solid NaOH in DMSO followed by borane reduction using condensed reagent vapor comfortably allows one to derivatize 1-10 μg of ganglioside for MS/MS analysis. A comparison of the CID spectra of the permethylated, permethylated-amide reduced and permethylated-ceramide-only reduced derivatives for the lower weight homologs of GM₁ are shown in Figures 1, 2 and 3, respectively. The FAB/MS spectrum of the permethylated compound exhibits ions resulting from protonation and cleavage of the glycosidic linkage (B and Y ions). The CID spectrum of the permethylated-amide reduced GM₁ offers stronger fragment ions that characterize the carbohydrate and aglycon portions at a glance, after one becomes familiar with the derivatives' fragmentation. Typically the ${}^n\text{A}_n$ ion, where n is the number of sugars in the longest chain, (m/z 1191 for GM₁) is the base peak in the spectra of these derivatives and reveals immediately the size of the carbohydrate moiety. The sequence of the sugars can be deduced from the B and C ions. Sialic acid strongly directs B ion formation in all derivatives, which is useful in confirming parallel or serial attachment of multiple sialic acids. There is also usually an ion, labeled E (m/z 1293 for GM₁), which is due to elimination of the long chain base beyond C2 and the entire reduced acyl chain. The fact that the ceramide amide is reduced more easily than the N-acetyl moiety is exploited to give one another choice concerning permethylated-reduced derivatives. Reduction at the ceramide only (Fig. 3) yields a complete series of X ions (which clearly show the carbohydrate sequence), a dependable U ion (which characterizes the base and fatty acid portion of the aglycon) and some selective eliminations in the sugars which can give clues pertaining to the substitutions about the rings. The conditions used to bring about these transformations, and the effects on all of the functional groups in the gangliosides studied are shown in the Table. As found with the CID spectra of peptides and with the reduced cerebroside and ceramides we initially studied, fragmentation is strongly influenced by the presence of basic sites. As the number of basic sites in a molecule increases, so does the number of directing influences and these factors alter the information in the CID spectra. Besides the effects on fragmentation, derivatization also brings about an improvement in sensitivity which is about 50-fold upon permethylation and becomes about 100-fold with subsequent reduction.

The availability of a number of derivatives for the analysis of gangliosides is necessary for a completely reliable determination. Many of the ambiguities present in the spectrum of one derivative can be answered by another. A combination of permethylation and reduction allows the flexibility to control the analysis of gangliosides by FAB/MS. The sensitivity increase observed and the ability to conduct the reactions on nmol amounts is extremely important since mass spectrometry often represents the only method to characterize these compounds at that level.

EXPERIMENTAL Gangliosides (5-10 μg) were permethylated by the method of Ciucanu and Kerek [Carbohydr. Res. (1984)131:209] and the samples were dried in the bottom of a melting point capillary. The tubes were placed inside an apparatus which allows the reaction of small amounts of sample with reagent vapor (ASMS 1988 p. 167). After the reactions were complete 10 μl of 10% NBA in 2:1 $\text{CHCl}_3/\text{MeOH}$ was placed into the capillary and the sample was concentrated *in vacuo*. An 0.5 μl aliquot of the above solution on the FAB probe of a JEOL HX110/HX110 high performance tandem mass spectrometer was used for analysis.



REACTIVITY OF CARBOXYSE FUNCTIONAL GROUPS	
First Reaction CH₃COOH + DMSO to form HT CH₃-CH₂-CH₂-CH₂-COOH CERAMIDE CH₃-CH₂-CH₂-CH₂-CH₂-COOH 16:0 Ceramide CH₃-CH₂-CH₂-CH₂-CH₂-CH₂-COOH 18:0 Ceramide	Second Reaction HT HT + HT⁺ 2 HT₂ at 25°C CH₃-CH₂-CH₂-CH₂-CH₂-CH₂-COOH HT⁺ CH₃-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-COOH HT⁺ CH₃-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-COOH HT⁺

This reaction is followed by catalysis with HCl/NaOH for 3 hr at RT and oxidation with 1:1 $\text{H}_2\text{O}_2/\text{NaOH}$ for 10 hr at 45°C. The entries shown reflect the final products after these additional steps.

2nd indication no (c)

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ANALYSIS OF CAROTENOID COMPOUNDS BY ELECTRON CAPTURE NEGATIVE ION MASS SPECTROMETRY

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INTRODUCTION

High consumption of certain foods abundant in carotenoids has recently been associated with a reduced incidence of several types of human cancer. Additionally, it has been suggested that carotenoids, along with vitamin E, protect the vascular system against development of arterial plaques. These considerations, among others, prompted an examination of methods for the analysis of carotenoid compounds.

While molecular weight data are available from electron ionization spectra of many carotenoids, there are classes of carotenoids, for example bis-fatty acyl esters of carotenediols, for which data indicative of the molecular weight are absent or extremely weak. Further, improvements in the sensitivity would be an advantage for analysis of biological samples where there is a paucity of available sample.

EXPERIMENTAL

Carotenoids bearing a variety of functional groups were obtained either from commercial sources or by extraction from vegetables and subsequent purification.

Electron capture negative ion (ECNI) spectra (one second cycle time) were obtained from a Finnigan-MAT model 4510 equipped with a direct exposure probe and a 5 KV conversion dynode. Except where noted, methane (0.25 Torr) was used as the reagent gas.

Under yellow light, samples were deposited onto the desorption probe wire loop and solvent (benzene) removed by a gentle stream of room temperature air. The probe was immediately inserted, heated by application of an increasing current (50 ma/sec) and negative ion spectra collected. Electron ionization and chemical ionization spectra were obtained using parameters detailed in table 1.

RESULTS

Under conditions for ECNI, hydrocarbons (alpha-carotene, beta-carotene), aldehydes (beta-apo-8'-carotenal) and ketones (canthaxanthin) yielded single ions corresponding to the molecular anions M^- . The increase in sensitivity (table 1), compared to electron ionization, ranged from 25 (beta-apo-8'-carotenal) to 490 fold (canthaxanthin). The low value for beta-apo-8'-carotenal can be rationalized based on its less extensive system of conjugation.

In addition to molecular anions, two carotenediols (lutein and isozeaxanthin) yielded ions reflecting the sequential elimination of two molecules of water. In the case of lutein which possesses both an epsilon ring and a beta ring, the molecular anion was the base peak. In contrast, for isozeaxanthin, which possesses two beta rings, the $M-2H_2O$ ion was the most abundant. A possible explanation for this inversion of abundance ratios is the resulting conjugation provided by elimination from the beta ring. ECNI provides an increase in sensitivity of approximately two orders of magnitude for the determination of molecular weight when compared to electron ionization.

ECNI mass spectral analysis of lutein using deuterio-ammonia (0.27 Torr) provided simultaneous deuterium exchange and electron capture, thereby demonstrating, at high sensitivity, the presence of two exchangeable hydrogens.

Two carotenoid dihydroxy epoxide compounds, lutein epoxide (a mono-epoxide) and violaxanthin (a di-epoxide), were examined. For both compounds, the molecular anion was the base peak. In the case

of lutein epoxide, however, the allylic hydroxyl group promotes an abundant ion reflecting the loss of water which was not observed in violaxanthin. Again, ECNI yields an increase in sensitivity of two orders of magnitude for the detection of ions indicative of molecular weight when compared to EI.

Under conditions of electron ionization, the di-ester isozeaxanthin dipelargonate yields a spectrum characterized by an abundance of low mass to charge ions. Absent are molecular ions and abundant ions diagnostic for the size of fatty acyl groups. In contrast, the electron capture negative ion spectrum provides not only ions indicative of the molecular weight, but also abundant ions reflecting the losses of fatty acyl groups as well as confirmatory carboxylate anions.

**SENSITIVITY^a FOR DETERMINATION OF MOLECULAR WEIGHT
(RELATIVE TO ELECTRON IONIZATION)**

COMPOUND	MW	EI ^b	NH ₃ ^c	CH ₄ ^d	ECNI ^e
BETA-APO-8'-CAROTENAL	416	1	1.3 ^c	1.1	25
ALPHA-CAROTENE	536	1	5.1 ^c	18.0	280
BETA-CAROTENE	536	1	3.2 ^c	15.0	290
CANTHAXANTHIN	564	1	3.0 ^d	30.0	490
ISOZEAXANTHIN	568	1	38.0 ^d	27.0	110
LUTEIN	568	1	13.0 ^d	30.0	254
LUTEIN EPOXIDE	584	1	24.0 ^c	67.0	210
VIOLAXANTHIN	600	1	35.0 ^c	10.0	540
ISOZEAXANTHIN DIPELARGONATE	848	- ^g	1.0 ^d	1.0	660 ^h

^a Chart compares relative sensitivity between ionizing methods, but does not compare relative sensitivity between compounds.

OBSERVED ION CONDITIONS

- ^b M⁺ 70 eV at source temperature of 150 °C
^c (M+H)⁺ 0.6T at source temperature of 60 °C
^d (M+NH₃)⁺ 0.6T at source temperature of 60 °C
^e (M+H)⁺ 0.3T at source temperature of 60 °C
^f M⁺ 0.25T at source temperature of 60 °C
^g No M⁺ ions were observed
^h Sensitivity calculated relative to methane positive ion

CONCLUSIONS

ADVANTAGES

1. Electron capture negative ion MS provides greater sensitivity for determination of molecular weight (ca. two orders of magnitude when compared to EI).
2. Non-electron capturing contaminants are invisible.
3. Presence of contaminating carotenoids readily apparent.
4. Number of exchangeable hydrogen can be determined at high sensitivity.
5. Size of fatty acyl moieties of esters easily determined.

DISADVANTAGES

1. Mode of introduction subjects sample to air oxidation.
2. Production of carotenoid anions quenched by gross amounts of contamination.
3. Lack of ions diagnostic for carotenoid end groups.
4. Sensitivity is highly dependent on source cleanliness.
5. Requires an instrument capable of detecting negative ions.

ETHANOL INDUCED CHANGES IN CENTRAL NERVOUS SYSTEM PROSTAGLANDIN PRODUCTION:

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Melvyn P. Heyes, Laboratory of Neurophysiology, NIMH, Bethesda, MD, 20892.

INTRODUCTION

In recent years, a relationship between the central nervous system (CNS) effects of ethanol and central production of prostaglandins has been suggested, based primarily upon the finding that behavioral effects of ethanol can be modulated by pre-administration of prostaglandin synthetase inhibitors. It has also been hypothesized that the etiology of alcoholism may be linked to aberrant fatty acid and prostaglandin metabolism. Direct evidence for ethanol induced changes in CNS prostaglandin production has been contradictory. We developed a sensitive and specific assay based on selected-ion-monitoring, electron-capture negative ionization GC/MS detection of the N-methyl methoxime, pentafluorobenzyl ester, tris-trimethylsilyl ether derivatives of PGE₂, PGD₂, PGE₁, PGF_{2α}, PGF_{1α}, TXB₂, and 6-keto-PGF_{1α}. We previously reported significant improvements in the assay procedure and results which indicated that the concentrations of PGE₂, PGE₁, PGF_{1α}, and 6-keto-PGF_{1α} were below 15 pg/mL in lumbar CSF of healthy humans and abstinent alcoholics. In order to investigate other means of measuring CNS prostaglandins, we explored both an *in vivo* microdialysis method and an *in vitro* tissue slice method.

METHODS

***In Vivo* Microdialysis:** Four male Sprague-Dawley rats, studied on separate days, were anesthetized with pentobarbital, immobilized in a stereotaxic frame, and the microdialysis probe inserted into the striatum following surgery. The dialysis membrane was perfused at 2 µL/min with Ringer's solution, and samples collected as six one hour aliquots. Eicosanoid levels were measured in the 120 µL samples, and expressed as pg / sample, from which extracellular levels can be calculated knowing the dialysis probe *in vitro* recovery.

***In vitro* Tissue Slices:** Male Sprague-Dawley rats were sacrificed by decapitation, their brains rapidly removed to an iced stage, and 1 mm slices of frontal cortex prepared and placed in ice cold 1 % bovine albumin in Krebs's buffer. The slices were then incubated at 37°C for 30 min, the buffer removed, and again washed with albumin for 30 min. Slices were then incubated in Krebs's alone (Control) or Krebs's plus 200 mg/dL ethanol for 30 min. Eicosanoid levels were measured in the buffer, and expressed as pg produced / mg tissue / hour.

Eicosanoid Assay: Prostaglandins E₂, D₂, F_{2α} and 6-keto-F_{2α} and thromboxane B₂ were determined by GC/MS using a slight modification of previous methods. Briefly, tetradeuterated standards were added to aqueous samples, which were treated overnight to produce the N-methoxime of the ketone containing eicosanoids, extracted with C₁₈ solid-phase cartridges using gravity flow, derivatized to the pentafluorobenzyl ester, trimethylsilyl ether, and dissolved in a final volume of 10 µL dodecane. All drying steps were accomplished using vacuum rotary evaporation. Samples were injected on-column at 215°C into a 1 m, 0.53 mm i.d. retention gap, attached to a 15 m, 0.25 mm i.d. DB-5 analytical column housed in a Carlo-Erba gas chromatograph. Heating to 315°C over 2 min allowed elution of measured eicosanoids in less than 4 min. The Kratos MS-80RF mass spectrometer was operated in negative electron-capture ionization mode, and set to switch between the predominant [M-PFB]⁻ ion in the spectrum of each eicosanoid using selected-ion-monitoring. Results are presented as mean ± SEM, and statistical analysis was done using a one-way analysis of variance and Dunnett's t-test.

RESULTS AND DISCUSSION

***In Vivo* Microdialysis:** Extracellular fluid levels of PGE₂, PGD₂, PGF_{2α}, 6-keto-PGF_{1α} and TXB₂ were sampled *in vivo* in the rat striatum (See Table 1 & Figure 1). Calculated extracellular concentrations ranged from 1.8 to 51 nmol/L. The lowest calculated concentrations were similar to levels reported in the literature for undamaged tissue. *In vivo* microdialysis may, therefore, allow sampling of baseline eicosanoid production in the brain. The results demonstrated that tissue damage induced by the probe's insertion resulted in elevated levels of PGD₂, PGF_{2α} and TXB₂ recovered from the area of damage in the first hour of sampling compared to later sampling times. This is consistent with the notion that arachidonic acid was rapidly released and metabolized to these specific eicosanoids. The increase in PGE₂ in the sixth hour of sampling relative to earlier time points suggests that selective production of this eicosanoid may occur as the damage process proceeds. Until the model is extended to chronically implanted animals, the study of ethanol's effects on the brain cannot be attempted by *in vivo* microdialysis, since exposure of anesthetized animals to ethanol would almost certainly kill the animals.

***In Vitro* Tissue Slices:** Study of brain eicosanoid production *in vitro* requires that tissues be sufficiently washed with albumin prior to incubation, in order to remove arachidonic acid released during decapitation and tissue preparation (See Figure 2). Ethanol at a level of 200 mg/dL induced a small decrease in eicosanoid production in central nervous system tissue *in vitro*, as measured in rat brain frontal cortex (See Figure 3).

TABLE 1: Eicosanoid Levels in Perfusate from *In Vivo* Microdialysis

Hours	PGD ₂	PGE ₂	PGF _{2α}	TXB ₂	6-keto-PGF _{1α}
1	432.4 ± 83.4 [†]	48.1 ± 15.3	203.9 ± 24.3 [†]	149.1 ± 23.8 [†]	20.8 ± 3.5
2	31.4 ± 6.1	24.2 ± 8.4	44.6 ± 12.8	22.3 ± 6.1	14.7 ± 2.0
3	31.4 ± 13.7	46.8 ± 10.6	31.2 ± 9.2	28.4 ± 16.7	24.4 ± 8.2
4	21.8 ± 11.3	79.2 ± 40.7	23.0 ± 5.9	17.0 ± 4.4	22.2 ± 5.9
5	24.8 ± 9.4	96.4 ± 35.3	29.6 ± 6.2	27.7 ± 11.0	27.2 ± 9.6
6	46.3 ± 18.2	162.5 ± 23.0*	51.9 ± 8.9	48.9 ± 2.2	24.5 ± 3.5

Values are pg/ one hour sample, presented as Mean ± SEM (n=4).

[†] Significantly different from all subsequent time points p < 0.0005.

* Significantly different from first four time points p < 0.01.

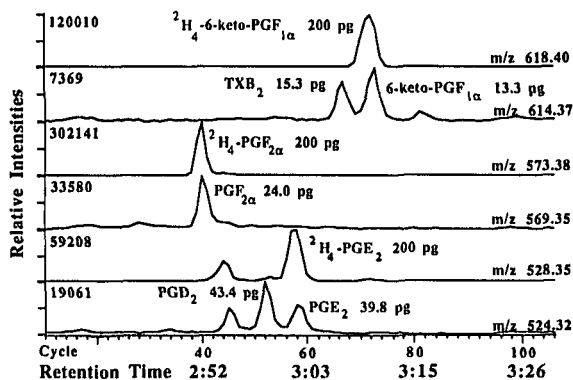


Figure 1: Selected-Ion-Chromatogram for GC/NCIMS Determination of Eicosanoid Levels from *In Vivo* Microdialysis perfusate of Rat Striatum.

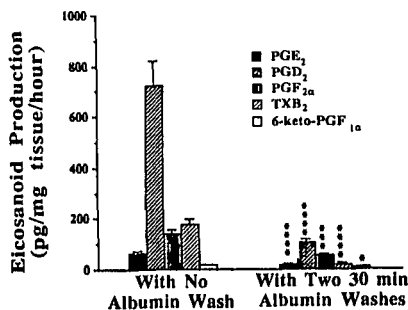


Figure 2: Effect of 1% Albumin Wash on Eicosanoid Production from Rat Brain Frontal Cortex Slices.

Values expressed as Mean ± SEM (n=6).
Significantly different from unwashed control:
**** p < 0.00001, *** p < 0.001, * p < 0.02.

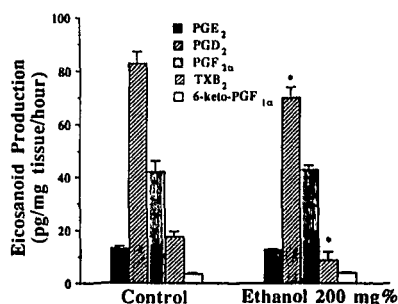


Figure 3: Eicosanoid Production from Rat Brain Frontal Cortex Slices
Values expressed as Mean ± SEM (n=8)
Significantly different from control:
* p < 0.05

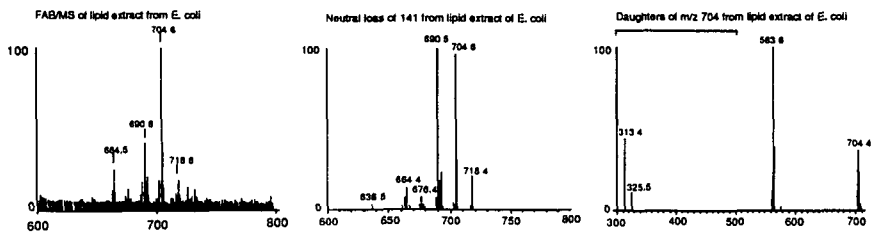
BACTERIAL PHOSPHOLIPID ANALYSIS BY FAB/MS/MS: CHARACTERIZATION OF THE EFFECTS OF GROWTH CONDITIONS

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Fast atom bombardment mass spectrometry (FAB/MS) shows promise for bacterial detection and identification based on analysis of desorbed phospholipids. Although providing information on phospholipid content and distribution, FAB/MS suffers from a high level of chemical background noise which produces a peak at every mass throughout the spectrum. This background noise can obscure important information useful for bacterial analysis, and raise the detection limit of the method. FAB in conjunction with tandem mass spectrometry (FAB/MS/MS) overcomes much of the chemical noise and detectability problems through the increased selectivity afforded by the tandem mass selection.

We are taking advantage of the increased scan modes available with MS/MS to develop a detection scheme based on the combination of conventional, neutral loss, and daughter spectra which fully profiles the phospholipid content and distribution in bacteria. An automated method is easily programmed into the instrument control to produce these profiles quickly, and in a single run. We are using these profiles to assess the effects of different growth conditions on the phospholipid content and distribution in bacteria.

When phospholipids undergo CID, they cleave at specific points which are common to all classes of phospholipids. These cleavages produce a neutral loss characteristic of the phospholipid class, and two fragment ions - each characteristic of a fatty acyl group contained in the phospholipid.



The phospholipid distribution in the bacteria showed wide variance over different growth temperatures. An example is shown in figure 2. In all of the bacteria studied, maintaining a constant growth temperature was found to be important to spectral reproducibility.

**LIPID ABUNDANCE VS. GROWTH TEMPERATURE
FOR B. SUBTILIS AFTER 1 DAY GROWTH TIME**

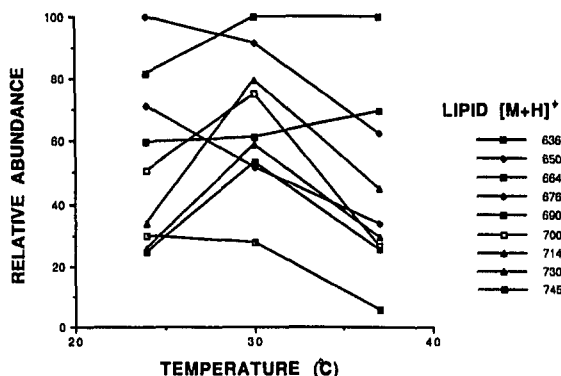


Figure 2

For bacteria not grown at their optimal growth temperatures, differences in growth time cause wide variability in lipid distribution (Fig. 3). However, we found that for bacteria grown at their optimal temperature, time differences between harvesting have little effect for most of the lipids (Fig. 4).

**LIPID ABUNDANCE VS. GROWTH TIME FOR
E. COLI AT 24°C**

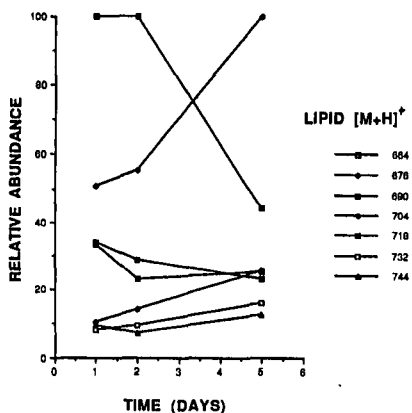


Figure 3

**LIPID ABUNDANCE VS. GROWTH TIME FOR
E. COLI AT 37°C**

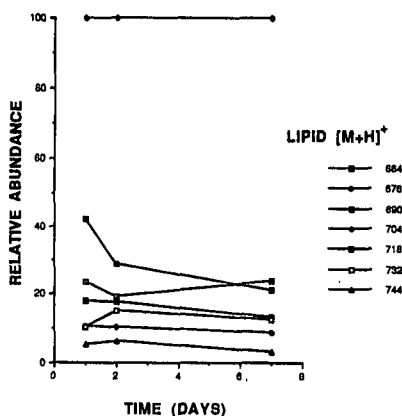


Figure 4

Therefore it is important, for spectral reproducibility, for bacteria to be grown at their optimal temperature. However, differences between harvesting times have a much reduced effect on reproducibility.

ANALYSIS OF BACTERIAL CELL WALL GLYCOLIPIDS BY LASER DESORPTION AND PLASMA DESORPTION MASS SPECTROMETRY

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N. Qureshi and K. Takakura, VA Hospital, Madison, WI 53705

INTRODUCTION

Glycolipids are important components in the cell walls of bacteria. The cell wall provides structural support and protection for the bacteria and the structures on their surface act as potentiators of immune responses in a host environment [1].

For the work presented here, laser desorption (LD) and plasma desorption (PD) ionization methods are used to analyze this class of compounds. The goal is to obtain both molecular weight and structural information about glycolipids.

Laser desorption mass spectra were obtained using a Tachisto (Needham, MA) model 2156 pulsed CO₂ laser with a CVC (Rochester, NY) model 200 time-of-flight mass spectrometer.

Plasma desorption mass spectra were obtained using a Bio-Ion Nordic (Uppsala, Sweden) model BIN-10K instrument with Californium²⁵² ion source. Samples were electrosprayed onto aluminized mylar foil.

RESULTS

(1) Trehalose Mycolates

Trehalose mycolates are glycolipids of the outer cell wall in bacteria. Mycolates (C₂₂-C₈₀, alpha-alkyl, beta-hydroxy fatty acids) have been found to play an important role in the immune response [1].

Corynebacteria frequently contain trehalose corynemylates on their outer cell wall surface. The positive ion laser desorption mass spectra shown in Figure 1 of trehalose monocorynemylate shows intense molecular ion peaks (MNa⁺, MK⁺) and little fragmentation. The molecular weight has been determined to within ± 0.5 amu with internal calibration standards.

Lithium cation attachment was shown in other studies with CO₂ laser desorption to result in increased fragmentation and structural information for oligosaccharides and peptides [2]. Here, LiBr was added to a glycolipid (trehalose monocorynemylate). The result shown in Figure 2 was an intense peak at 479 corresponding to the mycolate fatty acid acylium ion along with other fragments from the sugar (359, 376).

(2) T-Boc CAP Derivatives of Monophosphoryl Lipid A

Derivatization of hexaacyl monophosphoryl lipid A (MPLA) was performed with the goal of preparing intermediates with an amino group. The LD mass spectrum (Figure 3) of H₁ shows a molecular ion (MK⁺) at 1971 with corresponding weight of 1932. A peak 245 daltons less than the molecular ion corresponds to loss of t-Boc CAP+OCH₃. This loss indicates t-Boc CAP was added to the 6' position of the distal sugar. Loss of R₃ is the next major fragment at 1524, corresponding to a hydroxy myristate group from the reducing sugar. A number of other peaks appear at lower mass/charge resulting from neutral losses of fatty acids on the non-reducing (or distal) glucosamine. These peaks indicate the fatty acids on R₁ and R₂ are C₁₂ and C₁₄ groups. The plasma desorption mass spectrum of H₁ is characterized by neutral losses of R₃ and R₁. The PD mass spectrum shown here does not contain peaks corresponding to two bond ring cleavages in contrast to laser desorption.

(3) Plasma Desorption of Diphosphorylated Lipid A

Diphosphoryl lipid A (DPLA) analogs have now been isolated from *R. sphaeroides* and methylated at the hydroxy group of the phosphates. PDMS was used to determine where the second phosphate was added to monophosphoryl lipid A. The positive ion PD spectrum (Fig 4) of one of the two major fractions is shown below. The spectrum shows that the second phosphate is added to the reducing sugar of lipid A and not as pyrophosphate.

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- (1) G. Kubica, L. Wayne, *The Mycobacteria*, Marcel Dekker (1984), New York.
- (2) W.B. Emery, B. Spengler, R.J. Cotter, unpublished data.

Laser Desorption Mass Spectrum

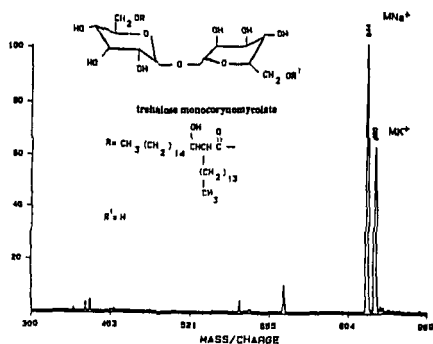


Figure 1

Laser Desorption
trehalose monooctamethylate plus lithium

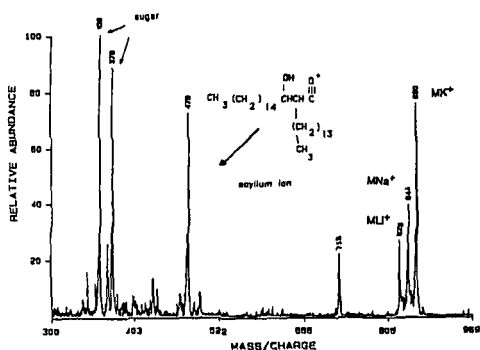


Figure 2

Laser Desorption Mass Spectrum
1-Dec CAP Lipid A Fraction II

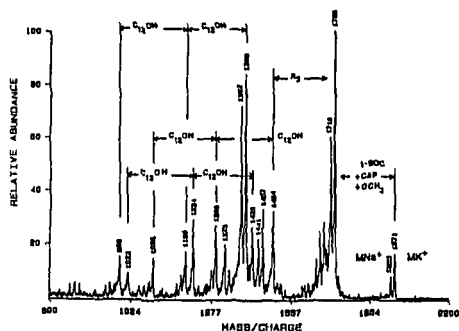


Figure 3

Plasma Desorption Positive Ion Mass spectrum
Diphosphoryl Lipid A from *R. Sphaeroides*
Fraction I

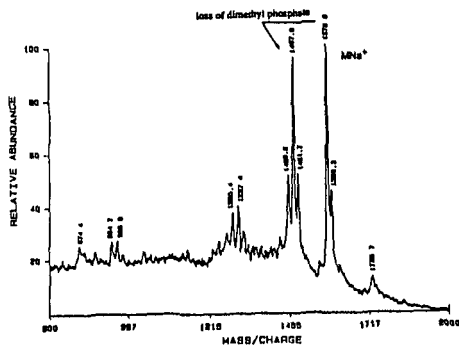


Figure 4

TANDEM MASS SPECTROMETRY OF ERGOSTEROL AND RELATED COMPOUNDS

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Fast atom bombardment (FAB) usually produces $(M+H)^+$, $(M+Na)^+$, $(M+K)^+$, $(M+H+X)^+$ (X ; molecule of matrix used for FAB ionization), etc. rather than $M^{+\bullet}$ itself in the positive ion mode. However, in the course of the study on FAB mass spectrometry, we have found that the positive ion FAB mass spectrum of α -tocopherol gave an unusual radical cation $M^{+\bullet}$ instead of the expected ion $(M+H)^+$.¹⁾ Recently, similar phenomenon was found in the FAB mass spectrum of ergosterol in 3-nitrobenzyl alcohol (3-NBA) which has been used as a matrix for FAB ionization of relatively low-polar compounds (Fig. 1a). The electron impact (EI) mass spectrum of ergosterol also showed $M^{+\bullet}$ as expected (Fig. 1b). Therefore, a comparison between $M^{+\bullet}$ ions produced by FAB and EI ionization methods was carried out by tandem mass spectrometry (MS/MS) for ionic structure study.

Fig. 2 shows the collisionally activated dissociation (CAD) spectra of $M^{+\bullet}$ ions (m/z 396) in the FAB and EI mass spectra of ergosterol. These two MS/MS spectra are very similar to each other. Furthermore, the MS/MS (CAD) spectrum of $M^{+\bullet}$ in the FAB mass spectrum of ergosterol obtained from an EB/EB type instrument (Fig. 3) well corresponds to the EI mass spectrum of ergosterol (Fig. 1b). Consequently, it must be presumed that the ionic structure of $M^{+\bullet}$ produced by FAB ionization of ergosterol is very similar to that of the corresponding $M^{+\bullet}$ generated by EI ionization. MS/MS spectra of other ions, such as m/z 397, 395, 377, 363 and 337 produced from FAB ionization of ergosterol were also measured to examine the fragmentation pathways of $(M-H)^+$, $M^{+\bullet}$ and $(M+H)^+$ ions.

7-Dehydrocholesterol also showed $M^{+\bullet}$ (m/z 384) in the FAB mass spectrum (Fig. 4). On the other hand, the FAB mass spectra in 3-NBA of cholesterol, desmosterol, fucosterol and cholestanol gave $(M-H)^+$ and $(M+H-H_2O)^+$ as the main peaks. The reason why $M^{+\bullet}$ ions predominate over $(M+H)^+$ and $(M-H)^+$ ions in the FAB mass spectra of ergosterol and 7-dehydrocholesterol must be due to the conjugated double bonds in the B-ring. The MS/MS spectra of $M^{+\bullet}$ ions in the FAB and EI mass spectra of 7-dehydrocholesterol were also very similar to each other.

From the above data, it must be assumed that the precursor ions with the same structure give almost same MS/MS (CAD) spectra, regardless of ionization methods.

- 1) T. Kinoshita, H. Nagaki and T. Nakamura, Ann. Rep. Sankyo Res. Lab., 39, 145 (1987).

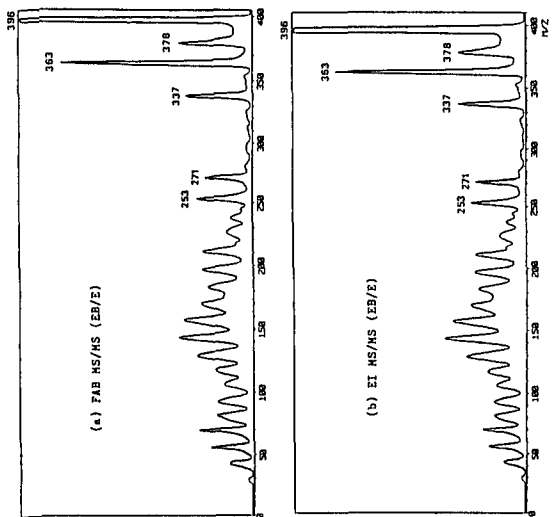


Fig. 2

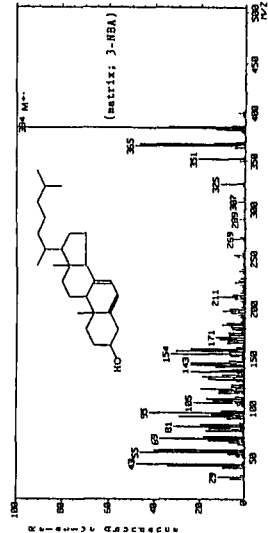


Fig. 4

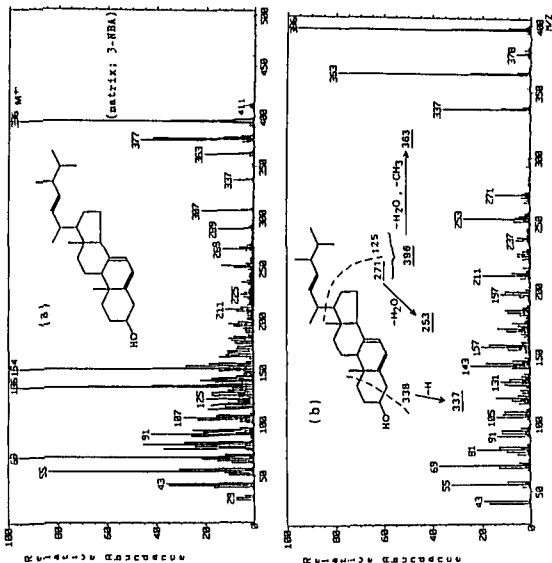


Fig. 1

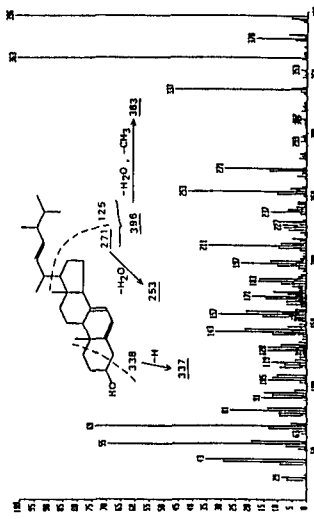


Fig. 3

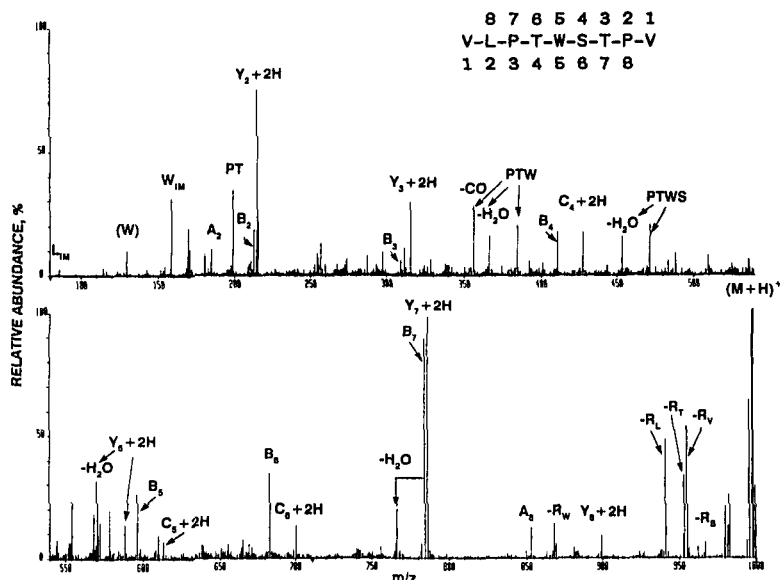


Figure 1. Tandem mass spectrum of COOH-terminal tryptic peptide of sCD4. The resolution of MS-1 was set to select only the monoisotopic $(M+H)^+$ and MS-2 was set at $R \sim 800$. The parent was attenuated by ca. 75% with He collision gas.

$(M+H)^+$ of Glycopeptide ^a	In-chain Mass of Carbohydrate ^a	Hex	HexNAc	NANA	Deoxyhex
Fraction 32 ^b					
3842.8	2352.1	5	4	2	1
3696.6	2205.9	5	4	2	0
3551.6	2060.0	5	4	1	1
3405.5	1914.8	5	4	1	0
3260.3	1769.6	5	4	0	1
3114.2	1623.5	5	4	0	0
Fraction 45 ^c					
5311.6	2352.1	5	4	2	1
5165.6	2205.9	5	4	2	0
5020.4	2060.0	5	4	1	1
4874.3	1914.8	5	4	1	0
4729.1	1769.6	5	4	0	1
4583.0	1623.5	5	4	0	0

^a Masses indicated are chemical average masses (ave); ^b attachment site peptide Asn³⁰⁰-Lys³¹², $(M+H)^+_{ave} = 1490.7$; ^c Attachment site peptide Lys²⁵²-Lys²⁷⁹, $(M+H)^+_{ave} = 2959.5$.

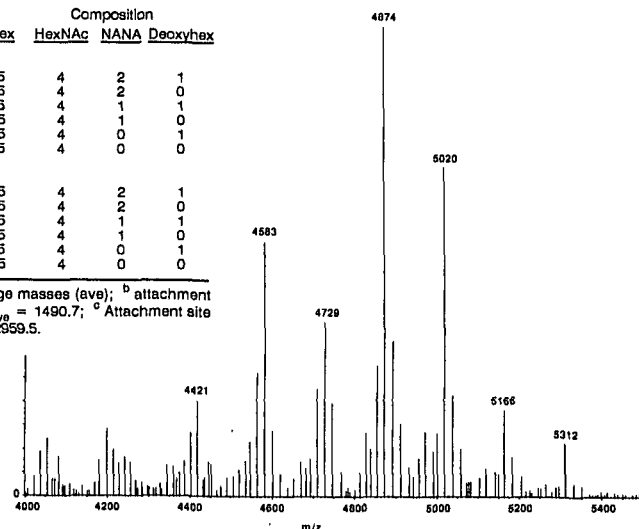


Figure 2. FAB mass spectra of glycopeptide-containing HPLC fraction 45 of the tryptic digest of RCM-sCD4. Masses are calculated as the chemical average values.

**CONTROLLED FRAGMENTATION OF OLIGOSACCHARIDES IN 252CF
PLASMA DESORPTION MASS SPECTROMETRY**

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The Rockefeller University, New York, NY-10021

The fragmentation of underivatized and derivatized malto-oligosaccharides containing four to seven glucose residues has been studied using positive ion plasma desorption mass spectrometry (PDMS). A rapid and simple method to control the nature and extent of fragmentation of derivatized oligosaccharides and related glycoscompounds has been developed.

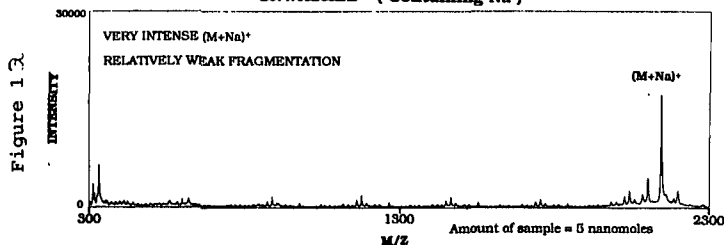
Peracetylation of the malto-oligosaccharides was carried out using a 2:1 (v:v) mixture of trifluoroacetic anhydride and acetic acid. Sample preparation for mass spectrometric analysis was done in two ways. 1) By electrospray deposition on to a metallized mylar foil. 2) By adsorption on a nitrocellulose backing. Underivatized malto-oligosaccharides prepared by electrospray deposition gave spectra containing an intense (M+Na)⁺ ion peak and weak fragment ion peaks. Peracetylation of malto-oligosaccharides resulted in a 10 to 100 fold increase in the mass spectral sensitivity compared with that for the underivatized compounds. Peracetylated malto-oligosaccharides prepared by electrospray deposition gave spectra consisting of a very intense quasimolecular (M+Na)⁺ ion and relatively weak sodium containing fragment ions. The spectrum obtained for an unwashed peracetylated maltoheptaose sample containing sodium is shown in figure 1a. A closer look at the fragmentation is presented in figure 2a. The fragmentation pattern is complex and involves many channels and the fragment ion peaks are sharp. Most of the fragments contain one Na. The most intense peaks are identified and marked in figure 2a. When the same sample is gently washed by dipping the sample foil in water the mass spectrum that results is shown in figure 1b. Water washing effectively removes sodium. The spectrum therefore does not show an (M+Na)⁺ ion peak. It is interesting the (M+H)⁺ ion is also absent. At the same time, the fragment ion intensities are enhanced. A closer look at the fragmentation (figure 2b) shows that there are only two fragmentation channels and the fragment ion peaks are broad and metastable. These fragment ions which do not contain sodium are identified and marked in figure 2b.

The results obtained for peracetylated maltoheptaose when prepared by adsorption on a nitrocellulose backing are similar to the above results of the electrospray deposited sample.

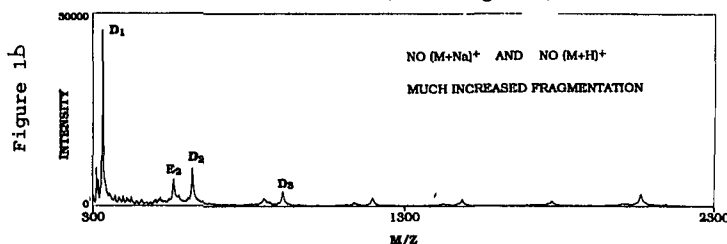
We have applied this method to a permethylated and reduced ganglioside Neu.Gc-GM3. The sample with added sodium gave a spectrum consisting predominantly of (M+Na)⁺ and sodium cationized saccharide fragment ions. Water washing removes these ions and enhances the non sodium containing fragment ions.

In summary, this rapid and simple method provides us a means of obtaining mass spectra consisting of either the intense (M+Na)⁺ ion peak or intense fragment ion peaks from the same sample by controlling the amount of sodium in the mass spectrometric sample. We can therefore, selectively enhance either the molecular weight information or fragment ion information from the same sample.

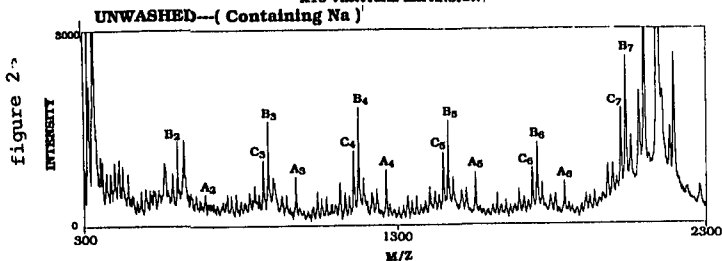
**ELECTROSPRAYED PERACETYLATED MALTOHEPTAOSE
UNWASHED---(Containing Na)**



**Same sample |
WATER WASHED---(Containing no Na)**

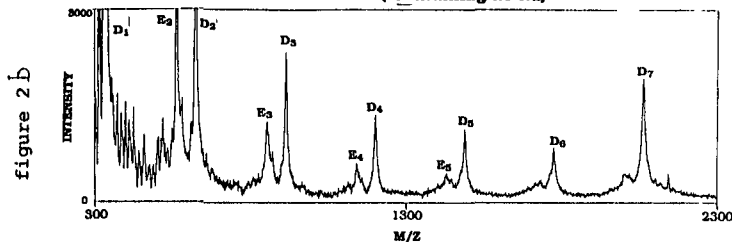


**A CLOSER LOOK AT FRAGMENTATION
X10 VERTICAL EXPANSION |**



$A_1 \rightarrow [M+Na-CH_2COOH-HCO-(7-GAc)]^+$
 $B_1 \rightarrow [M+Na-CH_2COOH-H-CH_2COOH-(7-GAc)]^+$
 $C_1 \rightarrow [M+Na-CH_2COOH-CH_2COOH-H-(7-GAc)]^+$
 Where GAc is an acetylated glucose residue

WATER WASHED---(Containing no Na)



$D_1 \rightarrow [M+H-CH_2COOH-(7-GAc)]^+$
 $E_1 \rightarrow [M+H-2CH_2COOH-(7-GAc)]^+$
 Where GAc is an acetylated glucose residue

Strategies to Distinguish Carbohydrate Sequence Ions

by

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New strategies for the rapid elucidation of carbohydrates are being developed, in which mass spectrometry and NMR are applied directly to the intact polymer, without recourse to chemical hydrolysis. Molecular weights obtained by mass spectrometry provide information about the complexity of a carbohydrate or glycoconjugate, and also about the heterogeneity of polymer mixtures. Plasma desorption, laser desorption and fast atom/ion bombardment have been used successfully for these purposes (1,2). More detailed structural information should be available from fragment ions, including achiral sequences. Tandem mass spectrometry coupled with collisional activation is expected to provide extensive fragmentation of the mass-selected parent ions. We are evaluating high energy collisional activation for this purpose.

This poster illustrates the use of tandem mass spectrometry for the structural analysis of minor components of mixtures of carbohydrates. Also, the sensitivity of this technique has been demonstrated to exceed 50 picomoles for several compounds. Several regioisomers have been found to be distinguishable from first principles, without the need for standards, see for example figures 1 and 2.

In addition, tandem mass spectra were obtained for both protonated and natriated molecular ions of carbohydrates. The collisionally activated fragmentation of these two species were found to be very different and provided complimentary information, as can be seen by the comparison of figure 3 (natriated) with figure 1 (protonated). It should be noted that these structural isomers can be distinguished in both the protonated and natriated spectra.

Experimental

All spectra were obtained on a JEOL HX 110/110 operated at full accelerating voltage (10 kV). Data were collected by a JEOL DA5000 data system. The FAB gun was operated at 6 kV with Xenon as the FAB gas. Conventional magnetic scans were performed on MS I. The Tandem experiments (MS/MS) were four sector experiments using a collision cell floated at 2 kV between MS I and MS II. Helium was used as the collision gas, with a precursor ion attenuation of 70%. All experiments were performed on positive ions.

Acknowledgement

The authors would like to thank Dr. Alan Bush for providing several of the samples.

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figure 1

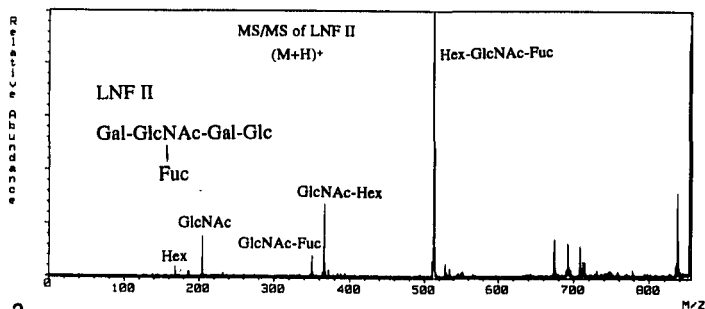


figure 2

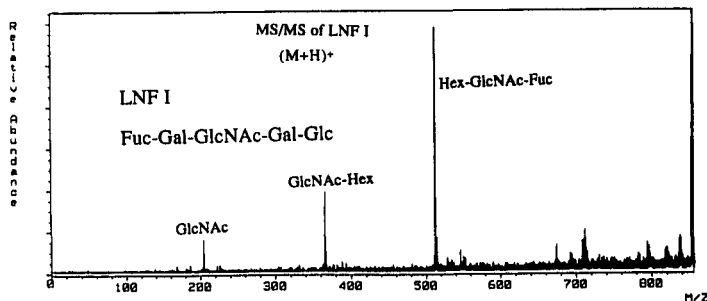
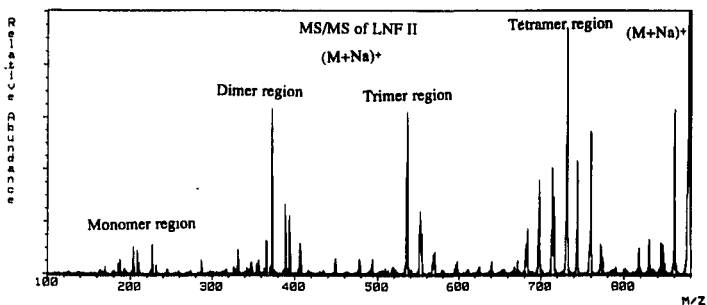


figure 3



LSIMS ANALYSIS OF OLIGOSACCHARIDES AS NEOGLYCOLIPID DERIVATIVES

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Introduction. The neoglycolipids produced by reductive amination of oligosaccharides with phosphatidylethanolamine dipalmitoyl (PPEADP) have been developed to study oligosaccharide recognition and have been used as solid phase probes in ligand binding assays with antibodies and with carbohydrate binding proteins of plant, animal and microbial origins [1-5].

The object of the present study was to assess the sensitivity of detection and degree of structural information obtainable by LSIMS of neoglycolipids on the conventional target probe and directly from the surface of silica plates following TLC.

Experimental. Neoglycolipids were derived from malto-oligosaccharides, chitin oligomers and a range of deoxyhexose-, hexose- N-acetamidohexose- and sialic acid-containing mammalian oligosaccharides by a procedure described previously [3] and represented in Fig 1. PPEADP conjugates were analysed by a VG Analytical ZAB2-E mass spectrometer (caesium gun: 35keV, 0.5 μ A emission) from the normal target using a DEA/TMU/NBA matrix (2:2:1) or from strips of silica (1.5 x 5mm) cut from the TLC plate together with the aluminium backing foil with addition of solvent (MeOH/CHCl₃/H₂O, 25:25:8) and the DEA/TMU/NBA matrix.

Results. a) Structural Information. Similar spectra were observed in both positive and negative mode but the latter were preferred due to lower background noise. Intense molecular ion species enabled molecular weight to be established and hence saccharide composition, while fragment ions, although of lower relative abundance, gave clear compositional and sequence information. Examples of negative ion spectra of PPEADP conjugates are shown in Fig 2 which are representative of the neoglycolipids studied. In addition to the ion series indicated, and formed with hydrogen rearrangement, were three other series at -18, -2 and +28 daltons to these ions that were confirmatory of saccharide sequence.

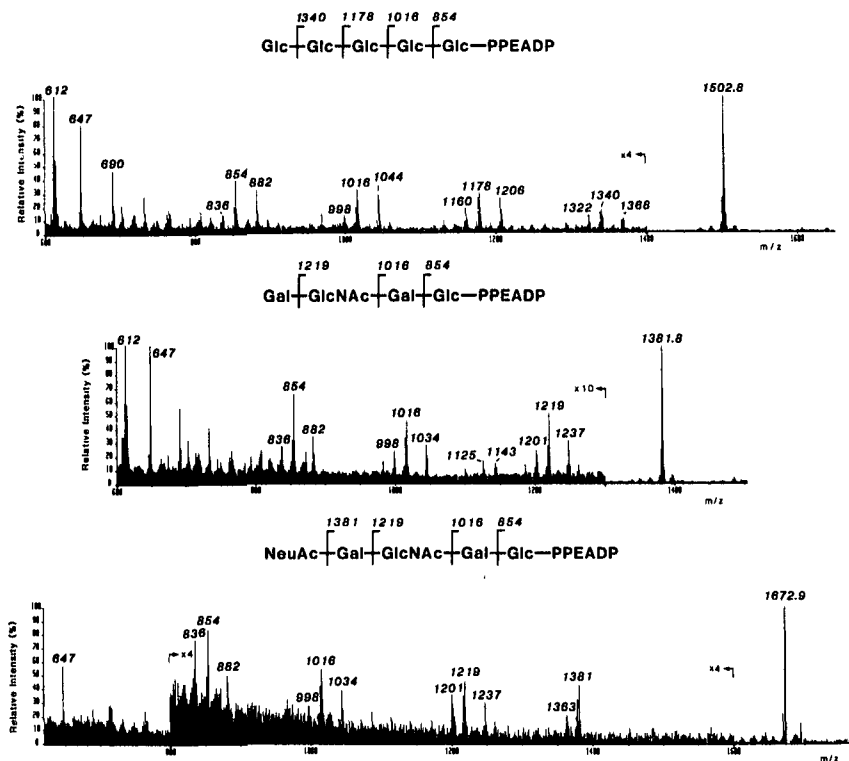
b) Sensitivity. Exceptionally high sensitivity was achieved for [M-H]⁻ detection of maltopentaose-PPEADP from the normal target. 100fmol produced a signal (S/N 12:1) from a complete mass scan. However, approximately 5pmol were needed to give clearly defined sequence-determining ions while ionization directly from the TLC silica surface to give fragment ions could be obtained from 50pmol. A biantennary octasaccharide PPEADP derivative required 200pmol for a full spectrum from TLC but gave a [M-H]⁻ ion from 2pmol (S/N 8:1) on the conventional target.

Conclusion. This study has demonstrated the useful structural information that can be obtained with very high sensitivity from neoglycolipids by LSIMS on both normal target and a TLC surface. The ability to provide compositional and sequence data on these oligosaccharide derivatives from a TLC surface permits individual components to be analysed from mixtures of oligosaccharides. This has immediate relevance to studies of oligosaccharide recognition.

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[illegible]

Figure 2.



CHARACTERISATION OF OLIGOSACCHARIDES FROM MUCIN GLYCOPROTEINS BY LSIMS USING AN
AUTOMATED TLC TARGET STAGE

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Introduction. Human gastrointestinal mucins are large glycoproteins which are thought to act as a defensive barrier of the mucosal epithelium against chemical and bacterial attack. The oligosaccharide structures are also differentiation and tumour associated antigens and hence are of potential use as tissue markers. Pronounced heterogeneity of mucin oligosaccharides, however, makes their structural elucidation difficult and can hinder assignment, particularly of minor components. The present report discusses the identification of oligosaccharides in partially purified fractions using the direct TLC/LSIMS analysis of phosphatidylethanolamine dipalmitoyl (PPEADP) derivatives.

Experimental. Following release by mild alkali/borohydride degradation from mucin glycoproteins, oligosaccharides were purified by Bio-Gel P4 chromatography and HPLC [1,2]. Tri- to hexasaccharide fractions were treated by mild periodate oxidation before conjugation to PPEADP. The reaction products were fractionated on silica TLC plates and then subjected to LSIMS (ZAB2-E mass spectrometer; caesium gun) using a VG Analytical automated TLC target stage. The TLC strip (5 x 75mm) was coated with a DEA/TMU/*m*-NBA (2:2:1) matrix and the target stage driven at 0.25mm/sec over a distance of 60mm with negative ion spectra acquired at 2 sec/decade.

Results. The ion profile of m/z 937 and 997, which represents the $[M-H]^-$ ions of the principal PPEADP derivatives obtained from a trisaccharide from meconium are shown in Fig 1 together with their spectra (scans 58 and 63). Fragment ions from loss of GlcNAc and Gal respectively allowed the carbohydrate branching sequences to be deduced together with the assignment of the core linkage position. These data were obtained by TLC/MS of approximately 2nmol of oligosaccharide and demonstrate the resolution of closely migrating bands and their integrity during analysis. The ion plot (m/z 997 and 1099) shown in Fig 2 was obtained from a tetrasaccharide fraction. The spectra of scans 34 and 64 shown gave fragment ions arising from loss of Gal.GlcNAc and Gal respectively and permitted selected components to be identified. Fig 3 includes the TLC chromatogram of a penta- and hexasaccharide fraction and the TIC output from direct ionization from silica surface. High quality spectra were obtained from all the carbohydrate related bands at scan numbers 22, 28, 40, 51, 64 and 70 which allowed the identification of oligosaccharide sequences. Reagent peaks (scans 96 and 111) were also readily distinguished.

Conclusions. It was possible in combination with the isolation and derivatisation strategy to identify by TLC/MS all the major and many minor components in the oligosaccharide mucin preparations studied. TLC proved to be an excellent means of separating mixtures of PPEADP derivatives and with the automated TLC target stage permitted data to be obtained on complete fractions providing individual spectra of components and the ability to detect the presence of selected oligosaccharides.

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Figure 1

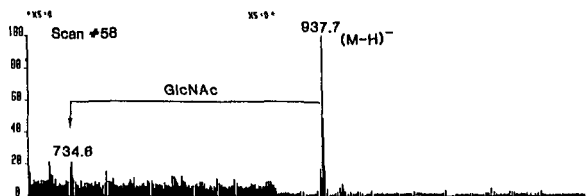
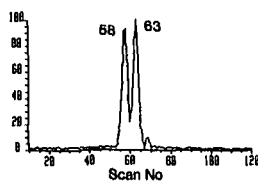


Figure 2

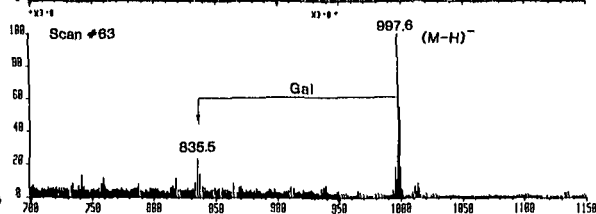
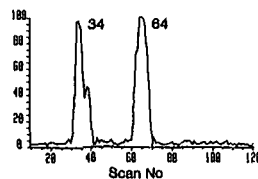
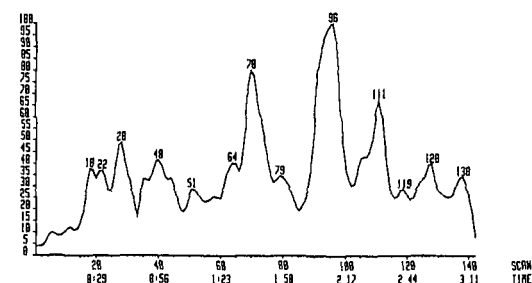
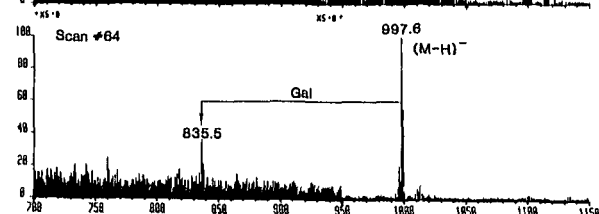
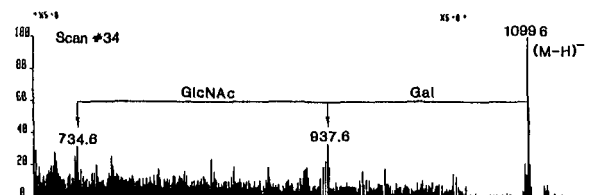


Figure 3



ORIGIN OF THE $[M-H]^+$ IONS IN THE FAST ATOM BOMBARDMENT MASS SPECTRA OF SOME CYCLIC SUGARS

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It is well established that FAB ionization of polar compounds usually produces protonated molecular ions $[M+H]^+$. These energetically stable species provide essential information for the determination of molecular mass and identification. It is worth mentioning that any unusual behavior in the generation of FAB mass spectra may cause incorrect assignment of molecular mass and therefore misinterpretation of the spectrum. Recently, considerable interest has been focused on the observations that for certain groups of compounds, the predominant peaks observed in the molecular ion region of positively charged FAB mass spectra are $[M-H]^+$ rather than $[M+H]^+$. This behavior has potential danger in the interpretation of the mass spectra of unknown samples. Two reaction pathways have been proposed to determine the origin of the predominant $[M-H]^+$ ions. It was suggested¹ that this phenomenon is a result of the transfer of redox equivalents between neutral molecular species during the bombardment desorption event, namely, the net loss of H $\cdot$ would result in the formation of $[M-H]^+$, or of the hydride abstraction from molecular ion, similar to that observed in gas-phase chemical ionization occurring from the hydrocarbon chain remote from the oxygen in long chain ethers².

To investigate the origin of $[M-H]^+$ ions formed in fast atom bombardment and to elucidate its reaction mechanism, derivatized cyclic sugars methyl-2,3,5-tris-O-benzyl-D-ribofuranose (1), S-phenyl-2-deoxy-3-O-methyl-5-O-benzyl-D-ribofuranose (2) and methyl-2,3-di-O-benzyl-erthiofuranose (3) were studied by FAB mass spectrometry. Gas-phase chemical ionization (CI) was also performed to compare with the FAB data. Positively charged FAB mass spectra of compounds 1-3 using thioglycerol as matrix are shown in Figure 1. These spectra can be characterized by: I) the predominant peaks observed in the molecular ion region for these compounds are $[M-H]^+$ rather than $[M+H]^+$, and the peak intensity of $[M+H]^+$ is very weak compared to that of $[M-H]^+$, II) besides the sodium adduct $[M+Na]^+$, there is also a unique adduct ion that can be observed at $[M+13]^+$, and III) the abundances of the parent-molecular and adduct ions are relatively low in comparison with those of fragment ions. Pertinent sections of the FAB mass spectra of these samples which detail the mass region of the molecular ion and adduct ions are shown in Figure 2. The CI (isobutane) mass spectra of the sugar derivatives also gave predominant $[M-H]^+$ ions except for compound 3 which showed abundant peaks at $[M-H]^+$ and $[M+H]^+$. However, no detectable adduct ion of $[M+13]^+$ was observed in CI.

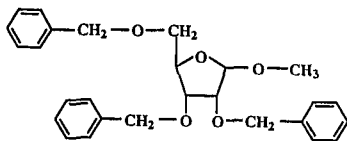
Relative abundances for peaks at $[M-H]^+$, M^+ and $[M+H]^+$ are listed in Table I and are compared with theoretically calculated isotopic distributions. In the FAB mode, the observed ratios of $[M-H]^+/M^+$ are very close to theoretical predictions. This seems to indicate that the M^+ ions are mostly due to isotopic contributions and that there is almost no molecular ion M^+ formed in our experiments. The residual abundances of $[M+H]^+$ ion are very low after subtracting isotopic contributions to this peak. This would imply that the formation of $[M-H]^+$ in the FAB spectra of these cyclic sugars is highly favored to the expense of $[M+H]^+$. An important feature which reveals the differences between FAB and CI regarding the formation of $[M-H]^+$ is obtained from the fragment ions. An isobutane CI spectrum of compound 1 is shown in Figure 3 where a peak at m/z 107 corresponding to benzyloxy ion $C_6H_5CH_2O^+$ is observed. In the FAB spectrum of this compound, the fragment at m/z 107 appears to be replaced by an ion at m/z 105. This peak at m/z 105 is observed in the FAB spectra of all compounds using glycerol, thioglycerol or in the absence of a matrix and is not formed via m/z 107 as demonstrated by MIKES experiments. A plausible assignment for the peak at m/z 105 would be the benzoyl ion $C_6H_5CO^+$ whose formation obviously involves rearrangement-dissociation processes.

The results described above indicate that the ether linkage in the side chain of derivatized cyclic sugars may be the source for the rearrangement-dissociation observed under FAB conditions. Furthermore, the ether oxygen which provides a site for protonation may also be significant in bringing about the formation of predominant $[M-H]^+$ ions. The formation of $[M-H]^+$ is not a simple hydride abstraction process but is a result of a protonation induced process. The molecular ions are firstly protonated under FAB conditions and the protonated molecular ions, being very unstable, rearrange immediately to form $[M-H]^+$ ions by loss of two hydrogens. That is why the $[M+H]^+$ peak is

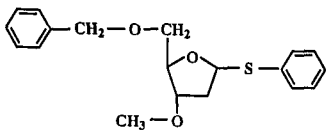
very weak and $[M-H]^+$ is the predominant ion in the FAB mass spectra of these compounds. This proposal may also apply to the formation of $[M+13]^+$, which is observed in the FAB spectra of these cyclic sugars with or without matrix. In this case, the attachment of a methyl CH_3 group to the molecule followed by the loss of two hydrogens can explain the formation of $[M+13]^+$, or more precisely, of $[M+15-2H]^+$.

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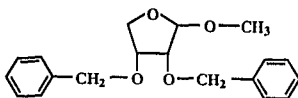
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1. (Mol. wt 434)



2. (Mol. wt 330)

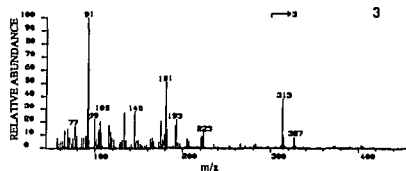
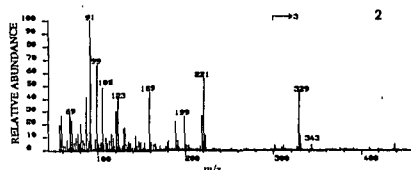
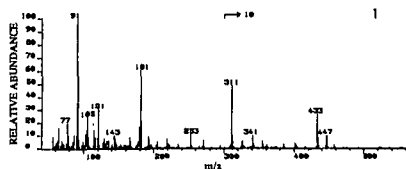


3. (Mol. wt 314)

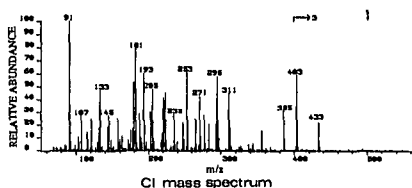
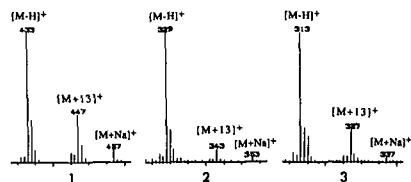
Table 1 Intensities (%) of peaks in the molecular ion region relative to most intense peak $[M-H]^+$.

	PAE (thioglycerol)	PAE (glycerol)	CI (isobutane)	THEORETICAL CALCULATIONS*
1				
$[M-H]^+$	100	100	100	100
M^+	32	33	35	30.30
$[M+H]^+$	9	10	14	5.44
2				
$[M-H]^+$	100	100	100	100
M^+	25	25	28	22.13
$[M+H]^+$	10	12	18	6.60
3				
$[M-H]^+$	100	100	100	100
M^+	26	27	46	21.33
$[M+H]^+$	20	16	79	2.97

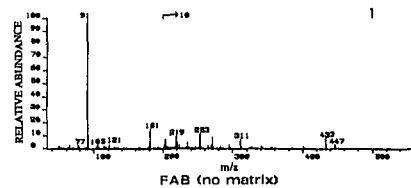
*Calculated based on the theoretical isotopic distributions assuming that the intensity of $[M-H]^+$ is 100 %.



FAB mass spectra



CI mass spectrum



FAB (no matrix)

SEQUENCE ANALYSIS OF HIGHLY SULFATED, HEPARIN-DERIVED
OLIGOSACCHARIDES USING FAST ATOM BOMBARDMENT MASS SPECTROMETRY

Larry M. Mallis

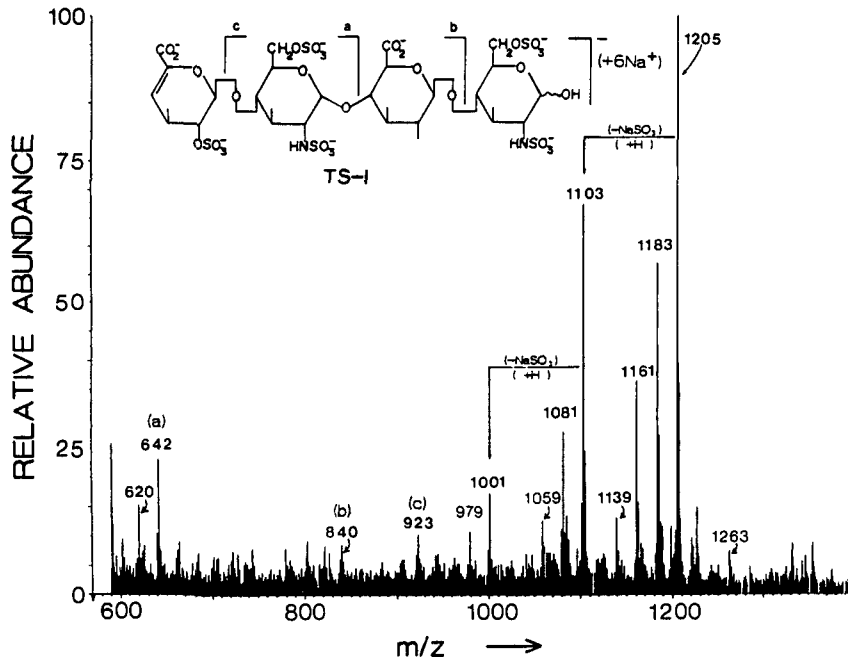
High Resolution Mass Spectrometry Facility
The University of Iowa
Iowa City, Iowa 52242

Hui M. Wang, Duraikkannu Loganathan and Robert J. Linhardt

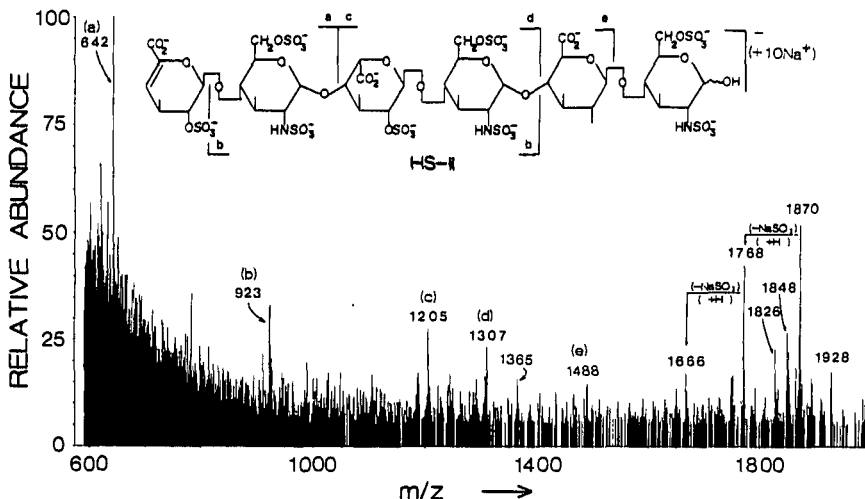
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Heparin, a polydisperse, sulfated polysaccharide of 1 $\rightarrow$ 3 linked glucosamine and uronic acid residues, has been used clinically as an anticoagulant for half a century, yet the exact chemical structure of heparin remains unclear.¹ The sequence of the polysaccharide chains comprising glycosaminoglycan (GAG)-heparin is of considerable interest (as this is heparin's drug form), thus heparin lyase is used to enzymatically depolymerize GAG-heparin into smaller oligosaccharides more suitable for structural analysis.

Reinhold and Green, et al., reported on the negative ion FAB-MS analysis of heparin-like oligosaccharides prepared by chemical synthesis.² The dominant molecular ions observed were of the type $[M + Na_x - H_{x+1}]^-$ (where M is the fully protonated, acid form of the oligosaccharide and x = 2-9) and in all cases the observed spectra contained little or no structurally significant fragment ions.²



The studies reported here were performed using a VG ZAB-HF, in the fast atom bombardment (FAB) ionization mode. Negative ion spectra were obtained using triethanolamine as the negative ion FAB matrix. Sequence specific fragment ions, of these highly sulfated enzymatically prepared (from porcine lung heparin) oligosaccharides, were observed. For example, the negative ion FAB-MS spectrum and structure of a heparin derived tetrasaccharide, TS-I, is shown in Figure 1. The molecular ion is observed at m/z 1205 and corresponds to a sodiated adduct ion of the type $[M+6Na-7H]^-$. The ion at m/z 923 is assigned as loss of the sugar residue from the nonreducing terminus of TS-I, whereas the ion at m/z 840 is assigned to loss of the sugar residue from the reducing terminus of TS-I. The ion at m/z 642 is the result of loss of two sugar residues nearest the reducing end of TS-I.



The utility of this technique is clearly dependent on the ability to aid in the prediction of unknown sample structures. The structure of hexasaccharide HS-II (Figure 2), isolated from a partial enzymatic depolymerization of bovine-lung heparin was determined, and confirmed by NMR experimentation, through the use of this mass spectral technique.³

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**PORPHYRIN PYRROLE SEQUENCING BY LOW ENERGY
COLLISION INDUCED DISSOCIATION OF (M+7H)⁺ GENERATED
IN SITU DURING AMMONIA CHEMICAL IONIZATION**

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High pressure NH₃ CI-MS promotes *in situ* reduction and decomposition of the porphyrin macrocycle producing a relatively complex pattern of mono-, di-, and tripyrrolic fragment ions that can be used, in principle, to determine the pyrrole sequence of the porphyrin (1). The CI mass spectra are complicated, however, by a plethora of peaks, most of which are not structurally informative (Figure 1a). A prominent peak in the CI mass spectrum is (M+7H)⁺, the reduced porphyrin species produced *in situ*. We have shown that high energy CID of (M+7H)⁺ on a BE geometry instrument produces a structurally informative daughter ion spectrum (2,3). The utility of such spectra is limited, however, because of poor daughter ion resolution and fragmentation of substituent groups from the individual pyrrolic units.

Low energy CID (30-100 eV (lab), argon target gas at 2 x 10⁻⁴ torr) of (M+7H)⁺, using a hybrid mass spectrometer of BEQQ geometry (VG-ZAB-EQ at the University of Tennessee), was found to yield simple, reproducible patterns of unit mass resolved daughter ions from which the mass of the pyrroles and pyrrole sequence of the porphyrin could be readily determined.

In contrast to the CI mass spectrum, the CID spectra of (M+7H)⁺ contain relatively few peaks, all of which are structurally significant (Figure 1b). Mono-, di-, and tripyrrolic daughter ions are observed and can be distinguished based on peak patterns and the nitrogen rule: monopyrroles occur as peak "triplets" of even mass, dipyrroles occur as peak "quadruplets" of odd mass, and tripyrroles occur as peak "quadruplets" of even mass. Fragmentation of the substituent groups on the individual pyrrolic units is observed in the 100 eV CID spectrum. The 70 eV CID spectrum contains all the daughter ions necessary to determine the mass of the individual pyrroles and deduce the pyrrole sequence of the porphyrin. The monopyrroles are of low abundance or not observed in the 30 eV CID spectrum.

The utility of the CI-MS/MS technique for pyrrole sequencing is best illustrated by the analysis of porphyrins with an asymmetrical pyrrole sequence. The CI mass spectra of porphyrins of this type are extremely complex and virtually impossible to interpret (Figure 2a). In contrast, the CID spectrum of (M+7H)⁺ from such a porphyrin is relatively simple and easy to interpret (Figure 2b).

A further advantage of the CI-MS/MS technique is the capability to pyrrole sequence porphyrins within a simple mixture of non-isobaric porphyrins. In such a case the EI mass spectrum is used to determine the molecular weight of the individual components present in the mixture. Ammonia CI of the mixture produces abundant (M+7H)⁺ from each of the porphyrins present (Figure 3a). The 70 eV CID spectrum from each of the (M+7H)⁺ species in the mixture (Figure 3b) is identical to the CID spectrum of (M+7H)⁺ acquired when analyzing the respective pure compounds.

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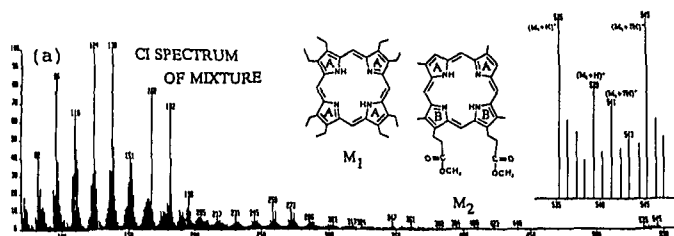
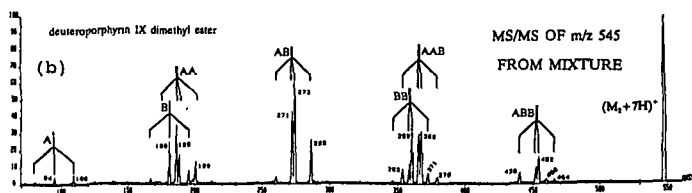


Fig. 3



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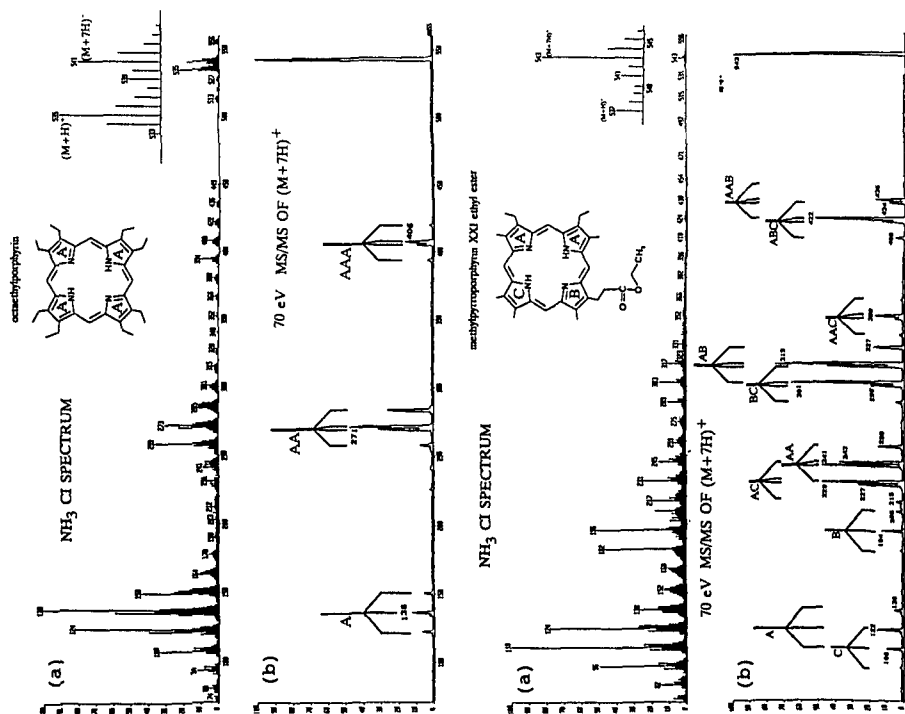


Fig. 1

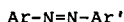
Fig. 2

AZO DYES: ENVIRONMENTAL CONCERNS, ANALYTICAL CHEMISTRY
AND MASS SPECTROMETRY

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Azo dyes, with the general structure I, are used as textile dyes, paint pigments, printing inks, food colorings, and for a variety of other applications. During the manufacture and use of this extremely diverse



I

WHERE: Ar and Ar' are phenyl, naphthyl, biphenyl, heterocyclic, etc.

SUBSTITUENTS: -R, -OH, -OR, -NHR, -NRR', SO₃H, -COOH, -CONHR,
- CONRR', M, -N=N-, -Cl, -Br, -CN, -NO₂, etc.

class of compounds, wastes are created and discarded as wastewater and solid residues. Azo dyes are made by the classical diazotization reaction, followed by coupling of the diazonium cation with reactive aromatic compounds. In recent years a number of azo dyes have been removed



from the list of substances approved for use in foods, drugs, and cosmetics, and a number of others are under study in regard to potential adverse health effects. In addition, azo dyes can be reduced in the environment and in vivo to produce amines that are known carcinogens.



WHERE RA = various organic and inorganic reagents
= anaerobic bacteria (sediments, flooded soil, landfills)
= reducing enzymes, intestinal bacteria, etc.

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

Azo dyes of particular concern are the water soluble Acid and Direct dyes that contain aromatic amine residues that are known or suspected carcinogens. Wastes from the manufacture and use of azo dyes present two

CARCINOGENS (SUBSTANTIAL EVIDENCE)	POSSIBLE HUMAN CARCINOGENS
benzidine	aniline
3,3'-dichlorobenzidine	4-chloro-2-methylaniline
3,3'-dimethoxybenzidine	2,4-dimethylaniline
3,3-dimethylbenzidine	2-methoxy-5-nitroaniline
4-(dimethylamino)azobenzene	2-methylaniline (probable)
1-naphthylamine	2-methyl-5-nitroaniline (mice)
2-naphthylamine	1-amino-2-naphthol

challenging and important analytical problems: (1) the determination of carcinogenic and suspected carcinogenic aromatic amines; and, (2) the determination of azo dyes in environmental samples.

The mass spectrometry of azo dyes has been investigated in recent years using some of the newly developed sample introduction and ionization techniques. Capillary column gas chromatography/mass spectrometry is applicable to certain azo dyes used in the petroleum industry (1). The separation and measurement of some water insoluble disperse azo dyes using liquid chromatography/thermospray/mass spectrometry has been studied (2,3). The desorption ionization spectra (SIMS) of several azo dyes have been reported (4). A liquid chromatography/ion spray/atmospheric pressure ionization (API)/negative ion mass spectrometry method for the determination of the acid soluble sulfonated azo dyes in environmental samples has been developed (5). And capillary zone electrophoresis (CZE) has been used in connection with the ion spray/negative ion API mass spectrometric method for acid azodyes (6).

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POSSIBLE WIDESPREAD ENVIRONMENTAL CONTAMINATION BY BROMO AND
BROMOCHLORO DIOXINS AND DIBENZOFURANS

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Flamingo Rd., Suite 120, Las Vegas, NV.

Major sources for brominated compounds in the environment may be the bromine containing flame retardant additives in plastics and combustion products of the plastic formulations, and the bromination, *in situ*, of materials during the combustion process. Plastics are ubiquitous in the environment because of their versatility, durability, and low cost. Many products were formerly made from such materials as wood, paper, metals, glass and ceramics. By volume used, plastics now exceed the amount of steel used in the United States. Plastic resin production has increased from 6.3 billion pounds annually in 1960 to 57 billion pounds in 1988. As the development, use, and availability of these materials to consumers have increased so have the rate and quantity of plastic articles entering the municipal solid waste stream also increased: from 800 million pounds in 1960 to 20.6 billion pounds in 1986. In 1960, plastics made up less than one percent by weight of the total waste stream, increasing to more than seven percent in 1986, with a projected rise to nine percent by the year 2000. Most plastic products degrade very slowly in the environment because they are in general biologically and chemically stable. Until recently, plastics waste was not seen as a threat to the environment.

Disposal of municipal solid waste has become an issue of increasing importance in the United States. The infamous 1987 voyage of the garbage barge from the town of Islip, New York, focused national attention on the difficulty encountered in disposing of municipal solid waste. Some major cities have run out of landfill capacity and must ship their wastes to remote sites at great cost. Incineration and subsequent landfilling of residues (fly ash, bottom ash) provide increased capacity for disposal of plastics and other solid waste. However, the environmental impacts of the residues and stack emissions, must be controlled. Leaching of potentially harmful chemicals is the other major pathway of concern for environmental release of plastics or plastic additives from landfills. Additives to plastics are used to provide enhanced strength, stability, color, resistance to fire, etc. Fiberglass, dyes, and other materials may be added for strength, color, or other commercially desirable properties. At least 36 million pounds of brominated flame retardants are used per year in the U.S. It is expected that plasticizers, flame retardants and other additives have the greatest probability of leaching from plastic products.

Brominated flame retardants such as polybromodiphenylethers (PBDPE) are routinely added to a variety of commercial products such as carpets, textiles, and plastics. Typical plastic resins into which PBDPE are blended include polyethylene terephthalate (PET), polybutylene terephthalate (PBT), high-impact polystyrene (HIPS) and acrylonitrile-butadiene-styrene (ABS). Generally 5-20% of flame retardant by weight is added to the plastic resin.

Concerns exist regarding the possibility of environmental contamination by polybrominated dibenzofurans (PBDF) from such formulations, either as a result of impure starting materials or

from reaction of the PBDPE during the extrusion processes, or upon other thermal processes including fires. We recently completed a study which determined that detectable levels of brominated furans were present in some typical commercial polymer products, prepared by heat extrusion manufacturing processes, and containing brominated diphenyl ether flame retardants. A pyrolysate of a PBT resin was found to contain PBDF and PBDD. Parts per billion (ppb) levels of PBDF were formed in the flame retardant materials and significantly higher levels of PBDF and PBDD formation were found in the pyrolysate of a PBT resin. A fume sample taken during resin extrusion was also analyzed and was found to contain low levels of PBDF. Thus, the possibility exists that manufacturing plant workers could be exposed to brominated flame retardants and PBDF by inhalation. The analytical methodology used for this study was based upon existing EPA methodology for chlorinated dioxins and dibenzofurans, RCRA method 8280 and CERCLA method IFB-WA84-A002. Modifications to these methods were made for extraction of analytes from the resin matrices and for GC/MS monitoring of the brominated analytes. The GC retention indices of PBDF and PBDPE are shown in Fig. 1. The GC separation of PBDF analytes from residual PBDPE interferences was important for confirmation of the PBDF because of their similar mass spectra with overlapping ion clusters.

Municipal incinerators have been extensively investigated as a source of chlorinated dioxins and furans. Our findings indicate that brominated polycyclic aromatic hydrocarbons (PAH) dioxins and dibenzofurans are also produced, usually as monobromopolychloro species. These cross-halogenated analytes were found at much lower concentration than the chlorinated species. Presumably, the flame retardants which are widespread among various domestic products serve as a major source of bromine during the incineration process. The negative ion chemical ionization (NCI) mass chromatogram of a fly ash extract for Br⁻ is shown in Fig. 2.

"Fluff" is the ground-up waste residue from junked cars and consumer appliances remaining after the bulk metals have been reclaimed. Three million tons of this waste are produced annually. Initial analysis of fluff extracts has shown the presence of polychlorinated biphenyls (PCB), other chlorinated aromatics such as naphthalenes and terphenyls, and brominated flame retardants in this highly complex matrix. We also initiated a leaching study to assist in determining whether land disposal of fluff is a potential source of environmentally significant compounds in ground water. Preliminary results indicate that significant levels of organics do leach from fluff.

Investigations of incinerator fly ash, of plastics which are also potential feedstocks for municipal incinerators and of the organic waste, "fluff", have shown a commonality in that brominated compounds including bromofurans and dioxins can be introduced into the environment by a variety of processes in general use today.

NOTICE

Although the research described in this article has been funded wholly or in part by the Environmental Protection Agency contract 68-03-3249 to Lockheed Engineering and Sciences Company, Inc., it has not been subject to the Agency's review, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute an endorsement for use.

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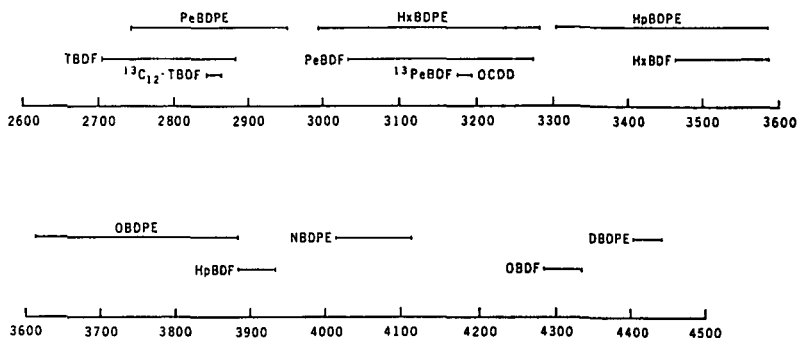


Figure 1. Retention Indices of PBDF and PBDPE.

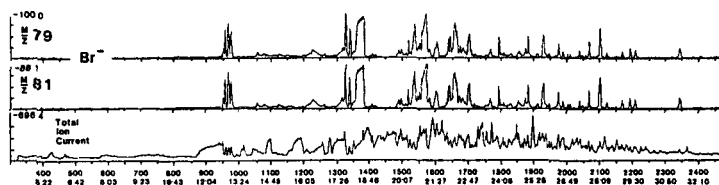


Figure 2. Reconstructed NCI ion chromatogram of fly ash sample.

Tributyltin; good for antifouling paint, not so good for oysters

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The organotins are a family of compounds that have found wide application in the chemical industry. Their uses include being intermediates in organic synthesis, heat and light stabilizers for PVC, and as biocides. It is this last use that has been implicated in environmental problems. The toxicity of the biocides is dependent on the number and nature of the substituent groups with the tri-substituted state being the most toxic. Trialkyltins (R_3SnX , where, typically, R = alkyl or phenyl and X = halide, -OH, -OSnR₃, or a polymer) have been used as preservatives for wood, textiles, leather and paper because of their activity as biocides against molluscs, bacteria, algae, insects and arachnids. The inclusion of TBT in marine antifouling paints has been the application with significant environmental impact. TBT in antifouling paint occurs either in free association where the TBT is mixed with the paint or covalently linked to the polymeric backbone of the paint. In both cases it is the leaching of the TBT, probably as TBT⁺, that provides the biocidal activity. TBT containing paints were introduced in the 1960s and their use increased until, by 1985, approximately 30-50% of vessels were painted with these paints.

Toxic effects of TBT have been reported for many species. Among the most obvious, and commercially serious, are the shell deformation of the oyster Crassostrea virginica and the induction of imposex in the dogwhelk Nucella lapillus. These effects occur at submicrogram/liter TBT concentrations. In oysters the shell becomes laminated and the organism becomes round or "balled" resulting in little space remaining for the soft tissues. In the dogwhelk the female develops a penis and vas deferens. The formation of the latter eventually closes off the oviduct thus preventing the reproduction of the affected individuals and populations.

The demonstrated link between environmental problems and TBT has led to the need to monitor the occurrence and behavior of TBT and its breakdown products dibutyltin (DBT) and monobutyltin (MBT) in the environment. Because of the low concentrations at which toxicity is encountered, analytical methodologies are required that measure TBT at the ng/L level in water and at the ug/kg level in sediments and biota. Capillary GC with flame photometric detection (FPD) and GC-MS are methods used at the Virginia Institute of Marine Science (VIMS). The GC-FPD uses a >600 nm pass filter to provide the needed specificity. GC-MS is by both full scanning and SIM with positive chemical ionisation (PCI). Preparation of aqueous samples is by extraction with hexane containing 0.2% tropolone. Sediments and biota are chemically dried and Soxhlet extracted with hexane. After volume reduction the sample is derivatised with hexyl magnesium bromide to form tetraalkyltins which are then cleaned up on a Florisil column. Triphenyltin chloride, which is added prior to extraction, is the internal standard. Extraction efficiencies from water for this method were TBT > 90%, DBT ~ 55% and MBT ~ 33%. The lower efficiencies for DBT and MBT are a result of the increased polarity of these compounds. Other laboratories which utilize GC or GC-MS are using similar techniques with variations such as tripropyltin chloride as the internal standard and derivatisation with pentyl magnesium bromide to form the pentyl derivatives. When atomic absorption is the analytical technique, hydride formation is the normal derivatisation technique.

The PCI mass spectra of the tetraalkyltins are dominated by the loss of an alkyl group. For hexylTBT this gives ion clusters around m/z 319 and m/z 291. These correspond to the loss of a butyl group and a hexyl group, respectively. The reason for the ion clusters is that tin has multiple isotopes, the most common of which have masses of 116 (14%

frequency), 118 (24%) and 120 (33%). There are seven other isotopes with frequencies of up to 9%. There is no quasimolecular ion, and the fragmentation favors the loss of the heaviest alkyl group. PCI with full scanning is the method of choice for confirmation of the identities of TBT and its derivatives in environmental samples. EI spectra show progressive removal of the alkyl groups until an $[\text{SnH}]^+$ ion is formed. There is no molecular ion. FAB-MS gives spectra that are the same as those obtained in PCI; again there is no molecular ion. The isotope clusters are useful for searching for organotin in complex mixtures such as environmental samples. The limited fragmentation in PCI means that there are ions of relatively high mass that are suitable for SIM, m/z 319 is used for monitoring TBT and DBT, and m/z 347 is used for DBT, MBT and the internal standard, triphenyltin. The two assay methods, GC-FPD and PCI GC-MS SIM have been compared using environmental samples. The results of the two techniques were directly comparable.

VIMS has monitored occurrence of TBT in the water column at a number of locations over the last four years and has surveyed the southern portion of the Chesapeake Bay for TBT in oysters and sediment. In addition, samples have been submitted from other parts of the country for analysis. TBT contamination is correlated with proximity to marinas and shipyards. Concentrations found were up to 1300 ng/L, well above those required for toxic effects. In the marinas it was noted that there were peaks in mean TBT concentrations in late spring and to a lesser extent in the fall. This corresponds to the times of year when recreational boats are painted and thus when there are highest inputs of TBT into the environment. Rapid fluctuations of TBT concentrations in marinas have been observed with concentrations varying by as much as an order of magnitude over a 1 week period. Reasons for this variation include factors such as tidal flushing and inputs from freshly painted boats. Multiple sampling is therefore required so that trends can be analysed.

In general, sediment concentrations are 10^3 greater than those of the surrounding water reaching up to 4000 ug/kg at ship repair facilities. This means that the sediments are acting as a sink for the TBT. It remains to be determined whether, as fresh inputs of TBT are reduced, the sediment will become a source of future contamination of the water column.

TBT has been found in all areas from which oysters have been obtained and tested. These areas include the Elizabeth, James, York and Rappahannock rivers and various stations on the Delmarva peninsula, both the bay and ocean sides. Concentrations encountered were up to 5600 ug/kg (dry wt.). Bioconcentration factors were of the order of 10^5 . TBT was encountered in oysters even in areas where it could not be found in the sediments.

In 1988 the use of TBT in antifouling paints was restricted. It was banned for vessels under 25 m, except for those with aluminum hulls. Its use on larger vessels is permitted provided that the leaching rate of the TBT is < 4 ug TBT/cm²/day. This should reduce the amount of TBT in the water, particularly around marinas, that is derived from recreational vessels over the next 2-3 years as vessels are repainted. There are, however, other sources to consider. In addition to the desorption of TBT from sediments mentioned above, a major source of TBT may be the paint chips that are scraped from vessels. In addition, the widespread use of organotins in applications other than antifouling paint and the low concentrations at which toxicity is encountered means that there is a need to be aware of these compounds as potential environmental contaminants in the future.

The tributyltin (TBT) project at VIMS has involved many individuals whose efforts must be acknowledged. These include Mike Unger, Bill MacIntyre, Francois Espourteille, Charles Rice, Donna Westbrook, Ellen Travelstead and Bob Huggett. In addition, though this brief review has been concentrated on the work conducted at VIMS, it is inevitable that

the work of other researchers has been mentioned. Below is given a list of references which the interested reader may wish to consult.

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THE ROLE OF MASS SPECTROMETRY IN THE ANALYSIS OF EXPLOSIVES

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Mass spectrometry, with its wide applicability, high specificity, and high sensitivity, has played, and will continue to play, an important role in the analysis of explosives. In addition to the fundamental study of the reactions that occur during an explosion, the analysis of explosives is important in environmental, clinical, forensic, and security applications. Explosives are of environmental concern at munitions facilities where the storage and manufacture of explosives has led to contamination of soil and water. Adverse physiological effects due to exposure to many of the commonly encountered organic high explosives has been documented making the analysis of explosives and their metabolites important in a clinical setting, particularly for munitions workers. The importance of explosives analysis in forensic investigations, such as the analysis of post-explosion debris, has long been recognized. And finally, the detection of hidden explosives for security applications has become a pressing problem since the bomb is now the terrorist's weapon of choice. Mass spectrometry has been employed in all of these areas. The mass spectrometry of explosives up through the early 1980s has been thoroughly reviewed [1,2]. This paper selectively reviews work described in the last seven years to illustrate recent applications of mass spectrometry in the analysis areas mentioned above with particular emphasis on LC/MS and MS/MS studies.

Table 1 shows some of the commonly encountered organic high explosives. Most high explosives in current and past use fall into one (or perhaps, two) of three compound classes, viz. nitroaromatics, nitrate esters, and nitramines. The most common nitroaromatics are 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT). The latter is frequently found as a contaminant in TNT and is often used as a sensitizer in smokeless powders. Nitrate esters, such as nitroglycerine (NG), are widely used in dynamites. Pentaerythritol tetranitrate (PETN) is commonly used in plastic explosives. The most commonly encountered nitramines are the heterocyclic nitramines RDX and HMX which are used in military explosives. It is important for explosives analysis that many of these compounds (e.g. PETN, RDX, HMX, and to a lesser degree, TNT) are involatile, thermally labile, and strongly adhere to surfaces. These properties can pose problems both in sampling from a matrix and in the introduction of the sample to the mass spectrometer ion source.

Results have been reported in explosives analyses using a wide variety of ionization methods. These include electron ionization, field ionization, field desorption, low pressure electron capture, high pressure electron capture, chemical ionization (both positive and negative), atmospheric pressure ionization (both positive and negative), and thermospray. One clear observation in surveying these results is the wide variety of different explosives-related ions that have been reported. Even when using the same nominal form of ionization, mass spectra are frequently not well reproduced between laboratories. This is due to a number of factors. Among these is the fact that many explosives readily fragment under collision-free conditions and the mass spectra can be very sensitive to temperature and electron energy. Another factor is that the highly polar explosives tend to form complexes under conditions where third body collisions can occur. This makes the mass spectra highly dependent upon the gaseous matrix in the ionization region. Different approaches to ionization employ different pressures, temperatures, residence times, reagent species, etc. The point here is that the literature is useful only as a guide as to what ions might be expected in the application of a new approach to ionization. However, if the new approach does not closely resemble the conditions used in other approaches, completely different explosives ions may be observed.

GC/MS is frequently used in explosives analyses and in many applications is clearly the method of choice, particularly for the volatile and semi-volatile explosives. A recent example has been described by Fetterolf et al. [3] where GC/MS using electron capture ionization was applied to the detection of firearms discharge residue (principally NG). However, HPLC is a more appropriate means for separating the less volatile explosives, particularly when they are present in water and biological fluids. Indeed, HPLC has been used extensively in explosives analyses with one of a variety of detectors. The relative strengths of mass spectrometry as a detector are obvious but only relatively recently has HPLC/MS been applied to explosives. The LC/MS interface has been a limiting factor in the sensitivity of this approach.

Early examples of HPLC/MS of some explosives involved the use of direct liquid introduction into the ion source [4,5]. For example, Yinon et al. [4] reported the HPLC/MS of TNT using methanol positive chemical ionization. The sensitivity of the approach, however, was limited by the fact that only 1% of the effluent could be directed into the ion source. A sample size of 1-10 µg injected onto the column was required. More recently, these same workers have shown improved performance using direct liquid introduction by using a micro-bore column [6,7]. The results were demonstrated with this system via application to TNT metabolites in body fluids of animals and humans.

Thermospray (TSP) has become a leading LC/MS interface and has recently been applied to the analysis of explosives [8,9]. For example, Berberich et al. [9] have analyzed the negative ions formed from a variety of low volatility explosives by HPLC TSP/MS in the filament on, filament off, and discharge modes. Detection limits at the picogram level were demonstrated. These results constitute the best limits of detection published thus far for explosives analysis by HPLC/MS.

MS/MS has also recently been applied to explosives analysis. One use for MS/MS is to obtain a better understanding of the unimolecular chemistry of the explosives related ions than can be obtained from a single stage of MS alone. MS/MS can provide parent-daughter ion relationships and information regarding the mechanisms for decomposition is often revealed by kinetic energy analysis of the fragment ions. Further information can sometimes be obtained by the use of labelled parent ions. For example, Carper et al. have acquired MIKE/CID spectra for the molecular cations of several labelled versions of TNT [10]. Yinon et al. [11] and our group [12] have also reported MIKE/CID data for a variety of explosives related ions formed by several ionization methods.

We have also studied the mechanisms for several of the commonly observed fragmentations of the nitroaromatic anions using MS/MS. For example, we have reported on the mechanism for the "ortho effect" loss of OH⁻ from nitroaromatic cations and anions [13] and have rationalized why some anions do not lose OH⁻ while the corresponding cations do. We have also discussed the loss of NO⁻ from nitroaromatic anions [14], the structures of the ions resulting from NO⁻ loss [15], and substituent effects in the CID spectra of nitroaromatic anions [16].

Perhaps the most important application for MS/MS in explosives analysis is in the detection of vapors of hidden explosives. This is a targeted compound analysis problem that requires speed, sensitivity, and specificity. A number of mass spectrometric approaches have been applied to explosives vapor detection but the most successful have employed some sort of direct air sampling ion source and MS/MS [17,18]. We have developed an atmospheric sampling glow discharge ionization source [19] which is well-suited to ionizing explosives in air. We have coupled this ion source to a quadrupole/time-of-flight instrument [20] and, more recently, to a Finnigan ion trap mass spectrometer [21]. We have demonstrated sub-picogram detection limits for explosives with these devices. The discharge source/ion trap

combination is still in an early stage of development. We therefore anticipate that explosives vapor detection via MS/MS will reach higher levels of performance.

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Common organic high explosives

nitroaromatics-

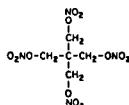


Table 1

nitrate esters-

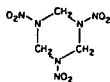


NG

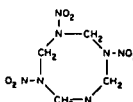


PETN

heterocyclic nitramines



RDX



HMX

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IDENTIFICATION AND DETERMINATION OF *TERT*-ALKYLPHENOLS IN CARP FROM THE TRENTON CHANNEL OF THE DETROIT RIVER, MICHIGAN, U.S.A.

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Introduction. We have previously reported on the analyses of four major classes of organic contaminants (polycyclic aromatic hydrocarbons, polychlorobiphenyls, polychloronaphthalenes, and polychloroterphenyls) in sediments of the Trenton Channel of the Detroit River (1). The purpose of the present study is to identify previously unrecognized pollutants in fish from the Trenton Channel and to compare these levels to those of nearby sediments. Since the most polluted site is located near the middle of the Channel, carp and surface sediment samples were taken from the north, the middle (near Monguagon Creek), and the south of the Trenton Channel and analyzed by gas chromatographic mass spectrometry (GC/MS).

Experimental. Fish were sampled in the fall of 1985 by electro-shocking. Whole carp were ground with a clean, commercial meat grinder. Four carp from the same location were combined into composites, which were stored in clean glass containers and frozen until analysis. A single surface sediment sample was collected at the middle and south fish sampling sites using a Ponar grab sampler. Sediment samples were homogenized and stored in clean glass jars at 4° C.

A thawed aliquot of ground carp (about 15 g) was mixed with pre-extracted Na₂SO₄ anhydrate until a powdery texture was obtained. This mixture was placed in a pre-extracted cellulose thimble. The mixture was Soxhlet extracted for 24 hours with 250 ml of a 1:1 solution of hexane and acetone. The final extract was concentrated by rotary evaporation and made up to 10 ml with hexane. Known amounts of d₂₁-2,4-di-*tert*-butylphenol and d₁₃-4-*tert*-butylphenol were added to 5 ml of this hexane solution for use as internal standards.

Gel permeation chromatography was used to separate the lipids from the low molecular weight, anthropogenic, chemicals (2). Fish extracts containing less than 500 mg of fat were loaded onto a Biobeads SX-2 (200-600 mesh) column (2.5 cm i.d. x 35 cm) and eluted with a 3:2 mixture of cyclohexane and dichloromethane at a flow rate of 4 ml/min using a FMI pump. An UV flow detector (Alltec) operating at 254 nm was used to differentiate between the lipids and anthropogenic chemicals. Lipids appeared in the first 130 ml of eluate which were not collected; the subsequent 80 ml, containing the anthropogenic compounds, were collected. The fractions were concentrated by rotary evaporation and chromatographed into two fractions on a silica gel column using dichloromethane and methanol as eluants.

Sediments were homogenized by stirring, and an aliquot was removed for percent water determination. Another aliquot was mixed with clean, anhydrous Na₂SO₄ to absorb sediment pore water. Samples were spiked with known amounts of the internal standards. Each sample was Soxhlet extracted for 24 hours with isopropanol, followed by dichloromethane for an additional 24 hours. Sample volumes were reduced by rotary evaporation, combined, and solvent-exchanged to hexane. Combined, hexane-exchanged extracts were chromatographed on columns containing 20 grams of 1% deactivated silica-gel, with HCl-rinsed copper at the bottom to adsorb elemental sulfur and a Na₂SO₄ "cap" at the top to absorb residual water. Samples were charged to the column in 3 ml of hexane and eluted sequentially with 45 ml of hexane, 45 ml of 10% dichloromethane in hexane, 45 ml of dichloromethane, and 45 ml of methanol. All fractions were collected separately, but prior to analysis, all fractions except the methanol were combined.

The identification of new pollutants was based on their electron impact mass spectra obtained on a Hewlett Packard 5995 GC/MS system, operating at an ion source temperature of 250°C. The temperature of both the GC injector and the transfer line was 280°C. A DB-5 fused silica column (30 m x 0.25 mm, J & W Scientific, CA) was used with He as the carrier gas. Quantification of the alkyl phenols was done using selected ion monitoring; the most abundant ions were used for quantification, and the second most abundant ions were used for confirmation.

Results and Discussion. PCBs and chlorinated pesticides, such as chlordane, were plentiful in fish from all three sites. This is not surprising; contamination of the Detroit River by organochlorine compounds is well documented (3-6). There were, however, several compounds which were not abundant in the fish sampled from the north and south of the Trenton Channel but were in those sampled from the middle site.

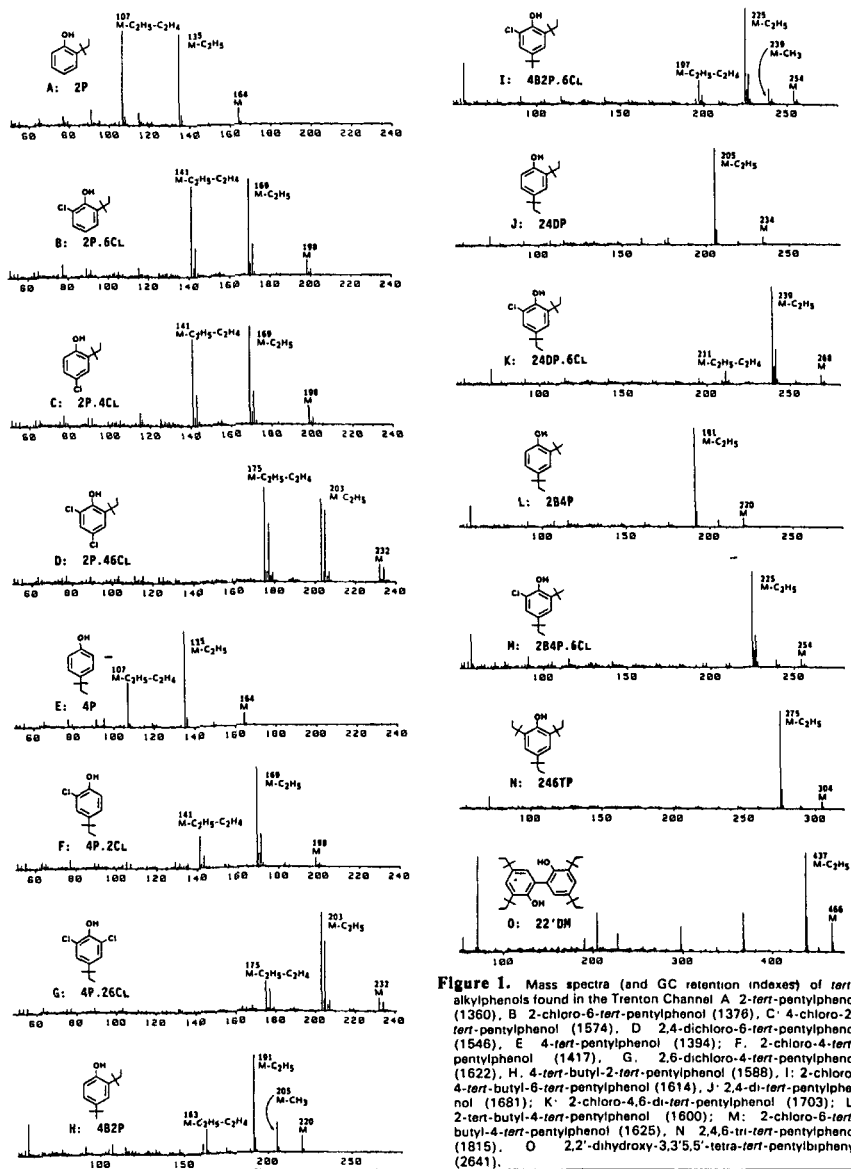
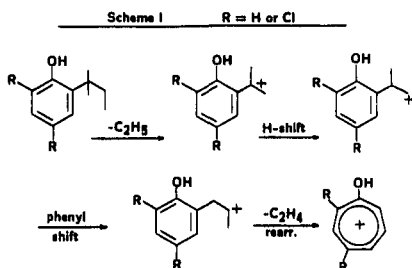


Figure 1. Mass spectra (and GC retention indexes) of *tert*-alkylphenols found in the Trenton Channel. A: 2-*tert*-pentylphenol (1360), B: 2-chloro-6-*tert*-pentylphenol (1376), C: 4-chloro-2-*tert*-pentylphenol (1574), D: 2,4-dichloro-6-*tert*-pentylphenol (1546), E: 4-*tert*-pentylphenol (1394), F: 2-chloro-4-*tert*-pentylphenol (1417), G: 2,6-dichloro-4-*tert*-pentylphenol (1622), H: 4-*tert*-butyl-2-*tert*-pentylphenol (1588), I: 2-chloro-4-*tert*-butyl-6-*tert*-pentylphenol (1614), J: 2,4-di-*tert*-pentylphenol (1681), K: 2-chloro-4,6-di-*tert*-pentylphenol (1703), L: 2-*tert*-butyl-4-*tert*-pentylphenol (1600), M: 2-chloro-6-*tert*-butyl-4-*tert*-pentylphenol (1625), N: 2,4,6-tri-*tert*-pentylphenol (1815), O: 2,2'-dihydroxy-3,3',5,5'-tetra-*tert*-pentylbiphenyl (2641).

The electron impact mass spectra of these compounds resembled each other; they are shown in Figures 1A to 1O. (The figure caption also gives their GC retention indexes.) $(M-C_2H_5)^+$ fragment ions were always very abundant. The spectra also showed fragment ions due to the loss of C_2H_4 from the $(M-C_2H_5)^+$ ion. Elemental compositions of the fragment ions were confirmed by high resolution mass

spectrometry on a Kratos MS-80 mass spectrometer. The $(M-C_2H_5-C_2H_4)^+$ ion was shown to result from two sequential losses by tandem mass spectrometry on a VG 30-250 triple quadrupole instrument operating in the parent and daughter ion modes. We suggest that these ions are formed by the route given in Scheme 1. These $(M-C_2H_5-C_2H_4)^+$ ions were of high to medium abundance when only one *tert*-pentyl group was present in the molecule (see Figures 1A to 1G). This ion was very abundant in the spectrum when the *tert*-pentyl group was substituted at the ortho-position (see Figures 1A to 1D); this is true regardless of the position and number of chlorine substitutes. The $(M-C_2H_5-C_2H_4)^+$ ion is much smaller or even missing when two alkyl groups are present (see Figures 1H to 1M); if it is of modest abundance when a *tert*-pentyl group is in the ortho-position but missing otherwise. Presumably, the presence of more than one alkyl group increases the abundance of ions due to losses, such as $(M-C_2H_5)^+$ and $(M-CH_3)^+$, relative to the $(M-C_2H_5-C_2H_4)^+$ ions. In effect, this suppresses the latter ion, in some cases, rather dramatically. Note that when there are three or more alkyl groups, the $(M-C_2H_5-C_2H_4)^+$ ion is not present at all (see Figures 1N and 1O).



With a few exceptions, the compounds identified here are not commercially available. Thus, these compounds were synthesized, and the identifications discussed above were confirmed by comparison of GC retention times and mass spectra. The concentration of 24DP in the middle fish was 140 ug/g fat; in the middle sediment it was 130 ng/g dry. 4P was 40 ug/g fat in the middle fish, and 18 ug/g dry in the middle sediment. The dimer compound (22'DM) was 2.4 ug/g fat in the middle fish, and 27 ug/g dry in the middle sediment. The other compounds were all less abundant. The total alkyl phenol concentrations were 1.9 ug/g fat in the north carp, 270 in the middle, and 0.11 in the south. In the sediments these values were 220 ug/g dry in the middle and 0.19 dry in the south.

The ratio of concentrations in the fish relative to those in the sediment crudely represents the partitioning of the alkyl phenols between sediment and fish. These ratios are all similar to one another (within a factor of 2 to 3) except for 246TP and 22'DM, which are highly hindered alkyl phenols. Clearly, the alkyl phenol levels in carp are affected by the contamination levels of the sediments, at least when the sediment levels are high. Why are the fish/sediment ratios for 246TP and 22'DM dramatically different from the other values (about 40 times lower, on average)? This may be another example of the dependency of biological availability on molecular weight: very high molecular weight, hydrophobic compounds are often less available to biota (7, 8). These highly lipophilic compounds may not be bio-available due to their loss of permeability through cell membranes (8).

The source of 24DP and 4P is apparently the chemical plant where these chemicals are manufactured. This plant is located very near where the middle fish were collected. The origin of the other compounds is still mysterious. There are two possible ways to form these compounds: (a) formation in the plant as reaction by-products from the production of 24DP or (b) formation in the aquatic environment by microbial and chemical degradation of 24DP. In order to differentiate these two possible sources, the commercial product (24DP, technical grade) was analyzed. 2P, 2B4P, 4B2P, 246TP, and 22'DM were found as impurities in this commercial product. We also found that the ratio of the two butylpentylphenol isomers (2B4P/4B2P) in the commercial product (0.19) was about the same as in the carp (0.25) and in the sediment (0.23) sampled near the manufacturing plant. We conclude that these unchlorinated alkylphenols in the Trenton Channel did not come from the degradation of 24DP in the environment but from the discharge of chemical wastes from the plant. The source of the chlorinated alkylphenols is unclear.

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LASER IONIZATION MASS SPECTROMETRY (LIMS): TECHNIQUE AND APPLICATIONS

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Introduction

Laser microprobe mass spectrometry is a microanalytical materials analysis technique employed in the characterization of a material's elemental and, in some cases, molecular composition.¹ The laser microprobe technique is based on performing a mass and intensity analysis of the ionic species formed in the high power density laser irradiation of a solid sample. The microanalytical feature of the laser microprobe technique is achieved by utilizing a finely focused (~ 1 micrometer in diameter) laser pulse to initiate the vaporization and ionization of a materials constituents. The laser microprobe technique generally employs a pulsed, Q-switched, frequency quadrupled Nd:YAG laser ($\lambda=266\text{nm}$) and a time-of-flight mass spectrometer (TOFMS). The combination of this finely focused, high irradiance (variable between $\sim 10^8$ and 10^{12} Wcm^{-2}) laser pulse with the "simultaneous" mass detection capabilities of TOFMS enables one to perform microanalytical survey analysis of a wide range of sample materials. The laser microprobe technique is referred to by several acronyms including LAMMA, LIMA, LAMMS, and one that has found favor in our laboratory, LIMS, which stands for Laser Ionization Mass Spectrometry.

In terms of analytical utility, the microanalysis of sophisticated materials is playing an ever important role in modern materials research and development. Analysis techniques which have sensitive elemental and/or molecular detection capabilities within analytical areas on the order of a few micrometers (or less) in diameter are being increasingly utilized for the characterization of materials such as semiconductor devices, integrated optical components, novel metallurgical alloys, ceramic composites, as well as biological materials. The LIMS technique is finding increasing utilization in these types of materials analyses because of its unique microanalytical capabilities which include the following:

1. Rapid, sensitive, elemental survey microanalysis
2. Ability to analyze electrically insulating materials, and
3. Potential of providing molecular or chemical bonding information from the analytical microvolume.

The only practical limitations to performing a qualitative laser microprobe analysis are the requirements that the sample material be reasonably vacuum compatible and that the Nd:YAG laser beam can be focused onto the analytical area of interest. In our laboratory, we have performed informative laser microprobe analyses on sample materials ranging from dehydrated sewage solids to liver tissues to a variety of sophisticated semiconductor materials and devices.

Figure 1 illustrates a schematic diagram of a reflection mode laser microprobe mass spectrometer. This particular instrument is the model LIMA 2A originally manufactured by Cambridge Mass Spectrometry, Ltd (Cambridge, England) which is now a part of Kratos Analytical. A similar instrument is manufactured by Leybold Heraeus and is referred to as the LAMMA 1000. Leybold also manufactured a transmission mode laser microprobe system (LAMMA 500). Vacuum Generators (East Grinstead, England) has recently introduced a TOFMS/SIMS system which can be equipped with a focused laser beam for laser microprobe analyses. The basic instrumental configuration permits one to identify the mass and measure the intensity of the ionic species produced in the high power density laser irradiation of a materials surface.

The laser microprobe can be operated under laser irradiance conditions which provide either or elemental or molecular microanalysis capabilities. Elemental analyses are generally performed at relatively high irradiance values ($\geq 10^9 \text{ Wcm}^{-2}$) and the ionization

produced at these irradiances is primarily composed of atomic and or atomic cluster ions. This mode of operation is referred to as laser ionization (LI) and part-per-million atomic (ppma) elemental detection sensitivities have been observed under these irradiance conditions.² "Molecular" LIMS analyses are typically performed at irradiances on the order of 10^8 Wcm^{-2} and this mode of operation is referred as laser desorption (LD). The ionization produced at these lower irradiances often includes molecular or pseudo-molecular (protonated, de-protonated, cationized) ions and structurally significant fragment peaks.³ Both the LI and LD ionization conditions can be employed to analyze for positive or negative ions emitted from the sample. The choice of analysis polarity depends on the sample composition and the efficiency of ion formation from the various analyte ions. For example, the elements in groups 1 through 13 form almost exclusively positive ions (with the notable exception of H) while the group 14 through 17 elements readily form negative ions.

Laser Microprobe Mass Spectrometry Applications

A large proportion of practical materials analyses are concerned with determining the materials' surface or in depth elemental composition. Examples of these survey analyses include measurements of the major, minor and trace element composition, chemical stoichiometry, and the presence or absence of chemical contaminants. Examples where these types of survey analyses are important include chemical certification of various starting materials used in semiconductor, optical, metallurgical, and polymeric materials processing, evaluation of the chemistry of various processing steps, failure analysis studies of products in which "chemical" problems are suggested as the causative factors of the failure and in the study of various environmental and biological processes. Microanalytical survey techniques address these same analysis areas while performing the analysis in analytical volumes on the order of 1 cubic micrometer or less.

Contamination Microanalysis

Figure 2 illustrates an example of contaminant microanalysis which is readily performed with the laser microprobe. This figure is a negative ion LIMS mass spectrum produced from the $4 \mu\text{m}$ square window etched through a photoresist layer deposited onto a HgCdTe substrate. An aluminum layer was deposited onto this window in order to make electrical contact with the substrate; however a number of the Al contacts on certain devices exhibited poor adhesion. The spectrum illustrated in Fig. 2 was produced from a window region on one of these devices which exhibited poor Al adhesion. The HgCdTe substrate in this spectrum contains relatively high Cl^- and Br^- signals which are not observed in spectra produced from similar regions on devices exhibiting good Al adhesion. Since this photoresist was etched with solutions containing Cl and Br, the analysis indicates that these contaminated surfaces were incompletely cleaned after the etching. This example illustrates several unique features of the laser microprobe technique including its ability to perform a near-surface microanalysis within an electrically insulating material (photoresist).

Organic Microanalysis

The laser microprobe technique has the potential for performing a sensitive molecular microanalysis of a vast number of organic materials including polymers⁴ and biological specimens.⁵ The characterization of a materials organic composition could also find extensive application in the analysis of a whole host of suspected organic thin film contaminants introduced during various materials processing steps. Organic LIMS analysis could easily become the most common application for the technique because of its unique combination of the microanalytical feature with the high mass detection capability of TOFMS. The recent very high mass capabilities ($m/z > 100,000$) of the laser microprobe technique demonstrated by Hillenkamp and Karas⁶ show exciting possibilities for the organic microanalysis of biological materials.

With regard to typical analyses performed in our laboratory, Figure 3 illustrates positive and negative ion spectra produced from a commercial photoresist (PR) layer on a

semiconductor device. The spectra produced from this type of polymeric sample is of particular interest to many of our clients because potential causes of device failure could originate with problems in the photoresist. Such factors as incomplete curing, non-uniform coverage, chemically contaminated materials, or incomplete photoresist removal could all lead to device performance problems. The spectra illustrated in Fig.5 were obtained by focusing a relatively high irradiance ($\sim 10^8 \text{ Wcm}^{-2}$) pulse onto the underlying device which produced the distorted crater shape at the photoresist surface. The positive ion mass spectrum exhibits a number of C based fragment ion peaks, several of which may be structurally significant signals of the photoresist. In particular, the peaks at masses 91 and 115 could be various reaction products of this particular PR formulation. The negative ion mass spectrum in this example exhibits an intense signal at mass 107 which is undoubtedly formed from the Novolak resin in the photoresist. This resin represents about 10% by weight of the PR composition. Although this is only one example of the qualitative organic microanalysis capability of LIMS, it represents a very pertinent one.

Acknowledgments

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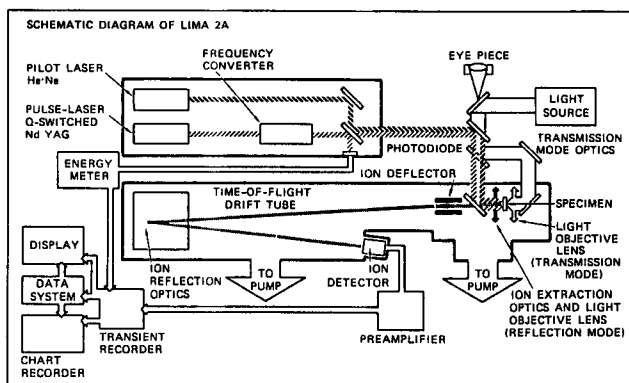


FIGURE 1

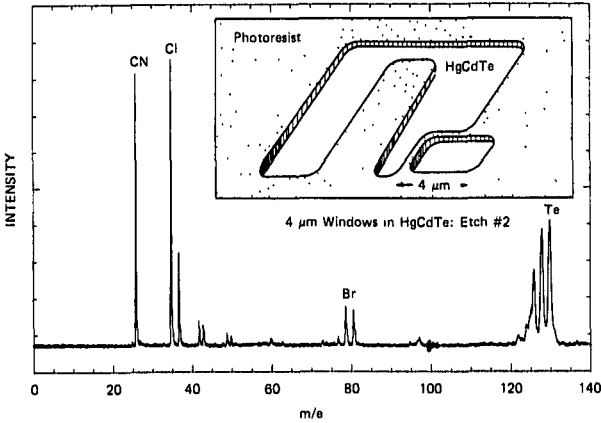
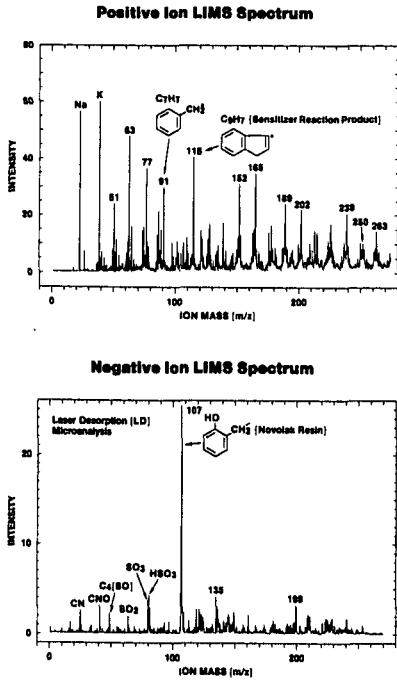


FIGURE 2

FIGURE 3

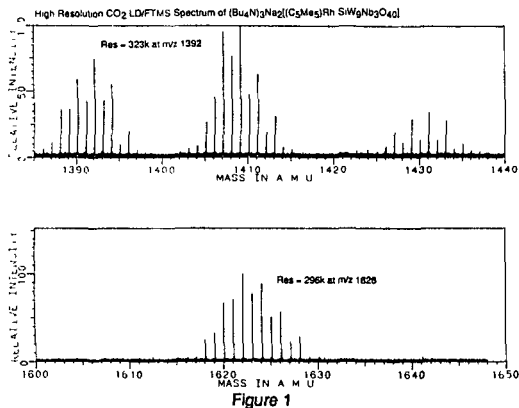


CO₂ AND EXCIMER LASER DESORPTION AND PHOTODISSOCIATION IN AN FTMS

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Laser desorption (LD) has certainly earned its place as a soft desorption/ionization technique amenable to high molecular weight, intractable, and thermally labile samples such as polymers, porphyrins, and biomolecules. Thus far, studies in our laboratory have utilized a pulsed CO₂ laser for desorption of samples up to m/z 9,700 with subsequent detection in a Fourier transform mass spectrometer (FTMS) (1). Recent literature reports describe the laser desorption of compounds up to m/z 100,000 (2)(3). Common to these studies is the use of a sample matrix to aid desorption and increase ionization yields of high molecular weight compounds. Accordingly, we have been investigating the effect of different matrices on the CO₂ and excimer laser (operating at 308 nm) desorption of various middle range molecular weight compounds.

CO₂ laser desorption of polymers and organometallic species with masses in the 1,000 to 11,000 dalton range have also been of recent interest. Exceptionally high mass resolution is obtained for some compounds as is illustrated in the partial spectrum of the tungsten-containing organometallic species presented in Figure 1. The resolving power of FTMS



can be used to advantage to obtain detailed distributions as is evident in Figure 2, which presents a portion of the CO₂ laser desorption spectrum of a methyl methacrylate-styrene

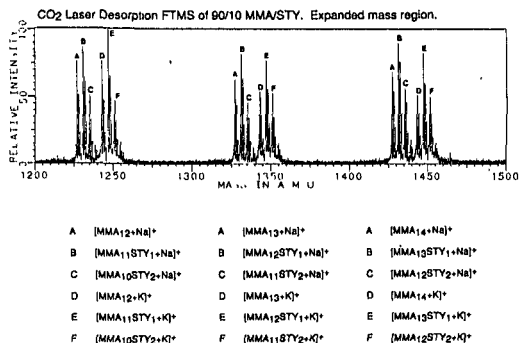


Figure 2.

copolymer. With respect to matrix effects, it is of interest that LD spectra of non-polar olefinic polymers can be obtained if a methanol-gold oxide solution is added to the sample on a stainless steel probe tip and the solvent removed, prior to laser desorption. In the absence of this treatment, no oligomer distribution spectra are obtained.

When a *p*-methoxycinnamic acid matrix of Gramicidin D was prepared and subjected to ultraviolet laser desorption using 308 nm excitation from a XeCl excimer laser, some evidence of matrix-assistance was obtained. Furthermore, use of carbon dioxide laser desorption to examine a 1:1 CsI-sucrose mixture resulted in results similar to those reported earlier by Hillenkamp using a frequency quadrupled Nd-YAG laser for a similar sample (Figure 3). Thus, there is some promise that matrix-assisted LD may be adaptable to FTMS conditions, although optimum parameters have yet to be established.

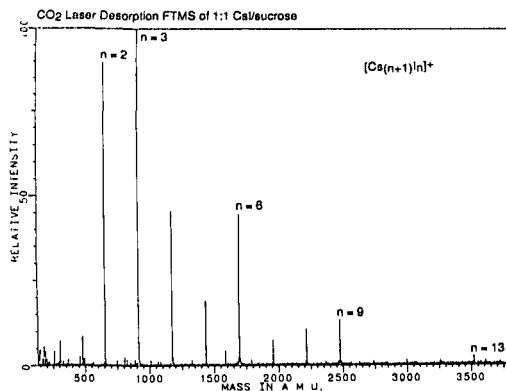


Figure 3.

A second set of studies has focussed on the use of an excimer laser for photodissociation of laser desorbed ions. Frequently, laser desorption alone does not afford enough useful fragments for structure determination. Preliminary studies have shown that laser desorption/photodissociation-FTMS may be a viable technique for analysis of various alkaloids and porphyrins (4). The use of metal ion attachment to aid photodissociation at 308 nm is currently being investigated.

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MECHANISTIC STUDIES IN LASER IONIZATION MASS SPECTROMETRY

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Ultraviolet laser light can be effectively utilized to ionize a variety of compounds in the source region of a mass spectrometer. Quantitative studies of ion yields are facilitated by the use of a gas chromatograph sample inlet system. This enables known amounts of different compounds to be introduced in a reproducible fashion and it separates impurities from sample compounds. The latter property is particularly helpful since impurities that laser ionize with high probabilities may provide larger ion signals than compounds that are of primary interest.

In a number of previous investigations, we have found that structurally similar molecules ionize with efficiencies that differ by up to two orders of magnitude. We hypothesized that this was due to variations in their gas phase absorption characteristics and excited state dynamics. An example of this variation is displayed in a laser ionization chromatogram of a mixture of polyaromatic hydrocarbons which is displayed in Figure 1A. A 10 nanosecond laser was used to record these data. In agreement with previous results, the naphthalene:biphenyl relative ionization efficiency at a laser wavelength of 239.5 nm is about 10:1, even though biphenyl's gas phase absorption coefficient is approximately six times larger than naphthalene's at this wavelength. This result suggests that biphenyl's poor ionization yield must be due to ultrafast excited state relaxation. This theory was tested by recording a chromatogram of the same mixture of compounds using the same laser wavelength but with light pulses of 20 picosecond duration. The result is displayed in Figure 1B. Biphenyl clearly ionizes much more efficiently than does naphthalene (due to its higher absorption coefficient) as long as it is not given enough time to relax from its excited electronic state.

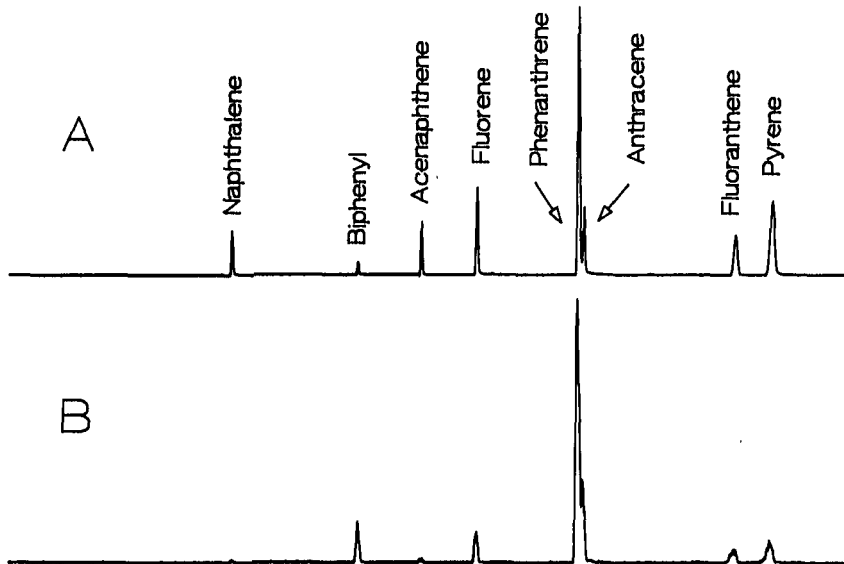


FIG. 1: Gas chromatograms of a mixture of polyaromatic hydrocarbons detected by laser ionization mass spectrometry. (A) 10 nanosecond light pulses (B) 20 picosecond pulses.

We are now developing a general rate equations model to predict the laser ionization efficiencies of different compounds as a function of their absorption coefficients, excited state lifetimes and the laser pulse duration. The results obtained for a model compound having an excited state lifetime of 10 nsec, and ground and excited state absorption cross sections of 10^{-17} and 10^{-19} cm², respectively, are displayed in Figure 2. The predicted time dependence of the excited state population and the ion yield are depicted for both 10 nanosecond and 10 picosecond light pulses. The most noteworthy result is that even though the photon fluence of the picosecond pulse is ten times smaller than that of the nanosecond pulse, almost five times more ions are produced in the former case. In summary, shorter laser pulses can effectively compete with excited state relaxation.

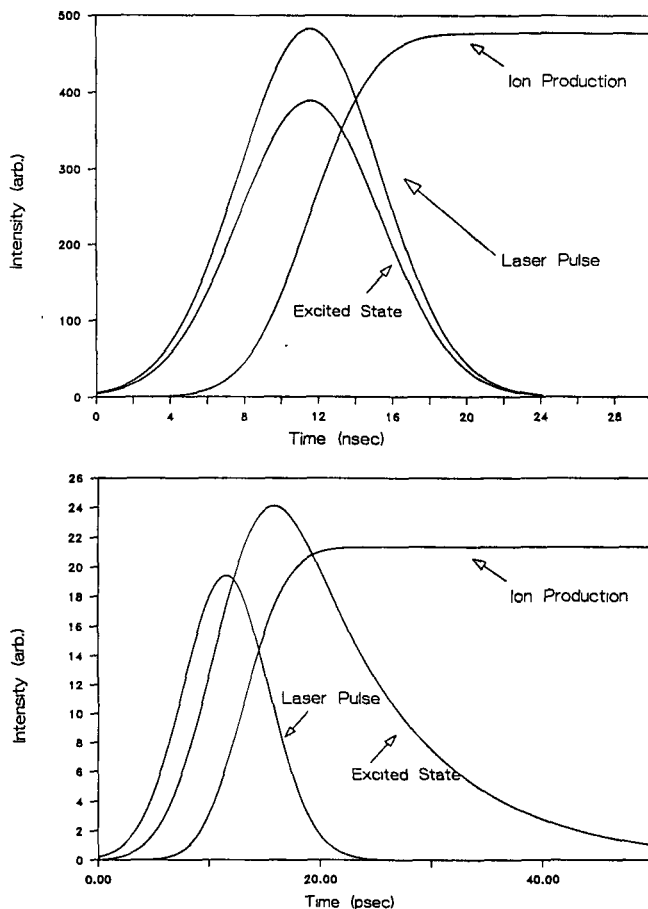


FIG. 2: The time dependence of excited state population and ion yield as calculated by a rate equations model. (A) 10 nanosecond light pulse (B) 10 picosecond pulse.

LASERS AND QUADRUPOLE FOURIER TRANSFORM MASS SPECTROMETRY
APPLIED TO PROTEIN SEQUENCE ANALYSIS

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New methodology development for protein sequence analysis continues to be a major goal of our research. The need for molecular weight determination at high mass, high mass resolution, and higher sensitivity are all important to protein sequence analysis. The ability to analyze oligopeptides for amino acid sequence at lower levels is becoming more and more challenging. Our approach to protein sequencing relies, in part, on the use of quadrupole Fourier transform mass spectrometry (QFTMS) for the mass analysis. QFTMS is routinely used to generate molecular weight determinations of oligopeptides up to 6 kDa at the pmol level (1,2). Lasers can now be employed as an integral part for ionizing extremely small amounts of very large proteins (3). The laser has also been used to directly photodissociate molecules, MS/MS/MS...(ad infinitum). Sequence analysis by photodissociation of peptides up to 29 amino acid residues in length and on as little as 10 pmol of sample using the QFTMS and an ArF excimer laser has been demonstrated (3,4).

Laser photodissociation by QFTMS has significantly contributed to the amino acid sequencing of proteins. However, complete sequencing information is usually only obtained when the oligopeptide cooperates, in part, by unimolecularly decomposing. A single laser pulse from an unfocused ArF (193 nm, 6.4 eV) excimer laser results in such efficient photodissociation of the oligopeptide, that most of the photofragments produced are the lower mass fragments (illustrated in Fig. 1). A study of photofragment yield vs. photon flux indicates a linear dependence with no change in ion distribution or fragment species. Experiments using surface induced dissociation (SID), pioneered by Cooks (5), were performed on several oligopeptides using the QFTMS (6). The ArF photodissociation spectrum and the SID spectrum yield virtually the same fragmentation, confirming the quantity of energy deposition and fragmentation pathways to be similar.

To enhance the higher mass sequence ions and better control the amount of absorbed energy, different wavelengths were tried. KrF (248 nm, 5 eV) gave very poor photofragmentation yields with no higher mass fragments and XeCl (308 nm, 4 eV) was not absorbed at all by the peptides. This is not surprising since the cross-section of peptides at these wavelengths is not very large. Excimer pumped dyes around 500 nm (2.5 eV) also proved unsuccessful. However, the photon flux was insufficient to pump up the ions using an unfocused laser beam. When the beam was focussed down to <1 mm, the photons no longer physically interacted with the majority of the ions stored in the ICR cell. Experiments using an unfocused Nd:YAG laser at 532 nm are planned.

Vibrational excitation of ions using a pulsed CO₂ (10.6 μ m, .11 eV) laser is also possible. Glycerol proton bound oligomers and small oligosaccharides had been dissociated using multiple CO₂ laser pulses (4). The pulsed CO₂ laser enhanced the dissociation of Gramicidin S (Fig. 2) yielding a series of the sequence ions but was unsuccessful on other oligopeptides. Increasing the photon flux by using a continuous wave (cw) CO₂ laser to pump up and dissociate small peptide ions has been demonstrated (7). Controlling the photofragment distribution and yield from large oligopeptides by pumping up the ions in small increments should be possible.

Ions formed by different ionization modes offer another means to control the net internal energy of the photo-excited oligopeptide ions. Laser desorption of high mass proteins from solid matrices has been successful (3) and should provide a reasonable ionization alternative to LSIMS. So far we have been unable to implement this ionization technique using the QFTMS due to difficulties in extracting and/or trapping the ions.

An alternative ionization mode, which provides internally "cold" ions, is electrospray ionization (ESI). It has been demonstrated that multiply charged ions can be successfully collisionally dissociated (8) and should also be amenable to photodissociation. Preliminary results interfacing ESI and QFTMS (9) are promising and are illustrated in Fig. 3. The left spectrum (Fig. 3a) has only one ion signal at m/z 571 and it is the doubly charged molecular ion from Gramicidin S. The right spectrum (Fig. 3b) results from a single unfocussed ArF pulse to photodissociate the $(M+2H)^{++}$ ion. Predicted sequence ions, as well as, a $(M-H)^+$ ion at m/z 1140 are observed.

Lasers may prove to be useful in a more abstract manor for dissociating ions. Collisional-activated dissociation of ions remains the most widely used means to generate fragment ions. Collision gas pressure, interaction time with the collision gas, and initial collision ion energy can all be controlled to finely tune the amount and type of fragmentation desired. The collision gas pressure for FTMS experiments is usually regulated by a series of pulsed valves, which only coarsely controls the pressure in the ICR cell. The collision gas pressure, necessary for collisional decomposition, in the pressure sensitive ion cyclotron cell can be rigorously controlled and placed using lasers. A liquid N_2 cold finger is used to condense a collision gas, such as carbon dioxide, near the ICR cell. A laser pulse or series of pulses are employed to desorb the solid carbon dioxide off of the cold finger to give a finely controlled burst of collision gas. This apparatus has undergone preliminary tests using the QFTMS and should provide an effective means of controlling collision gas pressure in the ICR cell.

Lasers offer an effective means of ionizing, as well as, dissociating oligopeptides and will continue to develop as an integral part of mass spectrometry.

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Laser PD of 50 pmol Rat Angiotensin III

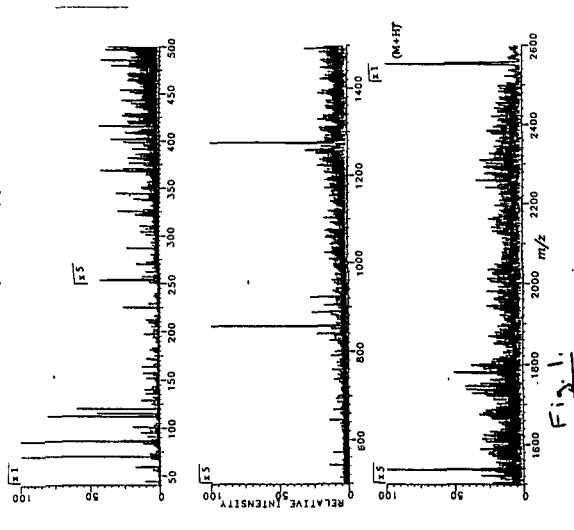


Fig. 1.

Gramicidin S - CO₂ Laser PD (40 pulses)

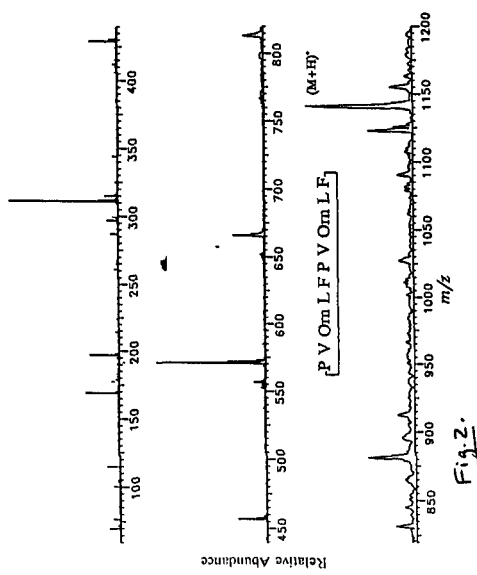


Fig. 2.

Electrospray Ionization of Gramicidin S
(Kent Henry, Evan Williams and Fred McLafferty)

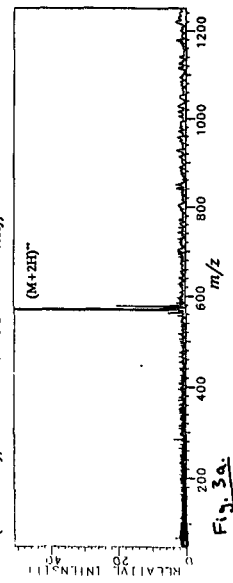


Fig. 3a.

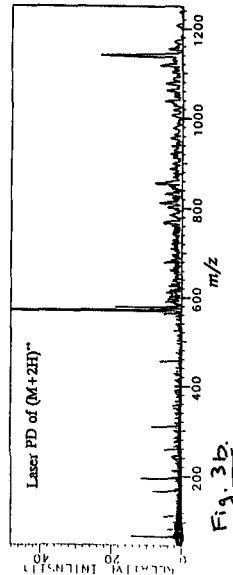


Fig. 3b.

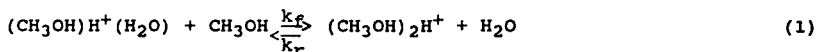
PHOTOINDUCED ION-MOLECULE REACTIONS IN THE GAS PHASE USING FTMS.

J.R. Gord and B.S. Freiser,

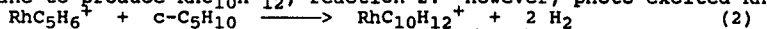
Department of Chemistry, Purdue University, W. Lafayette, IN 47907

In the mature area of solution phase photochemistry, a limitless number of examples exist in which a species, activated by the absorption of a photon, undergoes a reaction not observed in its ground state. In contrast, under the conditions used to study gas phase ion-molecule chemistry at low pressures (10^{-5} to 10^{-9} torr), examples of photo-induced or photo-enhanced reactions have been surprisingly sparse. In this environment, the time between reactive collisions is sufficiently long that internal conversion, photodissociation, or radiative and collisional relaxation of photo-excited ions significantly reduce the possibility of photo-induced reactivity.

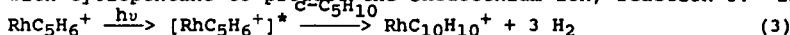
Only one example of a photo-enhanced ion-molecule reaction in this pressure regime has been reported to date [1]. Bomse and Beauchamp observed selective enhancement of k_r by $>10^3$ during an ion cyclotron resonance (ICR) study of the effect of infrared irradiation ($10.6 \mu\text{m}$) on reaction 1. In this



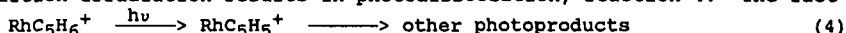
paper we discuss several strategies for generating long-lived intermediates and report the second and third examples of photo-induced reactivity in the ICR pressure regime and the first such examples involving organometallic ions. The first example involves RhC_5H_6^+ [2] which was prepared through reaction of laser-desorbed Rh^+ with cyclopentane. Thermalized RhC_5H_6^+ reacts with cyclopentane to produce $\text{RhC}_{10}\text{H}_{12}^+$, reaction 2. However, photo-excited RhC_5H_6^+



reacts with cyclopentane to produce the rhodocenium ion, reaction 3. In

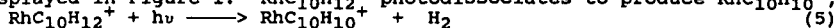


addition irradiation results in photodissociation, reaction 4. The fact that



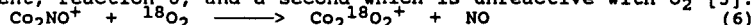
reaction 3 competes favorably with reaction 4 implies that the photodissociation pathway proceeds slowly, either because the photon energy is near the dissociation energy or because multiple photon absorption is required to produce photodissociation at this wavelength.

The mass spectra produced with and without laser irradiation are displayed in Figure 1. $\text{RhC}_{10}\text{H}_{12}^+$ photodissociates to produce $\text{RhC}_{10}\text{H}_{10}^+$,



reaction 5, so double resonance techniques were employed to ensure that the suspected photo-induced reaction product was not produced via this pathway.

The second example involves Co_2NO^+ . Klaassen and Jacobson recently identified two forms of this ion, one which reacts with $^{18}\text{O}_2$ by nitrosyl displacement, reaction 6, and a second which is unreactive with O_2 [3]. The



absence of isotope scrambling in reaction 6 suggests that the reactive form of Co_2NO^+ involves molecularly chemisorbed nitrosyl ligand. Further studies using H_2^{18}O indicate that the nonreactive form may involve the dissociatively chemisorbed ligand.

In this study we observed the reaction of Co_2NO^+ with O_2 as a function of time with and without irradiation. Figure 2 displays the relative abundances of Co_2NO^+ and Co_2O_2^+ under these conditions. Jacobson has demonstrated that the populations of the reactant and product ions approach an asymptotic limit with increasing time when no irradiation is present. However, when irradiated with 1064 nm (~ 27 kcal/mol) photons from a pulsed Nd:YAG laser operated at 10 Hz, the nonreactive isomer is observed to become reactive, presumably because the photo-excited species can access both the reactive and the nonreactive isomers. Relative abundances in Figure 2 do not sum to 100% due to a small photodissociation contribution not displayed.

Experiments using arc lamp irradiation at ~ 35 kcal/mol resulted in competition between the photo-induced reactivity characterized in Figure 2 and photodissociation, reaction 7. The 514.5 nm output from the Ar^+ laser



produced photodissociation products exclusively. In a continuing series of studies, we are using variable energy light sources in conjunction with this

photoinduced reaction to characterize the height of the Co_2NO^+ reactive/nonreactive isomerization barrier. In addition, photodissociation threshold studies are underway to determine $D(\text{Co}_2^+-\text{NO})$ for the reactive and nonreactive isomers as well as $D(\text{Co}_2^+-\text{O}_2)$.

Three mechanisms could explain production of the long-lived intermediate necessary to observe photo-induced reactivity: (i) generation of an ion in an electronically excited state which is forbidden to the ground state, formed directly, by intersystem crossing, or by some collisional or radiative process involving the initially photoaccessed excited state, (ii) generation of a vibrationally excited ground state ion by internal conversion, and (iii) generation of a different geometric isomer. Given the high density of low-lying electronic states characteristic of open shell organometallic species, (i) cannot be completely ruled out; however, we believe it is the least likely of the three. Photodissociation of an ion of this size, as observed in reaction 4, typically occurs following internal conversion, consistent with either (ii) or (iii). We believe reaction 3 involves a vibrationally excited ground electronic state intermediate, mechanism (ii), while the photoinduced reaction involving Co_2NO^+ proceeds by a novel photo-induced rearrangement, mechanism (iii).

Other systems currently under study in this and other laboratories suggest that rich and diverse photoinduced chemistries wait to be uncovered.

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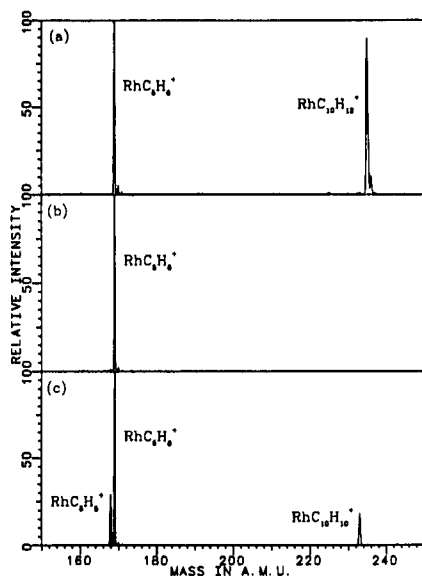


Figure 1. (a) Reaction of RhC_5H_6^+ and cyclopentane (-10^{-7} Torr) for 0.75 s with no irradiation, (b) with no irradiation and continuous ejection of $\text{RhC}_{10}\text{H}_{12}^+$, and (c) with 3-W 514.5-nm irradiation and continuous ejection of $\text{RhC}_{10}\text{H}_{12}^+$.

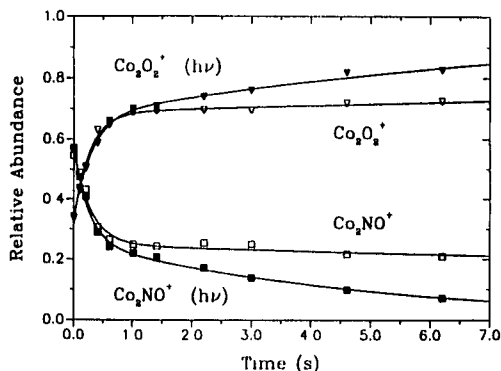


Figure 2. $\text{Co}_2\text{NO}^+ + \text{O}_2 \rightarrow \text{Co}_2\text{O}_2^+ + \text{NO}$ studied as a function of time with and without irradiation. The smooth curves represent optimized biexponential fits to the data.

DEPENDANCE OF CAR SPECTRA OF DIASTEROMERIC MH^+
ION UPON THE USED METHOD OF ION PREPARATION.

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Recently, various studies had been investigated on the different effects of initial internal energy on low energy CAD of selected protonated MH^+ ions^{1,2,3,4}. Relative daughter ion abundances are significantly dependent on the CI reagent used to generate the precursor MH^+ ion. Such low energy CAD processes occurred in the collision cell of triple quadrupole mass spectrometer. For relatively low energy collision (under $E_{lab} < 50$ eV) these studies shown that together (i) the initial internal energy of MH^+ and (ii) the protonated site on MH^+ ion played an important role on the orientation of competitive and consecutive decompositions. These factors yield large changes on the low energy CAD spectra. For diastereomeric protonated molecules, these effects enhance strongly the observed stereochemical effects⁴.

In this report, Collisionally-Activated-Reaction (CAR) spectra of protonated diastereomeric bicyclo-(2,2,1)-heptanol-2 (1a, 1b) and derivatives (e.g. endo and exo acetates (2a, 2b) and benzoates (3a, 3b)) are investigated according to the method to prepare the MH^+ ions (e.g. protonated by different CI reagent gases). In collision cell and under very low collision energy (<3 eV), the reactivity of diastereomeric protonated molecules (containing different internal energy) towards ammonia reagent are compared.

In the collision cell, different competitive processes occur from low energetic MH^+ ions under these conditions: Solvation (adduct ions, ammonium clusters), Nucleophilic Substitution ($(M+NH_4-ROH)^+$ ion, noted MsH^+) and Proton Transfer (yielding NH_4^+). Concerning the protonated isomeric benzoates (formed in CI/NH_3), weak stereochemical effects are observed (figure 1a and 1b) and disappear when they contain larger internal energy (figure 1c). Furthermore, solvation process yields decrease in particular for the produced MsH^+ ion. This behavior often takes place for reversible processes. This suggests that likely during flight through the first analyser, $C_{7}H_{11}-O-COH^+-C_6H_5$ isomerizes into $C_{7}H_{10}..H^+..HOOC-C_6H_5$ intermediate which reacts with NH_3 yielding of $C_{7}H_{10}..H^+..NH_3$ ion rather a covalent $C_{7}H_{11}-NH_3^+$ ion such as it is produced in CI source (via S_N2 and S_N1 according to the initial configuration). The protonated acetates are characterized by a different gas phase behavior since large stereochemical effects are observed (figure 2a and 2b). Furthermore, unusual stereospecific process can take place under these CAR conditions. The latter is due to a prior isomerization of bicyclic skeleton into a reactive protonated tricyclic hemiketal such as it is occurred in solution (lactone formation). From this tricyclic form, SN and methane elimination occur and lead to the unusual $(M+NH_4-R_2O-CH_4)^+$ formation (Scheme 1). The increasing of the initial internal energy of MH^+ leads to a "levelling" of CAR spectra. Likely, from the latter species, an isomerization in protonated norbornene-acetic acid complex is preferred to this yielding hemiketal intermediate prior reactive collisions. On the other hand, from protonated esters (formed via metastable NH_3 loss from selected $(M+NH_4)^+$ ion), consecutive isomerization into any intermediate is unfavored (shorter residence time in this region) (figure 2c).

In conclusion this work shows the role plays by (i) the function stereochemistry and (ii) the internal energy of the selected ions on reactivity towards ammonia. The increasing of precursor ion internal energy on CAR spectrum can be significant on the possible ion structures.

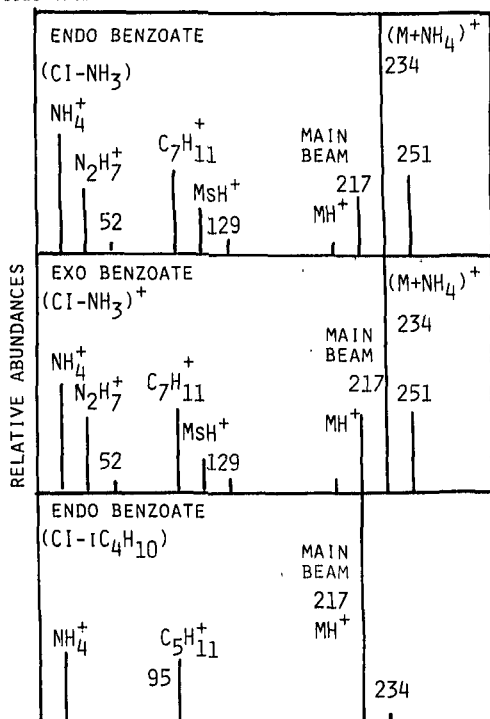


Figure 1. CAR spectra of (a) endo, (b) exo protonated benzoates formed in CI/NH₃ and (c) the endo MH⁺ ion formed in CI/IC₄H₁₀.

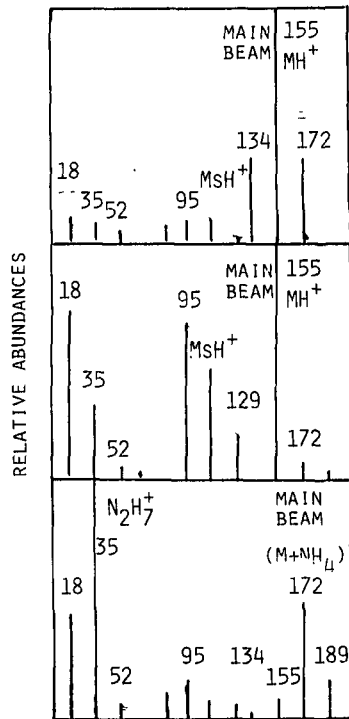
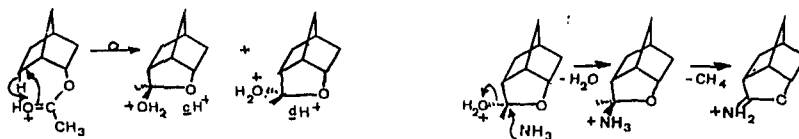


Figure 2. CAR spectra of (a) endo, (b) exo protonated acetates and (c) the (M+NH₄)⁺ (endo diastereoisomer) generated in CI/NH₃.



Scheme 1. Possible MH⁺ isomerization process and ion-molecule reaction induced by ammonia.

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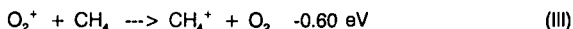
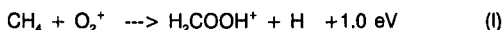
Effective Ion Temperatures in a Quadrupole Ion Trap

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Paul-type quadrupole ion traps are rapidly gaining recognition as simple and relatively inexpensive mass spectrometers that are well-suited, for example, for chemical ionization experiments, thermochemical studies, ion-molecule reaction studies and tandem mass spectrometry involving photo- or collision-activated dissociation of mass selected ions (1). These mechanically simple devices consist of a chamber formed between three electrodes with hyperbolic inner surfaces. Radio-frequency voltages, in combination with dc voltages, are used to trap, isolate, excite and eject ions. Typically, one mtorr of helium is introduced into the ion trap to dampen the motion of the ions, thereby improving the overall performance.

The lack of knowledge of the temperature of ions in a quadrupole ion trap limits the usefulness of this device. Knowledge of the internal and kinetic energies of the ions is very important for example for comparison of experimental results obtained using different instruments and for determination of kinetic and thermodynamic parameters, such as rate constants, equilibrium constants and bond energies. One can not directly measure the actual ion temperature. However, an "effective" temperature can be determined that is related to the internal energy distribution of the ions. The "effective" temperature is here determined as the temperature that would result in a specified rate (or product distribution) for a reaction in the absence of external electric fields.

In order to obtain information concerning the actual internal energy distribution of ions in a three-dimensional quadrupole ion trap, a number of experiments have been carried out in this lab. The reaction of O_2^+ with methane is one of the systems employed as a "chemical thermometer". This reaction has been the focus of a considerable research effort and is one of the best understood bimolecular gas-phase reactions. The rate constant as well as the product distribution are temperature dependent (2,3). There is only one exothermic pathway (I) available. At higher energies two endothermic channels ((II) and (III)) dominate the product distribution (4).



Under normal trapping conditions, the rate constant for the reaction of O_2^+ with methane was determined to be $8.2 \pm 3.4 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ second}^{-1}$. The ratio of exothermic to endothermic products (m/z 47 : (m/z 15 + m/z 16)) was 1 : 0.33 (when excess methane is present, m/z 15 and m/z 16 are detected as m/z 17 and m/z 29). Both the rate constant and the product distribution indicate an "effective" temperature of 600K - 700K for ions stored in the quadrupole ion trap in the presence of 5 mtorr of helium buffer gas. This temperature corresponds to kinetic energies of approximately 0.1 eV (3/2 kT).

Other systems studied to further investigate the "effective" temperature are shown in Table 1. The excess internal energy that these ions would gain upon a temperature increase from 298K to

600K is indicated together with the dissociation thresholds for each ion. The stability of these ions under normal trapping conditions was examined by storing the ions in the ion trap for approximately 3000 msec. Spontaneous decomposition was observed for tetraethylsilane and protonated cyclohexene oxide but not for the other ions studied. These findings support the estimated ion temperature of 600K. The general equation $k(T) = k_{\text{capture}} \exp(-E_a/RT)$, where E_a is the activation energy, may be used to approximate the "effective" ion temperature using an endothermic bimolecular reaction as a chemical thermometer. The dissociation of $(C_2H_5)_3Si^+$, upon collisions with helium, gives $k_{\text{dissoc.}}/k_{\text{AOD}} = 0.0003$ which results in an "effective" temperature of 720K.

The reaction of O_2^+ with methane was examined under more energetic conditions, i.e., the O_2^+ ion was isolated and a supplementary ac voltage was employed to increase the ions kinetic energy. The ratio of m/z 47 : (m/z 15 + m/z 16) varies with the ac voltage amplitude and is 1 : 5 under the most energetic conditions. This indicates that the "effective" temperature can be as high as 1300K for this system, corresponding to 0.4 eV internal energy for O_2^+ .

In conclusion, the "effective" temperature of ions in the quadrupole ion trap can be as low as 600K - 700K under normal trapping conditions. These results apply for m/z 30 - m/z 150, $q = 0.04$ - 0.50. The dependence of the "effective" temperature on the nature of the buffer gas, the mass of the ion, and the q value will be examined in detail.

TABLE 1

	$(H_{\text{AOD}} - H_{\text{ZPE}})$ - $3/2 kT$	dissociation threshold:	reaction observed
$(CH_3CH_2)_3Si^+$	0.92 eV	0.8 eV	YES
 OH ⁺	0.53 eV	0.5 eV	YES
$(CH_3O)_2P(O)H^+$	0.34 eV	0.7 eV	NO
$(CH_3CH_2)_3Si^+$	0.69 eV	1.5 eV	NO
 OH ⁺	0.53 eV	1.6 eV	NO

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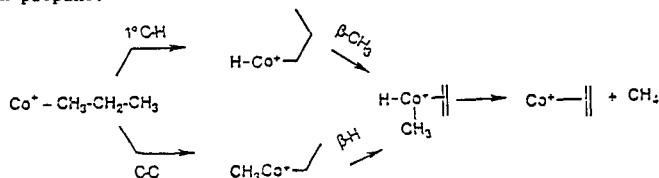
Acknowledgements:

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MECHANISTIC AND ENERGETIC IMPLICATIONS OF KINETIC ENERGY RELEASE DISTRIBUTIONS FOR REACTIONS OF TRANSITION METAL IONS WITH ALKANES

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The growing number of investigations probing aspects of organotransition-metal reactions stem in part from the importance of organometallics as widely proposed intermediates in many catalytic transformations. In particular, considerable effort in recent years has been directed at understanding the mechanisms and energetics of C-H and C-C bond activation of alkanes by transition metal ions. Such exothermic ion/molecule reactions typically result in elimination of molecular hydrogen, alkanes, and alkenes, cleavages which are initiated by oxidative addition of a metal ion across a C-H or C-C bond. The insertion site has yet to be determined for some of these alkane elimination reactions, for example CH_4 loss from Co^+ and Fe^+ reacting with propane.



Product kinetic energy release distributions (KERD) have proven valuable in deducing the mechanisms as well as in elucidating details of potential energy surfaces for metal-mediated reactions. Statistical theories such as phase space theory have been used successfully to model translational energy release distributions for reactions on a surface where there is no barrier, excluding a centrifugal barrier, for the the reverse association reaction. Recently, we have examined a number of reactions, including methane loss from Co^+ and Fe^+ complexes with propane, where the kinetic energy releases are narrower than predicted by statistical theory. These are unusual and interesting results since non-statistical kinetic energy release distributions are generally broader than the statistically predicted ones. The results are explained in terms of a rotational energy barrier on the potential energy surface prior to the exit channel. Labeling studies indicate that the initial C-H bond activation energy barrier is responsible for these observations.

Metastable kinetic energy release distributions were measured using a reverse geometry double focusing mass spectrometer (VG Instruments ZAB-2F) with a home built variable temperature EI/CI source. Metal ions were formed by electron impact (150 eV) of $\text{Fe}(\text{CO})_5$ and $\text{Co}(\text{CO})_3\text{NO}$. The bare metal ions react with the organic neutrals in the ion source to form the organometallic adduct ions. The ions are accelerated to 8 kV and mass analyzed using a magnetic sector. Metastable ions decomposing in the second field free region are energy analyzed by scanning the electric sector.

The KERD for methane loss from Co-propane^+ is shown in Figure 1 along with the statistically calculated distribution. The experimentally measured distribution is much narrower than the one modelled using phase space theory. This translationally cold distribution derives from the influence of angular momentum conservation on reaction dynamics. Examination of the potential surface shown in

Figure 2 reveals that the presence of an insertion barrier close to the asymptotic energy of the reactants dictates the outcome of capture collisions involving varying amounts of angular momentum. For collisions involving large angular momentum, it is possible that the system can cross the centrifugal barrier but yet be unable to approach closely enough to undergo insertion. Thus, the Co^+ /propane adduct may be formed but unable to proceed to final products. This discrimination against large angular momentum collisions is manifested as a reduction in the high kinetic energy release portion of the product kinetic energy distribution, since large values of angular momentum contribute to the higher energy portion of the distribution.

It is possible to model translationally cold kinetic energy release distributions using statistical theory by simply restricting the total angular momentum of the orbiting transition state for the reactants. The kinetic energy release distribution obtained using the restricted model is shown in Figure 1, and yields a good fit to the experimental distribution.

To determine the site of initial insertion for the demethanation of the Co^+ /propane complexes, deuterium labelled propane was used. The abundance of the loss of CH_4 from complexes formed using 2,2-propane- d_2 is the same as for the undeuterated complexes. Conversely, the loss of CD_4 from Co^+ /propane- d_8 is virtually negligible, indicating that insertion into a primary C-H bond rather than a C-C bond is the initial step for the demethanation of these organometallic complexes.

Figure 1

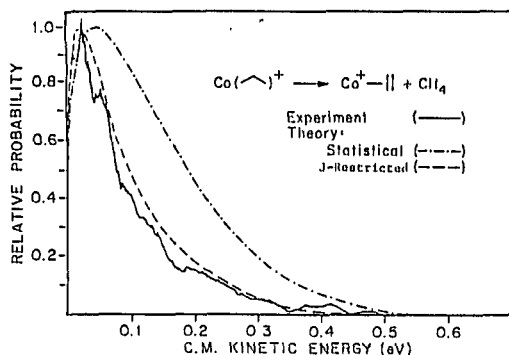
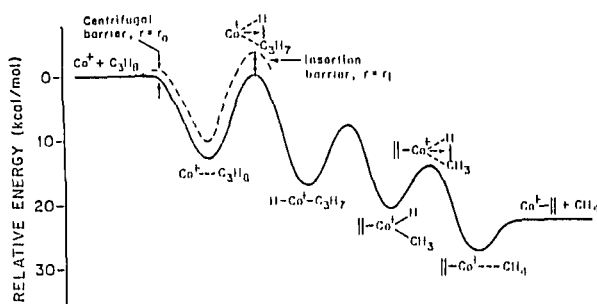


Figure 2



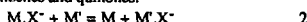
HYDROGEN-BONDED, σ -BONDED AND π -BONDED COMPLEXES $M \cdot X^-$ FROM M AND X^- WHERE M = SUBSTITUTED BENZENES AND QUINONES AND $X^- = F^-, Cl^-, Br^-,$ AND I^- .

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The equilibrium constants K and complexing energies, $-\Delta G^0 = RT \ln K$, were obtained at 150°C for the complexes $M \cdot X^-$ by determining the ion-molecule equilibria:

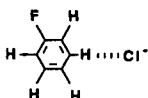


with a pulsed-electron high pressure mass spectrometer. These complexing free energies were complemented by the measurement of X^- transfer equilibria between the substituted benzenes and quinones:



which serves as a cross-check for the complexing energies. An agreement within 0.3 kcal/mol between these two types of measurements was observed. Combining the results from the present (~fifty) measurements using principally Br^- (1) with earlier work from this laboratory (2,3) gives an insight into the variety of bonding that can occur in these complexes.

Previous theoretical work on Cl^- bonding to fluorobenzene (2) showed that hydrogen-bonding leads to the most stable complex:



The same type of bonding is expected also for $X^- = Br^-$. Further information on the nature of bonding in $M \cdot X^-$ from present measurements, where M is benzene and singly substituted benzenes and X^- is Br^- , can be gained using methods developed by Taft (4) for the separation of the substituent effects, $-\Delta G^0$, into resonance (R) and field-inductive (F) contributions by:

$$-\Delta G^0 = \rho_R \sigma_R + \rho_F \sigma_F \quad 3$$

In this case $-\Delta G^0$ is the experimentally-determined relative free energy change in bromide affinity between each substituted benzene and benzene ($-\Delta G^0 = 0$ kcal/mol for substituent = H); σ_R and σ_F are system-independent parameters compiled by Taft (4) which rank resonance and field effects for each substituent; ρ_R and ρ_F are coefficients particular to this system which indicate the magnitude of R and F effects and are determined by multiple regression analysis of equation 3 applied to each benzene substituent. Figure 1 shows a good correlation of $-\Delta G^0_{calc}$, determined with these evaluated ρ_R and ρ_F values, with $-\Delta G^0_{BFA,423K}$. Thus the substituent effects are well described by established resonance and field-inductive terms of the substituent.

Table 1

	ρ_R	ρ_F	ρ_R/ρ_F	
	6.8 ± 2.0	8.5 ± 0.5	0.8	
	14.6 ± 0.7	15.0 ± 0.6	1.0	Acidity data from (4)
	49.0 ± 1.5	18.6 ± 0.5	2.6	" " " "

The ρ_R/ρ_F ratio, see Table 1, which provides a means to compare the relative stabilizations by resonance and field effects for different systems, is similar for $Br^- \cdots H-C_6H_4Y$ and $CO_2-C_6H_4Y$ stabilization but larger for $O-C_6H_4Y$. This is to be expected from the resonance structures in Figure 2 where direct conjugation of the negative charge occurs only in $O-C_6H_4Y$ stabilization which leads to a larger resonance contribution and consequently a bigger ρ_R/ρ_F ratio. The smaller ρ_R/ρ_F ratio therefore supports the theoretical prediction of the bromide ion being hydrogen-bonded to singly substituted benzenes where resonance stabilization is a relatively small effect.

Progressive substitutions of electron-withdrawing groups on benzenes and quinones were found to cause a continued increase in the bromide affinities. Very strong bonding was observed for Br^- with perfluorobenzenes and quinones which contain no aromatic hydrogens at all. For example, the bond energy increases by ~10 kcal/mol from benzoquinone. Br^- to tetrafluoroquinone. Br^- . SCF calculations on the tetrafluoroquinone. Br^- complex (5) predict that Br^- is σ -bonding to the aromatic ring forming a so-called

Meisenheimer complex. Confirmation of this bonding can be again be obtained using the Taft methods (4) applied this time to the experimental $-\delta\Delta G_{BrA,423K}^{\circ}$ and $-\delta\Delta G_{ClA,423K}^{\circ}$ values for the singly substituted perfluorobenzenes. The results are shown in Table 2.

Table 2

	ρ_R	ρ_F	ρ_R/ρ_F
	7.4 ± 3.2	4.1 ± 1.0	1.8
	9.0 ± 2.8	2.8 ± 0.8	3.2

The larger ρ_R/ρ_F ratios obtained show, as for the phenols acidities in Table 1, that the negative charge is directly conjugated into the ring as would be anticipated for the σ -bond formation as shown in Table 2. This type of σ -bonded complex has also been previously predicted by ab initio calculations for $C_6F_6-F^-$ (6).

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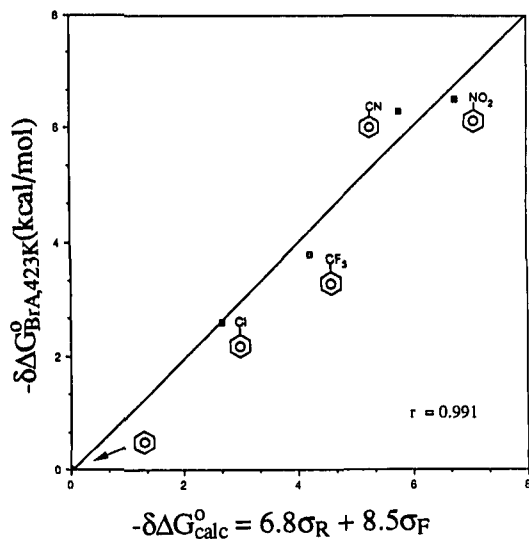


Figure 1

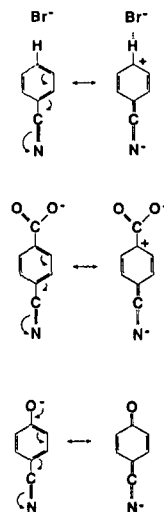


Figure 2

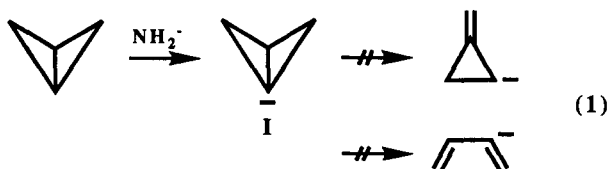
UNIMOLECULAR REARRANGEMENTS IN THE GAS PHASE: RING OPENING OF CYCLOPROPYL ANIONS

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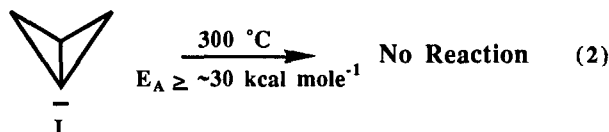
Theoretical and experimental investigations of unimolecular rearrangements are a productive and vast area for research. The elucidation of reaction mechanisms and information regarding the relationship between structure and reactivity has provided some of the impetus for these studies.

Rearrangements of positive and negative ions have been extensively examined in condensed media, but gas phase studies offer some distinct advantages. An obstacle impeding such work is that unimolecular rearrangements lead to isomeric species, which cannot be distinguished simply on the basis of their mass. Another difficulty is that energetically well defined systems are needed if rates of reaction are to be measured and activation energies obtained. These latter quantities are of fundamental importance but are generally not accessible. We now wish to report that a variable temperature flowing afterglow apparatus can be used to probe unimolecular rearrangements.

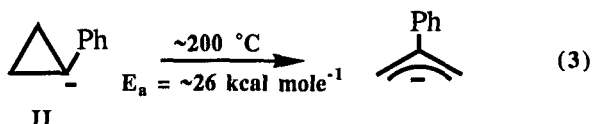
It has previously been shown by several research groups that cyclopropanes do not undergo ring cleavage upon reaction with strong bases.^{1,2} More recently, we have found that even the highly strained bicyclobutyl anion (I) can be formed without any accompanying rupture (eq 1).³ These results are consistent with liquid phase studies and molecular orbital calculations which suggest that a significant barrier exists for the ring opening of cyclopropyl anions.^{4,5}



Remarkably, the bicyclobutyl anion is unaltered by heating to temperatures as high as 300° C (eq 2). In sharp contrast to the



behavior of I, phenylcyclopropyl anion (II) begins to undergo thermolysis at a temperatures approximately 200 °C (eq 3). The



activation energies for the rearrangement of these two ions are clearly quite different. They can be estimated if one assumes a typical value, 10^{13} - 10^{14} , for the A factor in the Arrhenius equation. The results are in reasonable agreement with theory, and reveal that structural effects are of critical importance.

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A THEORETICAL STUDY OF NUCLEOPHILIC SUBSTITUTION
REACTIONS IN THE GAS PHASE

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Measurements¹ of the rate constants for the exothermic gas-phase nucleophilic substitution reactions $\text{OH}^- + \text{CH}_3\text{X} \rightarrow \text{X}^- + \text{CH}_3\text{OH}$ (where $\text{X} = \text{Cl}, \text{Br}$ or I) over the temperature range 200–500 K reveal two unusual features: reaction does not occur on every collision, and the rate constants decrease with increasing temperature. We report here the results of theoretical calculations of the reaction efficiencies, and show that good agreement can be obtained with the experimental observations.

These calculations are based upon a model² which assumes a double minimum potential energy surface of the type shown in Figure 1 for the generalized ion-molecule reaction $\text{A}^- + \text{B} \rightarrow \text{C}^- + \text{D}$. Briefly stated, this model assumes that the reactants, attracted by long range ion-dipole forces, form the reactants adduct A^-B at the collision rate, k_1 . This adduct can either reform the reactants with a rate constant k_{-1} , or pass over the central barrier E_0 with a rate constant k_2 to form the product adduct C^-D . For sufficiently exothermic reactions, it can be shown that the predicted reaction efficiency is given by $k_2/(k_{-1} + k_2)$. The model further assumes that the ratio k_{-1}/k_2 (and hence the reaction efficiency) can be calculated as a function of temperature from the RRKM theory of unimolecular reactions if the properties of the relevant transition states are known and if a value is known or assumed for E_0 .

For all three reactions, the geometry of the transition state along the pathway leading to products (TSB) was optimized using AMPAC,³ a semiempirical method of optimization. For the reaction with $\text{X} = \text{Cl}$, the transition state geometry obtained from AMPAC was very similar to that reported by Ohta and Morokuma⁴ from ab initio quantum mechanical calculations. (Ab initio results have not been reported for the reactions with $\text{X} = \text{Br}$ or I .) Vibrational frequencies for TSB were then obtained from the computed structure by using the BEBOVIB IV program.

For the pathway leading back to reactants, the transition states were assumed to be orbiting transition states; the reactants were fixed at the separation corresponding to the maximum in the centrifugal barrier, and AMPAC was used to optimize the remaining degrees of freedom. Vibrational frequencies were then derived using BEBOVIB IV.

The height E_0 of the central barrier (or more directly, the energy difference ΔE between the two transition states) was treated as an adjustable parameter. Using the transition state properties obtained from AMPAC, RRKM calculations⁵ were carried out to find the value of ΔE required for the calculated efficiency to equal the experimental efficiency at room temperature. Using this "empirical barrier height," RRKM calculations of the reaction efficiencies were carried out at a number of temperatures over the range 200–500K. The results obtained for the reaction with $\text{X} = \text{Cl}$ are compared with the experimentally obtained reaction efficiencies in Fig. 2. Similar results (not shown) were also obtained for the reactions with $\text{X} = \text{Br}$ and I .

From the good agreement obtained between calculated and measured efficiencies for these three reactions, it would appear that the double minimum model proposed by Brauman and co-workers can provide a useful explanation for the rather unusual temperature dependence exhibited by nucleophilic substitution reactions in the gas phase.

A more complete account of this work is being prepared for publication elsewhere.

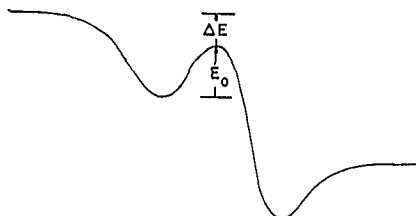
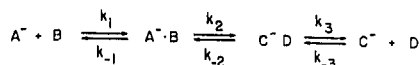


Figure 1. Schematic representation of the double minimum potential energy surface for the generalized ion-molecule reaction $A^- + B + C^- + D$.

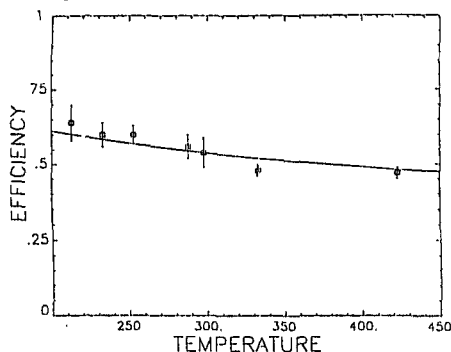


Figure 2. Reaction efficiency vs. Temperature (K) for the reaction $OD^- + CH_3Cl + Cl^- + CH_3OD$. The squares represent the ratio of the measured reaction rate constant to the calculated collision rate constant. The solid line represents the results of the RRM calculations discussed in the text.

References

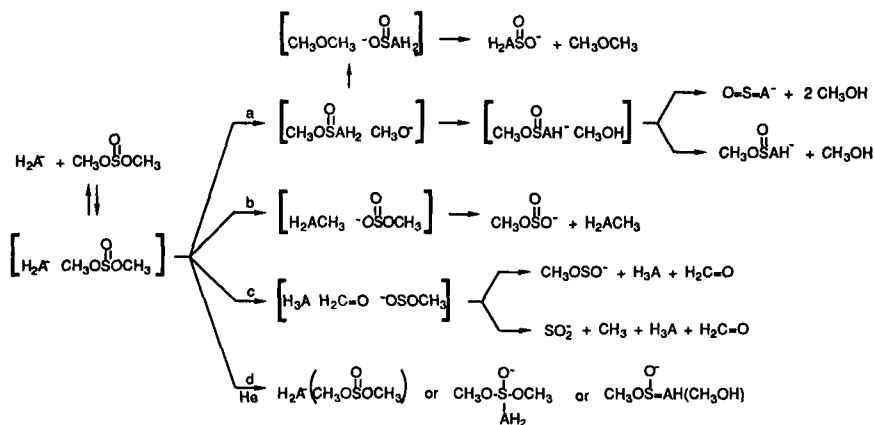
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SELECTIVITY AS A FUNCTION OF ANIONIC BASE PROPERTIES IN THE REACTIONS OF DIMETHYL SULFITE

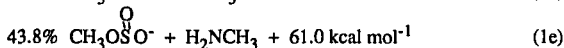
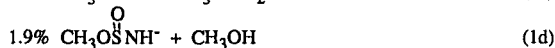
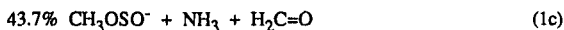
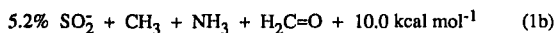
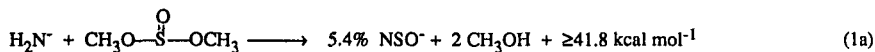
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As part of our continuing interest in understanding the intrinsic reactivity of organic sulfur compounds,¹ especially in relation to the utility of such functionality by bio-organic systems and in relation to the fate of sulfur in the atmosphere, we report here our detailed anion-molecule studies of dimethyl sulfite. The related molecule dimethyl carbonate ($\text{CH}_3\text{OCO}_2\text{CH}_3$) has been briefly examined by the ion cyclotron resonance (ICR) technique² and has been found to undergo nucleophilic attack at both $\text{sp}^2\text{-C}$ and at $\text{sp}^3\text{-C}$. In this flowing afterglow study, we report the products observed and our mechanistic interpretations of a series of anions interacting with dimethyl sulfite at 300K. We are interested in isolating the factors that define the site of reactivity (C or S) and the type of reaction ($\text{S}_{\text{N}}2$, E2 or $\text{B}_{\text{AC}}2$) that occurs. The studies have focussed on the competitive chemical processes that can occur within an ion-neutral encounter, and to a much lesser extent, the absolute rate of reaction.

We can account for the observations reported herein with four general pathways by which an anion might reaction with dimethyl sulfite (Scheme): (i) nucleophilic attack at sulfur, path a; (ii) nucleophilic substitution at the methyl group, path b; (iii) elimination initiated by proton abstraction from the methyl group, path c, and (iv) adduct/cluster formation, path d.



Consider the data from the amide reaction shown in eq 1. The major ion at m/z 79, CH_3OSO^- , can be envisioned as arising from elimination across a carbon-oxygen bond (Scheme, path c) while the m/z 64 ion, SO_2^- , is formed by a minor fragmentation during the elimination of those CH_3OSO^- ions that obtain enough of the reaction exothermicity to surmount the fragmentation barrier. Also produced in 44% overall yield from the reaction of amide with dimethyl sulfite is $\text{CH}_3\text{OSO}_2^-$, m/z 95, which arises from bimolecular substitution at saturated carbon. The two minor products are m/z 62 and 94, NSO^- and $\text{CH}_3\text{OSONH}^-$, both of which arise from initial addition of amide to sulfur to form a tetravalent species. Breakdown of the tetravalent species by extruding methoxide, followed by either elimination or proton transfer gives ultimate rise to the respective products noted.



HA ⁻	PA (kcal/mole)	Elimination	Substitution at sp ³ -C	Reaction at S
H ₂ N ⁻	403.7	48%	44%	8%
H ₂ C=CH-CH ₂ ⁻	390.8	0%	44%	56%
HO ⁻	390.8	19%	← 81% →	
Ph-CH ₂ ⁻	380.7	0%	12%	88%
CD ₃ O ⁻	381.0	0%	50%	50%
	368.5	0%	94%	6%

Several reactions are summarized in the Table, according to the type of reaction noted, E2, S_N2 and B_{AC}2, as have been discussed for amide. Inspection of the data in the Table reveals that the structures of the anionic nucleophile is of primary importance in determining selectivity and only secondarily does one need consider basicity. Strongly basic, localized heteroatomic nucleophiles yield larger amounts of elimination products compared to carbon bases of comparable basicity. While all anions yield substitution products, localized bases are much more nucleophilic at carbon than delocalized carbon bases. For nucleophilic attack at sulfur, the opposite trend is found, delocalized carbon bases being most nucleophilic at this site. The new level of chemical understanding developed from the overall reaction scheme and reactivity patterns noted above will help design future gas-phase experiments to probe specific reaction mechanisms.

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GAS-PHASE NUCLEOPHILIC ADDITION REACTIONS OF NITRILES: A CA MS/MS STUDY OF THE THORPE REACTION.

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The study of gas-phase negative ion-molecule reactions has become a significant area of research in gas-phase ion chemistry. Specifically, conducting an ion-molecule reaction in the gas phase provides an opportunity to study the intrinsic properties of a possible adduct without the complicating effects of solvent. Furthermore, an adduct that is formed with excess energy under conditions of higher pressure [e.g., chemical ionization (CI)] can often be collisionally stabilized by either a buffer gas (e.g., CS_2) and/or unreactant neutrals.

In two previous studies we employed tandem mass spectrometry (MS/MS) techniques to isolate and characterize adducts formed in a CI source by gas-phase base-initiated aldol and Reformatsky condensations.^{1,2} The enolate anions $\text{CH}_2=\text{CHO}^-$ and $\text{CH}_2=\text{CC}(\text{OEt})\text{O}^-$, respectively, were shown to react with acetaldehyde and acetone to produce "stable" adducts. Differences in the relative abundances of characteristic losses from the gas-phase adducts compared to appropriate model compounds pointed to the existence of at least three adducts; viz, ion-dipole, proton-bound, and tetrahedral complexes. The major constituents are loosely-associated ion-dipole and proton-bound complexes.

In this study, we report on the gas-phase base-initiated condensations of nitriles. Nitrile condensations in solution, as a class, are known as Thorpe reactions, and are analogous to the well-known aldol reaction of carbonyl compounds.³

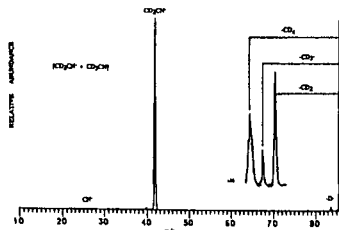
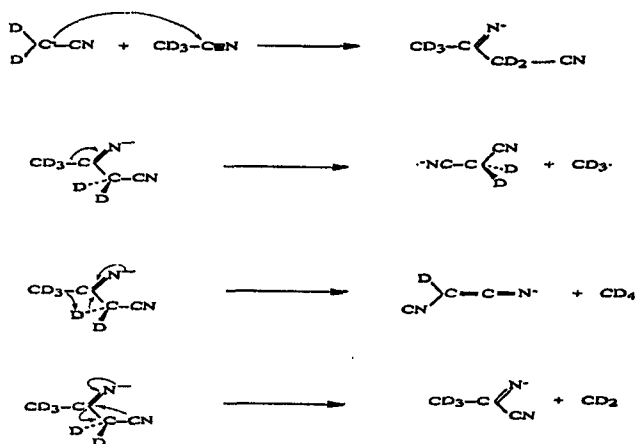


Figure 1.

The acetonitrile ($\text{M} - \text{H}$)⁻ anion condenses with acetonitrile to form a "stable" adduct. Upon collisional activation this adduct decomposes primarily to yield CH_2CN^- . Minor fragment ions are formed by losses of H^- , CH_2 and CH_4 . Similarly, an adduct formed by condensation of CD_2CN^- with CD_3CN undergoes the losses of D^- , CD_2 and CD_4 , and a retro reaction to reform CD_2CN^- (Figure 1).

The charge reversal spectrum of the gas-phase adduct shows that a portion of the adduct is covalently bound as evidenced by the multiple dissociation processes. Significant reversion to starting materials is also observed. Ion-dipole complex are typically transparent to charge stripping, and thus, that fraction of adduct undergoing reversion is likely to be a proton-bound complex.

A possible reference ion, $\text{NCCH}=\text{C}(\text{CH}_3)\text{NH}^-$, formed by deprotonation of the condensed phase Thorpe product of CH_3CN , undergoes fragmentation in a manner distinct from the gas-phase adduct. Presumably, the covalent portion of the gas-phase adduct that loses D^- , CD_2 and CD_4 does so not in the enamine form, but rather in the imine conformation as shown in Scheme 1.



Scheme 1

The propionitrile anion reacts with propionitrile to form an adduct that fragments upon CA in a manner analogous to that described for the acetonitrile condensation homologue. Specifically, the adduct eliminates CH_3CH and reverts to CH_3CHCN^- .

A mixed adduct of acetonitrile and propionitrile fragments in a manner that indicates that the dominant conformation of the adduct is that formed by reaction of the acetonitrile anion with neutral propionitrile. This is to say that the major fragmentation process is reversion to CH_2CN^- . A minor loss of CH_2 is also observed that is consistent with such an adduct.

The dinitrile, 1,4-dicyanobutane, was considered a potential candidate for intramolecular cyclization. We were, however, unable to show that indeed cyclization had occurred. Specifically, the major fragmentations that occur upon CA are consistent only with a linear anion.

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GAS-PHASE REACTIONS OF FIRST-ROW TRANSITION METAL IONS (Sc^+ - Zn^+) WITH PHENYL HALIDES.

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INTRODUCTION

The reactions of Fe^+ with phenyl halides in the gas phase have been characterized (1-3) and were found to be of particular interest for two reasons. First, the iron-ligand bond energies, $D^0(\text{Fe}^+-\text{C}_6\text{H}_4)$ and $D^0(\text{Fe}^+-\text{C}_6\text{H}_5)$ could be estimated. Second, the initial steps in the metal catalyzed polymerization to produce polyphenylenes could be observed when oligomers of the form $(\text{C}_6\text{H}_4)_n^+$ were produced with $n=1-5$ from $\text{Fe}(\text{C}_6\text{H}_4)_n^+$, which in turn were produced in successive steps of dehydrohalogenations.

The reactions of the other first row transition metal ions (Sc^+ - Zn^+) have now been studied and considerable variations have been observed in the behavior of the different metals.

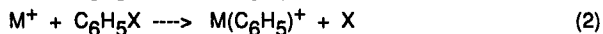
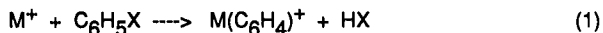
EXPERIMENTAL

The experiments were carried out, by using a Nicolet FTMS-2000 Fourier transform mass spectrometer. Metal ions were made by focusing a laser pulse onto a target of the pure metal, placed on the direct insertion probe of the mass spectrometer. Following isolation of the metal ion, using double ejection pulses, it was allowed to react with the sample molecule, which was incorporated through the gas inlet system and maintained at a static pressure of about $2 \cdot 10^{-7}$ torr. Reaction time was varied to elucidate reaction paths, and double resonance techniques were used for their verification. Nitrogen or Krypton was used in several cases as a buffer gas and maintained at pressures ten times that of the sample gas.

RESULTS

Sc^+ , Ti^+ , V^+ , Co^+ , and Ni^+ were found to form $\text{M}(\text{C}_6\text{H}_4)_n^+$ in dehydrohalogenation reactions with the phenyl halides, each to a different extent (up to $n=6$), depending on the metal and the halide. This behavior puts them in a group with Fe^+ , but unlike iron, was the absence of oligomer ion formation. Cr^+ , Mn^+ , and Cu^+ were relatively unreactive towards the phenyl halides and at best formed metal/molecular ion cluster ions or formed C_6H_5^+ and MX . Zn^+ , due to its high ionization potential only participated in charge transfer reactions with the phenyl halides.

Initially, two reaction channels are available:



The second reaction channel is never observed in the case of fluoro-, chloro-, or bromobenzene. Variations are observed in the case of iodobenzene. Sc^+ and Ti^+ only react with iodobenzene according to reaction 1, whereas Fe^+ and Co^+ only react via channel 2. V^+ and Ni^+ react through both channels.

The reactions of Fe^+ , Ti^+ , and Co^+ with naphthalene halides have also been studied. Dehydrohalogenation is also observed in those reactions and metal-catalyzed oligomer formation as well. Reactions of Fe^+ , for example gave products of the form $Fe(C_{10}H_6)_n^+$ with $n=1-6$, and the smaller of these were observed to lose the iron atom producing oligomers ions of the form $(C_{10}H_6)_n^+$ with $n=2$ and 3.

These studies were performed with instrument access provided through the courtesy of Nicolet Analytical Instruments, which is gratefully acknowledged. Studies are continuing with a larger selection of metal ions and a wider variety of polymerization processes.

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**ELECTROSPRAY IONIZATION MASS SPECTROMETRY AND TANDEM MASS
SPECTROMETRY OF SMALL OLIGONUCLEOTIDES**

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We have carried out a preliminary evaluation of electrospray ionization (ESI) mass spectrometry for the analysis of small oligonucleotides. In addition to the qualitative and quantitative potential suggested by the success of ESI of peptides and proteins, ESI tandem mass spectrometry may also be amenable to the sequence determination of oligonucleotides. Sequence analysis of large oligonucleotides (n 10 to 10^3) is usually performed by chemical degradation according to Maxam and Gilbert¹ or by the chain termination technique of Sanger *et al.*². Smaller oligonucleotides have been analyzed by mass spectrometry with advantages in speed and simplicity of analysis³. Tandem mass spectrometry has been effectively employed for the singly charged molecular anions of oligonucleotides to a polymerization level of $n = 6^4$. Fenn and co-workers have reported the ESI of nucleotide mono-, di- and triphosphates⁵. A ESI mass spectrum of a deoxynucleotide 14-mer has also been recently reported⁶.

Mass spectra were acquired on a tandem quadrupole mass spectrometer with a sheath flow ion source which has been described elsewhere⁷. Samples prepared in deionized water at a concentration of 1-2 $\mu\text{g}/\mu\text{l}$ were introduced by continuous infusion at 0.25 $\mu\text{l}/\text{min}$. These were obtained from Sigma Chemical Co., St. Louis, MO, Pharmacia LKB Biotechnology Inc., Piscataway NJ and colleagues acknowledge below. Concentrated biochemical buffers required ethanol precipitation of the sample prior to analysis. Daughter ion experiments were performed with Ar collision gas at a thickness of 100×10^{12} atoms/ cm^2 . Lab frame collision energies were ca $100 \cdot q$ eV (where q = number of charges). Calculations of M_r were performed on peak maxima (at unit mass precision) utilizing a computer program which accepts a group of m/z values from a multiply charged distribution and combines these to calculate charge state and M_r from the maximum number of pairs for best precision.

We have examined the ESI mass spectra of a number of synthetic and biological oligodeoxyribo- and oligoribonucleotides ($n = 2-76$). We have observed the efficient collisionally induced dissociation (CID) of multiply charged molecular anions of small ($n = 2-8$) oligonucleotides. These show differences in lab frame energies required for efficient CID and minor differences in preferred modes of fragmentation.

The ESI behavior of larger oligodeoxynucleotides is illustrated in Figure 1 for the synthetic 21-mer d(AAATTGTGCACATCCTGCAGC). These spectra illustrate the increasing contribution of sodium salts to the backbone phosphate with increasing level of polymerization. Up to this level we observe that the lowest m/z member of the peak cluster for a given charge state is invariably the free acid. The ESI mass spectrum of the largest oligonucleotide so far examined, the phenylalanine isoacceptor transfer ribonucleic acid isolated from Brewers yeast (76 nucleotide residues, M_r 24,952) show three overlapping distributions of multiple charged molecular ions corresponding to the intact tRNA and apparent degradations of the native structure.

The ESI mass spectra of oligonucleotides ($n = 2-76$) show abundant multiply charged molecular anions which significantly extend the range of application of a quadrupole mass spectrometer of limited m/z range and allow the precise determination of molecular weight. These multiply charged ions are efficiently dissociated by collision with a neutral gas and afford qualitatively informative daughter fragments. These results highlight significant new capabilities for the mass spectrometric analysis of oligonucleotides. Further studies of the ESI of oligonucleotides and the CID of the multiply charged molecular ions produced by CE/ESI MS are proceeding in our laboratory.

Acknowledgment:

We acknowledge Dr. T. Keough and colleagues at the Proctor and Gamble Company, Cincinnati, OH and Dr. L.K. Fritz, Division of Biology and Chemistry, Pacific Northwest Laboratory for kindly providing samples for analysis and helpful discussions. We thank the U.S. Department of Energy, Office of Health and Environmental Research, for support of this research under Contract DE-AC06-76RLO 1830. *Pacific Northwest Laboratory is operated by Battelle Memorial Institute.

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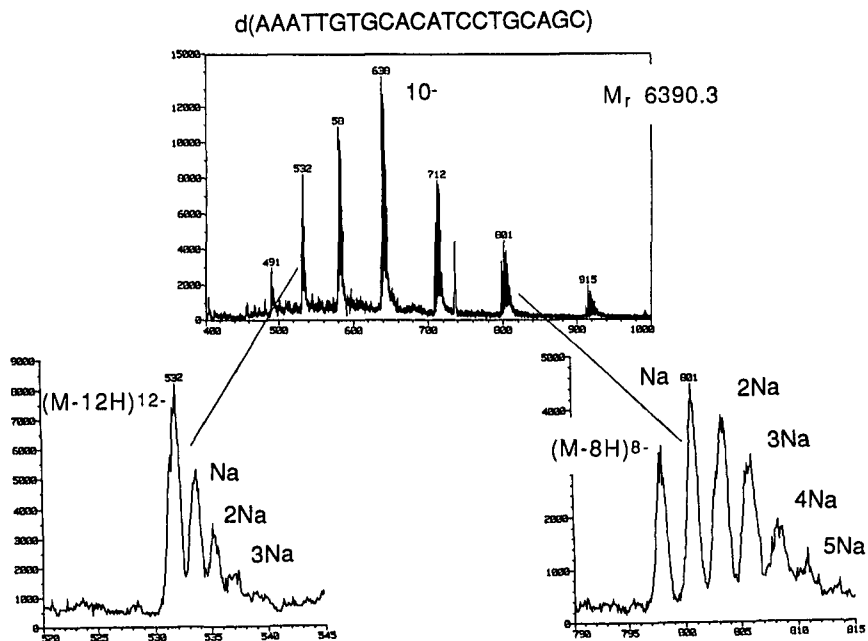


Figure 1. ESI mass spectrum of the synthetic oligodeoxynucleotide, d(AAATTGTGCACATCCTGCAGC), M_r 6390.3. Inserts below show the molecular anion and the accompanying sodium salt ions for the $(M-12H)^{12-}$, m/z 532 and $(M-8H)^{8-}$, m/z 801.

FAB TANDEM MS OF PROTEASE AUTOLYSIS PRODUCTS

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Despite dramatic increases in the mass range of FAB mass spectrometry, most proteins are still large and must be cleaved into smaller peptides for analysis. The cleanest most reproducible way to effect selective cleavage is to hydrolyze specific amide bonds with suitable enzymes. Trypsin, chymotrypsin, and *Staphylococcus aureus* V8 protease are the most widely used proteases for this purpose. Their molecular weights range from 23,900 to 27,000 Daltons. Generally, the protein to enzyme ratios used are between 10/1 and 200/1 (wt/wt). Unfortunately, proteases also catalyze their own hydrolysis (autolysis). The peptides produced by protease autolysis are presumably mixed in with the peptides from target proteins and thus are part of the background in HPLC analyses and in FAB mass spectra of digested mixtures. We are identifying the major peptides produced by autolysis and attempting to ascertain the extent of their background participation.

Trypsin [EC 3.4.21.4] is the most frequently used protease. It catalyzes hydrolysis of amide bonds whose carbonyls come from either arginine (R) or lysine (K). Trypsin's sequence (1) of 223 amino acids includes two arginines and 14 lysines making it a substrate for itself. When trypsin [bovine pancreas, TPCK treated, Sigma] is dissolved in 0.1 M NH_4HCO_3 /0.1 mM CaCl_2 (1 mg/mL), it completely autolyzes in two hours at room temperature (See Figure 1.) When the lyophilized reaction mixture is subjected to FAB mass spectrometry, ions accounting for most (79%) of its 223 amino acids are observed (See Figure 2) especially if the mixture is partially separated by HPLC before FAB/MS (See Figure 3.) Ions at 659 (See Figure 4.), 805, and 1154 have been sequenced using MS/MS confirming the assignments based on mapping.

In our hands, calcium chloride stabilizes an ice cold aqueous solution of trypsin. However, trypsin dissolved in 0.1 M NH_4HCO_3 alone, completely autolyzes within two hours at room temperature giving the same HPLC trace and the same mass spectrum as it does in the presence of calcium chloride.

All of the mass spectra were obtained on a JEOL HX110/110 (with a JEOL DA5000 data system) at the full accelerating voltage of 10 keV. The FAB gun was operated at 6 keV with xenon as the FAB gas. Helium was the collision gas in the MS/MS experiments.

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Figure 1. Trypsin Autolysis - HPLC (Aquapore RP-300)

A = 0.1 % TFA/water, B = 0.1 % TFA/acetonitrile

Gradient = 10-60 % B 20 minutes

- a) 2 hours, 0.1 M NH_4HCO_3 + 0.1 mM CaCl_2 , pH 8,
room temperature, 1 mg trypsin/mL
b) 1 minute
c) baseline

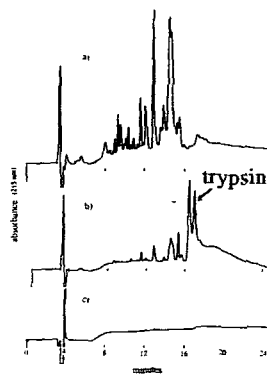
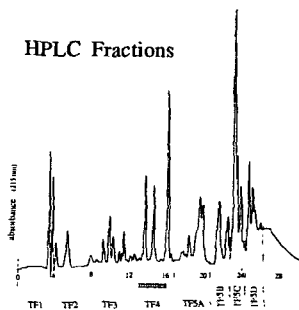


Figure 3. Trypsin Autolysis

HPLC Fractions



(step gradient 10-60 % B)

Figure 4. MS/MS of $[M+H]^+ = 659$ m/z

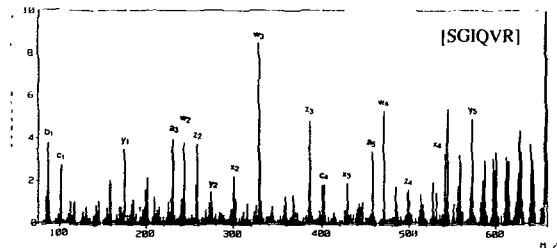
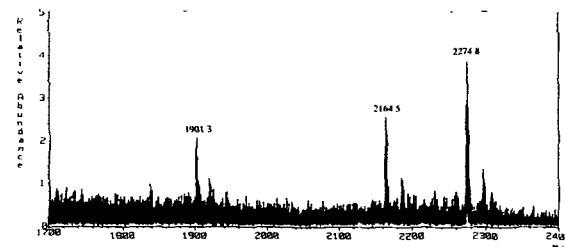
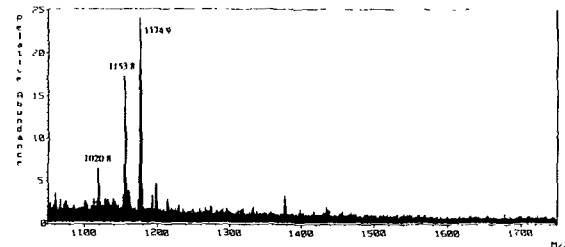
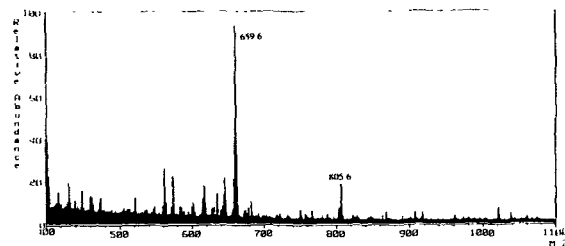


Figure 2. FAB/MS of Trypsin Autolysis Mixture
[scan 0-4500 amu, thioglycerol, 2hour]



SEQUENTIAL MASS SPECTROMETRY ON A HYBRID TANDEM INSTRUMENT OF BEqQ CONFIGURATION

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The use of tandem mass spectrometry together with fast atom bombardment has become increasingly important in the structure elucidation of peptides. A standard technique employed on a double focusing sector/quadrupole instrument is to select a specific parent ion beam with the magnetic and electric sectors and subsequently use the quadrupole to obtain a spectrum of daughter ions derived from the chosen parent. Analyses of these type yield improved information which might be obscured by high background components in a conventional spectrum.

In principle, further improvements in structural elucidation may be achieved by incorporation of additional stages of mass spectrometry to give sequential MS.^{1,2} Modifications to our instrument (a VG ZAB SEQ) have enabled us to implement this technique in our laboratory. The ESA voltage depends on the value of its 0-10V program. Under normal operating circumstances this is linked to the 0-10V accelerating voltage program. The accelerating voltage and ESA voltage therefore usually maintain a fixed ratio. We have incorporated the option of uncoupling this link and operating the ESA supply via an independent 0-10V supply derived from a 5k ohm resistor in series with a 10-turn 10k potentiometer connected between a 0V and 15V rail. In the sequential daughter ion experiment, the initial parent ion beam is selected by the magnetic sector and subjected to collisional activation in the cell between B and E.. A specific daughter ion fragment is then transmitted through the electric sector and collisionally activated in the rf-only quadrupole. The quadrupole mass filter is scanned to obtain a spectrum of "granddaughter" ions produced by fragmentation of the transmitted daughter.

As an example of this sequential MS approach, we have determined the origin of the "internal" fragment at m/z 205 (assigned as $(B_4Y'_3)_2$) in the daughter ion spectrum derived from leucine-enkephalin MH^+ ions^{3,4} (Figure 1.). It was not possible to predict from the daughter ion spectrum whether the ion was formed via an initial B_4 fragmentation (m/z 425) or a Y'_3 fragmentation (m/z 336). However, this ambiguity was resolved by setting the magnetic sector to transmit the parent ion beam at m/z 556 and using the electric sector to select either the m/z 425 (Figure 2, upper spectrum) or m/z 336 (Figure 2, lower spectrum) daughter ion beam prior to scanning the quadrupole filter over a range to include m/z 205. It was clearly seen that the major origin of m/z 205 ions was the Y'_3 fragment at m/z 336. It can also be seen from Figure 2 that the internal fragment $(A_4Y'_3)_2$ at m/z 177 also arises via the initial Y'_3 fragment at m/z 336.

Daughter ion spectra of metal-cationised peptides have been shown to provide structural information which complements that provided by CAD of protonated species.^{5,6} The conventional FAB spectrum of the europium adduct of leucine-enkephalin, formed by the addition of $Eu(NO_3)_3$ to the liquid matrix, includes doublets at m/z 705 and m/z 707 indicating the incorporation of both ^{151}Eu and ^{153}Eu .

Tandem MS and sequential MS analyses were performed on each isotopic variant and the complementary daughter ion spectra and sequential MS data from $[M-H+^{151}Eu]^+$ and $[M-H+^{153}Eu]^+$ were recorded. Both C- and N-terminal fragments were observed, each retaining the europium. The complementary MS/MS and MS/MS/MS data suggest a multiplicity of sites of interaction between peptide and metal cation.

This work was supported in part by NIH (AI 26916).

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FIGURE 1

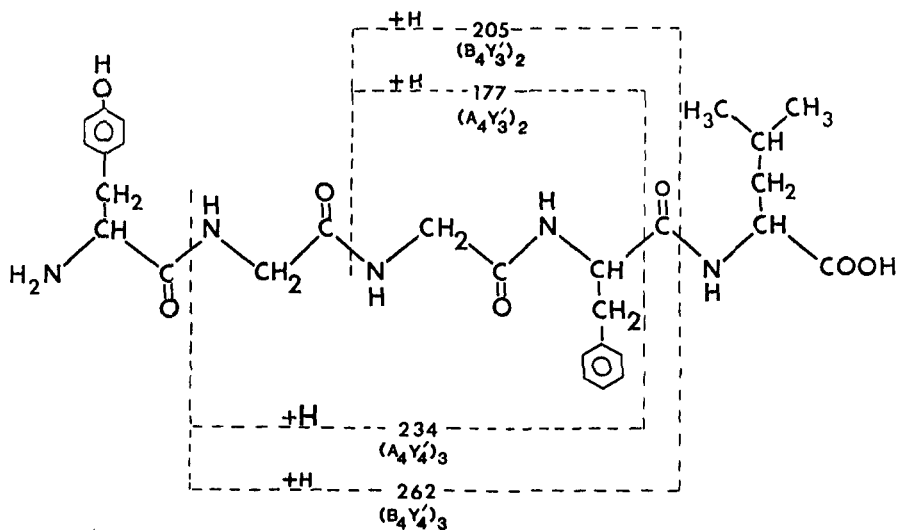
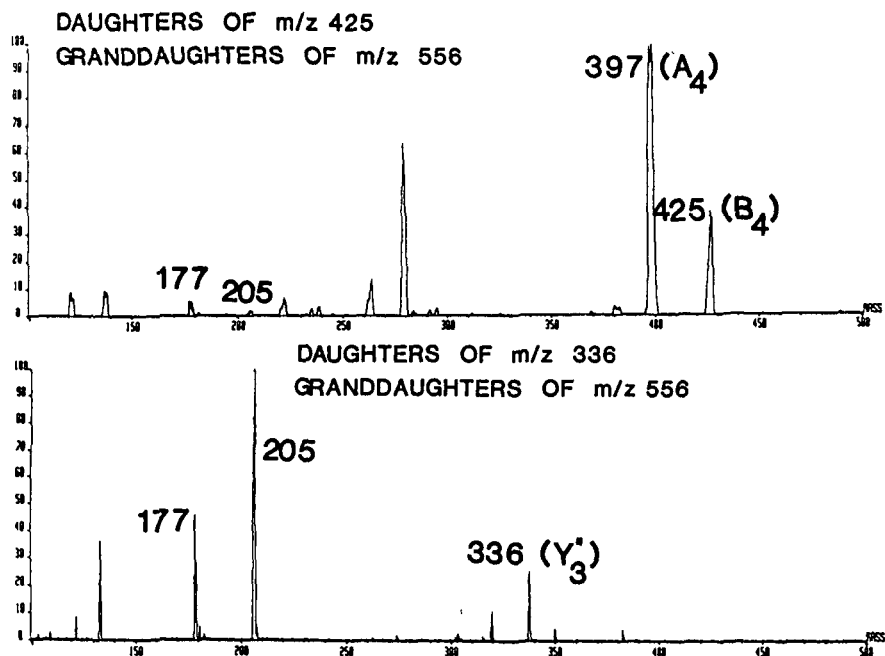


FIGURE 2



APPLICATIONS OF STABLE ISOTOPES AND TANDEM MASS SPECTROMETRY TO THE
CHARACTERIZATION OF REACTIVE METABOLITE-PEPTIDE INTERACTIONS.

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Covalent modification of protein or peptide amino-acid residues by electrophilic species, generated metabolically from drugs or other xenobiotics, may represent an initial event in the expression of chemical-induced toxicity. Understanding the nature of the chemical events and the target amino-acid residues involved, allows the sites of modification in toxicologically important target proteins or peptides to be predicted.

A major metabolite of the investigational anti-tumour agent, N-methylformamide (NMF) in rodents is S-(N-methylcarbamoyl)glutathione (SMG) which possesses similar structural features to reactive glutathione and cysteine conjugates of allyl- and benzyl isothiocyanate¹. Consequently, SMG may be a reactive metabolite and display the potential to covalently modify free thiol functionalities in peptides.

The objective of the present investigation was to evaluate the reactivity of two cysteine analogs of SMG *viz* S-(N-methylcarbamoyl)cysteine (SMC) and S-(N-trideutero-methylcarbamoyl)cysteine (D₃-SMC) towards oxytocin, a model thiol-containing peptide. The structures of the oxytocin adducts were established by fast-atom bombardment tandem mass spectrometry (FAB/MS-MS).

Experimental: SMC or trideuteromethyl-SMC (10 equiv.) were incubated with reduced² or oxidized oxytocin, in aqueous triethylamine-formate buffer (pH 7.7) for 6 hr, and the lyophilized incubations were analyzed directly by FAB / MS. Collision-induced dissociation (CID) daughter ion spectra were recorded on a VG 70 SEQ hybrid tandem instrument of EBQ geometry, equipped with an Ion-Tech fast atom gun, and a VG11/250 data system. CID was performed in the first (rf-only) quadrupole employing argon as a collision gas (3.5×10^{-6} Torr) and collision energies of 35 eV. Daughter ion spectra were recorded via the data system in the multi-channel-analysis mode.

Results and Discussion: The CID daughter ion spectra of reduced oxytocin, was characterized by a structurally informative (Y_n) and (B_n) series ions, with an intense B₆ ion (m/z 725) diagnostic for the reduced Cys-1 and Cys-6 residues. The assignment of sequence ions was confirmed by shifts in m/z of (B_n) series ions upon acetylation or by comparison with oxidized oxytocin. Upon reaction of SMC with reduced oxytocin, two adducted peptides were recognized by FAB/MS consistent with the formation of mono- (MH⁺ = 1066) and bis-N-methylcarbamoylated (MH⁺ = 1123) peptides. In incubations of reduced oxytocin with D₃-SMC a shift in m/z values to 1069 and 1129, for the mono- and bis-carbamoylated species respectively was observed. A CID spectra of MH⁺ = m/z 1066 revealed the presence of two mono-adducted peptides which arose from carbamoylation of either Cys-6 or Cys-1 in a ratio of 5:1; an isotopically induced shift in mass was observed for the Y₄⁺ and B₆⁺ ions when D₃-SMC was used in place of SMC.

A daughter ion spectra of the bis-adduct obtained by CID on MH⁺ = 1129 (figure 1) revealed a series of sequence ions diagnostic of modification of oxytocin at Cys-1 and Cys-6. No carbamoylation of the terminal glycinamide was observed based upon a shift in B₆⁺ ion at m/z 725 to 845, and the presence of the unchanged Y₃⁺ ion at m/z 285. In addition, the Y₄⁺ ion at m/z 448, when related to the unaltered Y₃⁺ ion (m/z 285) reports specifically upon modification of Cys-6; in each case an appropriate isotopically induced shift of 3 or 6u was observed. Reaction of oxidized oxytocin with SMC resulted in the appearance of mono- and bis-adducted species of identical mass to those observed for reduced oxytocin. The CID daughter ion spectra of these adducts compared closely with the spectra obtained upon carbamoylation of reduced oxytocin by SMC.

In conclusion, the results indicate that SMC is a reactive S-linked conjugate which may covalently modify oxytocin by specific carbamoylation of cysteinyl thiols. In addition we have demonstrated the value of MS/MS techniques and stable isotope methodology to study the interactions of a reactive metabolite with a model peptide.

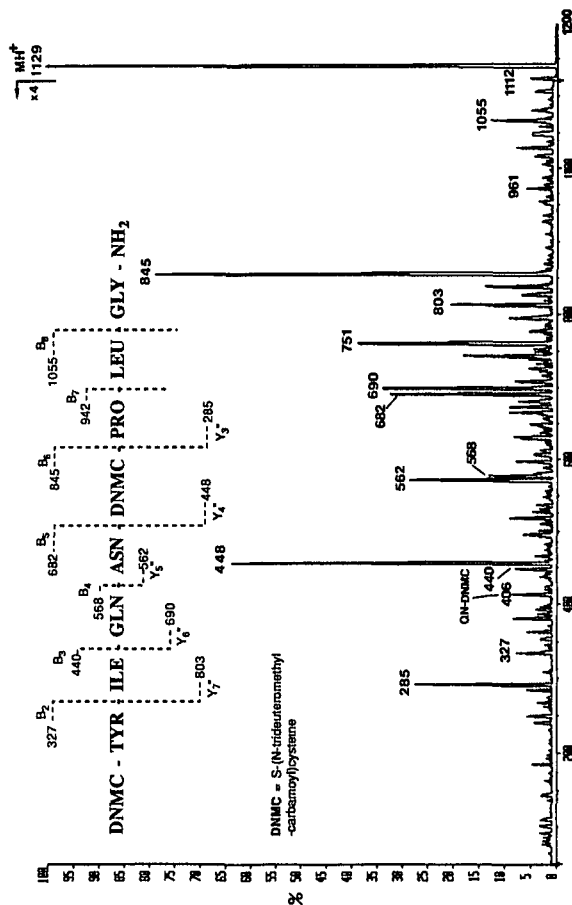


Figure 1. Daughter ion spectra of bis-S-(N-trideutero-methylcarbamoyl)oxytocin obtained by CID on MH⁺ = 1129. The proposed structure of the adduct and the origin of the daughter ions, according to Roepstorff and Fohlman³ nomenclature, is as indicated.

This work was supported by grants from NATO and NIH (DK30699).

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ALTERNATE MASS SPECTROMETRIC METHODS FOR PEPTIDE SEQUENCE DETERMINATION

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Introduction In recent years the utility of tandem mass spectrometric methods for the determination of peptide sequence has been clearly demonstrated. In our laboratory we have had to sequence naturally occurring peptidic antibiotics that did not yield useful structural information using our MS/MS instruments (a VG-ZAB-HF and Finnigan TSQ 4600). Fortunately, there are other mass spectrometric techniques that aid in sequence determination. These techniques are:

1) Amino acid analysis by DCI MS/MS of an acid hydrolysate, 2) Amino acid analysis and chirality determination by GC-MS, 3) Peptide mapping of mild acid hydrolysates by FAB, 4) Identification of Edman degradation products by FAB and CI MS and MS/MS.

Amino acid analysis by DCI allows rapid identification of residues obtained by total acid hydrolysis. Daughter ion spectra are obtained from each of the molecular ions and compared to the library spectra for positive identification of the amino acids.

Since peptides produced by micro-organisms may contain amino acids in both the D and L form, GC-MS is useful for determining not only the identity of amino acids, but also their chiralities.

Knowledge of amino acid content greatly aids in interpreting FAB mass spectra obtained from a mild acid hydrolysis of a peptide. The overlapping peptide fragments obtained make it possible to determine large portions of the original sequence.

The products from an Edman degradation of the peptide can be positively identified by examination of the cleaved PTH-amino acid by CI MS/MS and by analyzing the residual peptide by FAB-MS to determine mass loss.

Amino Acid Chirality Determination by GC-MS

Leu-Leu-B-OH-Phe-B-OH-Leu-Leu-Arg-Ser-Thr-Gly-B-OH-Asn-Ser

LYSOBACTIN

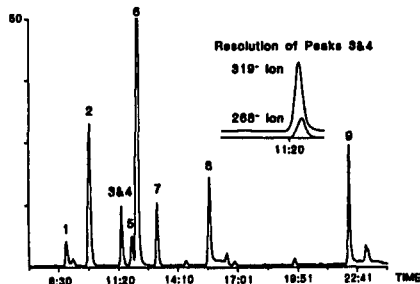
Hydrolysis Conditions

1) 5N NH₄OH 37°C 10 min.

2) 5N HCl 110°C 24 hrs.

Amino Acid Derivative
N,O trifluoroacetyl-isopropyl ester

GC-MS TOTAL ION CHROMATOGRAM NEGATIVE CHEMICAL IONIZATION



EXPERIMENTAL

FAB: VG ZAB-HF

Matrix: dithiothreitol/dithioerythritol

HIGH RESOLUTION: VG ZAB-HF

Peak matching: Polyethylene glycols (1:8000)

MS/MS: Finnigan TSQ-4600

CAD: 30 eV with argon

GC-MS: Finnigan TSQ-4600 Negative chemical ionization

Reagent Gases: CH₄/N₂O

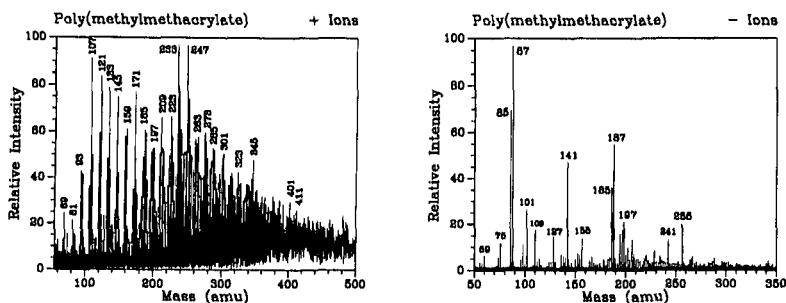
GC Carrier Gas: Helium

Column: Chirasil VAL III (Alltech Associates) 25m X 0.31mm i.d. Temperature program: 60°C for 5 minutes then 4°C/min final temperature 200°C for 10 min

Peak	Retention Time (min)	Amino Acid	Chirality
1	8:52	Gly	-
2	9:59	B-OH-Leu	L-threo
3	11:30	Ser	L
4	11:32	Ile	L
5	12:00	Leu	D
6	12:15	Thr	L-allo
7	13:11	Leu	L
8	15:40	B-OH-Asp	L-threo
9	22:16	B-OH-Phe	L-threo

lation site and attach to nascent neutrals. In the TOF experiment, the strong electric extraction field clears all charged particles as they evolve, so no such attachment can take place. The phenomenon in the FTMS experiment is similar to *chemical ionization*. 3) Most spectra show at least one of three distributions of C clusters. Polyimide is the most general case, in which low (C_{9-25}), intermediate ($C_{20-40}H_{1-12}$) and high mass (C_{2n} , $25 < n < 225$) clusters are observed. The intermediate range is the most common. 4) Ion series consistent with the general formula $+HA_mB_n$ appear. That is, stable subunits which may or may not be present in the original polymer seem to condense to form series of (protonated) ions. In some cases, "B" appears to be bare carbon. 5) Spectra often evolve as a function of laser fluence and number of laser shots to the same spot. This observation is important for laser modification of polymers as well as for analytical purposes. The positive ion spectrum of poly(methylmethacrylate) (PMMA) presented here shows both protonated rearrangement products and intermediate mass C clusters.

Negative ion spectra: These spectra often exhibit far more structural ions than corresponding positive ion spectra. This is particularly true for poly(phenylene sulfide), poly(vinyl acetate), and is illustrated for PMMA.



In closing, we cite the following observations. All polymers studied by us to date can be easily distinguished based on their FTMS-LAMMS spectra, which are radically different from literature TOF spectra. From these differences and a consideration of the experimental arrangement, we can propose a number of mechanisms to explain observed the observed ions. These proposals await confirmation by high mass resolution studies which are in progress.

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SELECTIVE IDENTIFICATION OF DISULFIDE-CONTAINING PEPTIDES BY ELECTROCHEMICAL HPLC AND FAB/MS

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Peptides containing disulfide bonds are important constituents of the extracts of many organs, and are often present in digests of many enzymes. Because of their importance, it is desirable to have rapid and definitive methods for detecting and identifying disulfide-containing peptides. A general procedure for identifying peptides containing disulfide bonds in complex mixtures involves fractionation of the mixture by HPLC and analysis of all chromatographic fractions, before and after reduction of disulfide bonds, by FAB/MS. This is not an efficient approach when only a few of the fractions contain peptides with disulfide bonds. We have extended this general method by using a dual electrode electrochemical detector to denote those fractions which contain peptides with disulfide bonds. As a result, only the fractions of interest are analyzed by FAB/MS.

Disulfide-containing peptides can be detected by using two Hg/Au electrodes in series such that the first one (upstream) is used as a generator to reduce the disulfide bond, and the second one (downstream) is used to detect the thiol produced by the upstream electrode. Chromatograms of the bovine pituitary extract recorded with tandem UV and electrochemical detectors are shown in Fig. 1. Chromatogram A was obtained by monitoring the UV absorbance at 230 nm. Chromatograms B and C were obtained with the EC detector in the two- and one- electrode modes, respectively. Since peaks 2 and 3 are not present when only one electrode is used, these peptides most likely contain disulfide bonds. Peak 1 elutes in the void volume.

FAB mass spectra of peak 3 from the chromatograms of the pituitary extract, taken before and after treatment with performic acid, are shown in Fig. 2. The spectrum in Fig. 2a has a strong peak corresponding to a protonated molecular ion at m/z 1007. The small peak at m/z 1115 is a 1-thioglycerol adduct of the molecular ion at m/z 1007. Upon treatment of this fraction with performic acid, a strong single protonated molecular ion m/z 1105 was found. This shift of 98 m/z is consistent with oxidation of the disulfide bond to two cysteic acid moieties. It was concluded that the substance comprising peak 3 is oxytocin, and peak 2 is [Arg⁸]-vasopressin. This assignment was confirmed by comparing their chromatographic retention times and FAB mass spectra to those reference samples of oxytocin and [Arg⁸]-vasopressin.

Chromatograms of the catfish pancreas extract recorded with tandem UV and EC detectors shown that it has many disulfide-containing peptides (Data are not shown.). FAB mass spectra of fraction 10 and 11 have no peaks from 2000 m/z to 3200 m/z . Upon reduction, fraction 10 gave one pair of peaks (A), which is insulin 1 chain A (m/z 2390), chain B (m/z 3119), whereas fraction 11 gave another pair of peaks (B), which is insulin 2 chain A (m/z 2381), chain B (m/z 3175). It was concluded that the substance in fraction 10 is catfish insulin 1 with average mass (MH^+) 5502, and fraction 11 is catfish insulin 2 with average mass (MH^+) 5549. These assignments were confirmed by amino acid sequence analysis, and performic acid oxidation followed by FAB/MS analysis.

In summary, the combination of RPHPLC/electrochemical detection and FAB/MS analysis may be used to identify cystine-containing peptides. This is attractive because it is rapid, specific for disulfide-containing peptides, and applicable to a variety of complex biological mixtures.

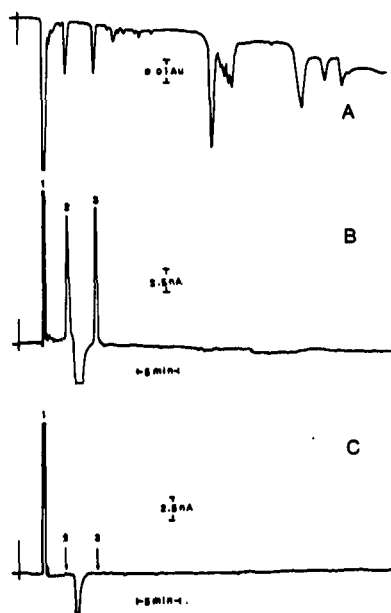


Fig. 1 Chromatograms of bovine pituitary extract recorded with tandem UV and EC detectors. (A) UV detection. (B) and (C) EC detections in the two- and one- electrode modes.

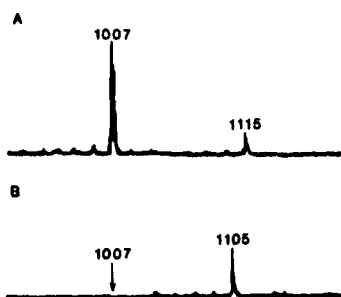


Fig. 2 FAB mass spectra of peak 3 from the chromatograms of the pituitary extract. (A) before oxidation. (B) after oxidation.

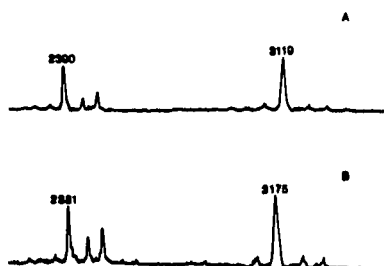


Fig. 3 FAB mass spectra of fraction 10 and 11 from the chromatograms of the catfish pancreas extract after reduction. (A) insulin 1. (B) insulin 2

Amino-Terminal Derivatization of Peptides Yields Improved CAD Spectra

John T. Stults, Roger Halualani, and Ronald Wetzel

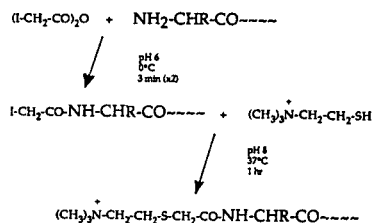
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Mass spectrometry is now an integral tool in the protein sequencing laboratory. Peptide sequencing by tandem mass spectrometry (MS/MS) in particular is proving extremely useful for peptide sequencing. The complexity of the ion fragmentations, however, often makes data interpretation difficult and time consuming. Sophisticated computer programs to aid interpretation are generally required to determine the correct sequence in a timely manner. An alternative approach we have pursued is to modify the peptide to produce simplified, predictable fragmentation.

Attachment of a fixed positive charge to the peptide amino-terminus, to favor production of N-terminal fragment ions, is especially attractive for simplifying the spectrum [1]. The reaction described here consists of two steps (see Scheme 1) [2]. In the first step, 1-30 nmol of peptide were dissolved in 200 μ l of 0.01M MES (2-[N-morpholino]ethanesulfonic acid), pH 6, and cooled to 0°C in a 1.5 ml Eppendorf tube. To this solution was added 2 μ l of 300 mM iodoacetic anhydride (Aldrich) in dry THF. The sample was vortexed. A second aliquot of the anhydride was added after 3 min. To add the quaternary ammonium group in the second step, the pH of the solution was raised to pH 8 with 20 μ l of 0.5 M N-ethylmorpholine. Thiocholine iodide (Pfaltz and Bauer) was added and the sample incubated at 37°C for 2 hrs. The reaction was terminated by drying, *in vacuo*, or by injection of the solution into an HPLC system. The use of nonvolatile buffer and excess reagent necessitates sample cleanup after the reactions. However, if the reaction is carried out immediately after enzymatic digestion, the cleanup and separation of the proteolytic fragments are accomplished simultaneously.

The first step selectively modifies the N-terminus of an unblocked peptide by exploiting the differences between the pKa values of its α -amino group (pKa~8) and the side chain amino groups of Lys (~10.5) and Arg (~12). Although different N-terminal amino acids affect reactivity and degree of specificity, the responses of twenty model hexapeptides (X-GAKQA, X = each of the 20 common amino acids) are sufficiently similar that conditions were found which should modify any peptide containing 0-1 Lys residues with at least 80% selectivity for the N-terminus, with essentially complete conversion of starting peptide with no detectable side reactions. Because the iodoacetyl functionality readily reacts with free sulfhydryl groups, free cysteines must be protected prior to reaction. Conventional reduction and alkylation of the peptides is sufficient. The use of a two-step reaction allows the quaternary ammonium compound to be changed without altering the specificity of the α -amino attachment. For example, use of a compound with a longer hydrocarbon chain should increase the hydrophobicity of the peptide to make its desorption from a liquid matrix more favorable.

The CAD spectra were obtained with a JEOL HX110HF/HX110HF tandem double focusing mass spectrometer, operating with a JEOL DA-5000 data system. Ionization by FAB with 6 keV xenon atoms or LSIMS using 15 keV Cs⁺ ions produced positive ions that were analyzed at 10 keV accelerating voltage, 3000 resolution. Nitrobenzyl alcohol was preferred as the matrix because degradation products were observed in glycerol, thioglycerol, and DTT:DTE (5:1). The



SCHEME 1.

^{12}C -isotope peak of the precursor M^+ ions was selected at unit resolution with MS-1, the ions passed into the collision cell containing a pressure of helium sufficient to attenuate the parent ion beam to 25% of its original intensity, and the fragmentation resulting from collision-induced dissociation was measured with MS-2.

The CAD spectra of the N-terminally derivatized peptides (Fig. 1 and 2) show only fragment ions derived from the amino-terminus (a_n , c_n , d_n), the a_n and d_n ions predominating. The strong $\text{M}^+-\text{N}(\text{CH}_3)_3$ peak, and several a_n -trimethylamine ions, are likely formed by gas-phase nucleophilic attack of the sulfur on the amino carbon to form a cyclic sulfonium ion with loss of trimethylamine.

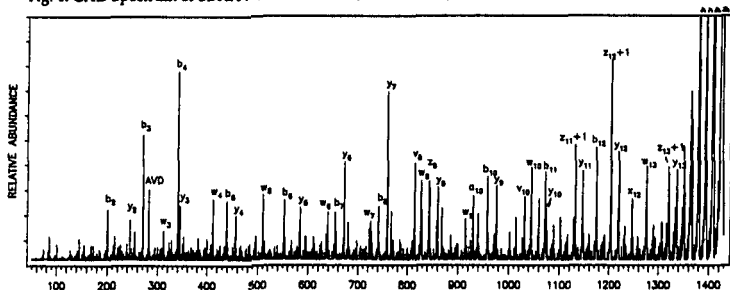
The utility of the derivative may be summarized as follows:

1. Simplified interpretation -- fewer fragments, all derived from the N-terminus, are produced.
2. Enhanced signal -- fewer fragment ion types mean ion intensity is spread among fewer channels.
3. Lower resolving power required for daughter ions -- sequence ions solely from the N-terminus produce well separated peaks.
4. Distinction of Ile and Leu -- abundant d_n ions are produced that differentiate these residues [3].

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3. R.S. Johnson, S.A. Martin, K. Biemann, Int. J. Mass Spectrom. Ion Processes, **86**, 137-154 (1988).

Fig. 1. CAD Spectrum of SDAAVDTSEITTK ($\text{MH}^+=1424.7$)



INVESTIGATION OF THE FRAGMENTATION OF PEPTIDES AS A FUNCTION OF COLLISION
ENERGY IN A FOUR-SECTOR TANDEM MASS SPECTROMETER.

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Over the course of the last few years we have investigated the type and extent of fragmentation of peptides undergoing high energy collisions (10000 eV). These studies lead to the characterization of three fragmentation series; d_n , v_n and w_n , which are apparently not observed in low energy collision spectra of peptides acquired with triple quadrupole mass spectrometers (1). Since these ion series are important in determining the covalent structure of peptides, we have investigated the collision energy (CE) regime in which the relative abundance of these fragment ions change. In the process of defining these transitions we have evaluated the overall information content of the MS/MS spectra of peptides as a function of CE. This information is important not only for understanding the mechanisms of fragmentation but also for determining the CE to be used with focal plane detectors which can simultaneously integrate 20-30% of the mass range. In these experiments very low collision energies are required in order to transmit the product ions, which vary in both mass and energy through the electric sector which typically has an energy bandpass of approximately four percent.

All experiments were performed on a JEOL HX110/HX110 tandem mass spectrometer in which the collision energy (E_{coll}) is continuously variable from 10000 to 150 eV. The initial energy of the precursor and final energy of the product ions is 10000 eV (V_{acc}). The resolution of MS-1 was sufficient to transmit only the $^{12}C(M+H)^+$ ion into the collision region. The peptides chosen for this study, renin substrate (1757.9), LHRH (1181.6) and β -lipotropin (950.5), represent all the major fragmentation processes observed at high collision energies: a_n , b_n , d_n , v_n , w_n , x_n and y_n . The product ion spectra were recorded at more than ten CE values ranging from 10000 to 130 eV employing both helium and xenon as the collision gas. The product ion spectra for all the peptides examined with helium gas were very similar for CE= 10000 to 2000 eV at which point the relative abundance of the d_n , v_n , and w_n ions began to decrease and were absent below 500 eV. When helium was replaced with xenon the same trend was observed, however, the relative abundance of these ion series did not begin to decrease until CE= 500 eV and were almost completely absent in the range of 150 to 200 eV. The distribution of fragment ions as a function of CE with xenon gas is shown in Figure 1 for renin substrate at E_{coll} = 750 and 130 eV, respectively. At E_{coll} = 750 eV the relative abundance of product ions is almost identical to that at E_{coll} = 10000 eV with very abundant a_n and d_n ions throughout the MS/MS spectrum. This is in contrast to the MS/MS spectrum of the same sample at E_{coll} = 130 eV (Figure 1). At this CE all the d_n ions are absent and several ions which were not observed at the higher collision energies, i.e. b_n-NH_3 , b_6 , are very abundant. This transition from one type of ion series to another as a function of CE is illustrated in Figure 2 for three product ions of renin substrate. This plot indicates that the transition occurs around E_{coll} = 400 eV, therefore, to obtain collision spectra which retain the characteristic side chain losses associated with high energy collisions an E_{coll} > 400 eV is required with xenon as the collision gas. These studies indicate that it is feasible to acquire collision spectra with E_{coll} from 10000 to 130 eV in a tandem magnetic sector mass spectrometer and that the transition from high to low energy type MS/MS spectra is dependent on the collision gas and, therefore, the center of mass collision energy.

Acknowledgement. This work was carried out at the NIH Mass Spectrometry Resource in the Department of Chemistry, MIT, Cambridge, MA with support from NIH (RR00317) to K. Biemann.

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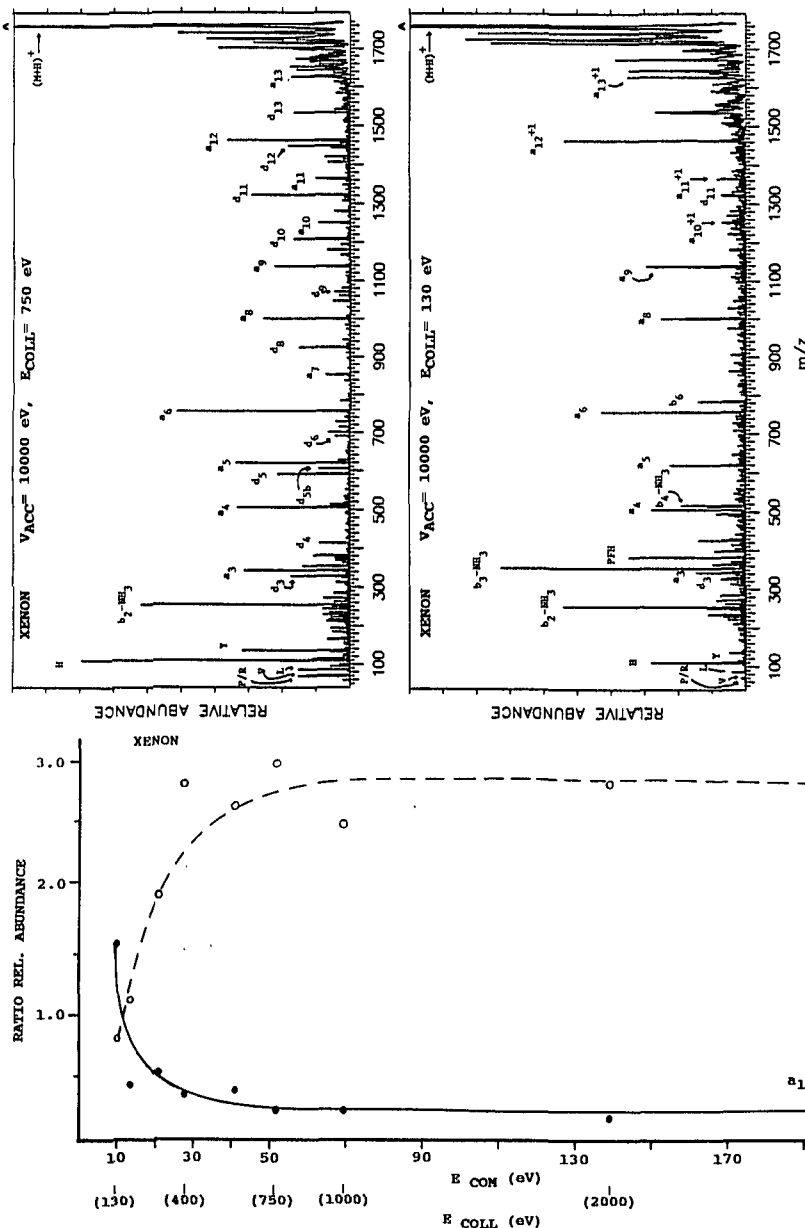


FIGURE 1. Comparison of the product ion distribution in the FAB MS/MS spectrum of the $(MH)^+$ of renin substrate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser, M_r 1757.9) as a function of collision energy (V_{coll} , 750 eV vs. 130 eV, with xenon collision gas. The initial energy of the precursor and final energy of the product ions is 10000 eV. Each MS/MS spectrum is the sum of 5 scans.

FIGURE 2. Plot of the ratio of the relative abundance of product ions (a_{11} , $a_{11}+1$ and d_{11}) as a function of the center of mass collision energy (E_{COM}) and instrument collision energy (E_{COLL}) in the FAB MS/MS spectrum of renin substrate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser, M_r 1757.9) for xenon collision gas, each data point represents the sum of at least 5 scans.

EFFECT OF TRIFLUOROACETIC ACID ON THE REDUCTION OF DISULFIDE BRIDGES IN PEPTIDES ANALYZED BY FAB/MS

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The analysis of peptides by fast atom bombardment mass spectrometry is a good example of the extensive use and utility of desorption-ionization techniques for the characterization of polar, involatile and higher molecular weight compounds. This technique produces parent-molecular ions, which along with their isotopic clusters, can be useful in determining the molecular weight and empirical formula of the compound. Both these types of information are essential for the characterization of a peptide. However, a common problem that can be encountered when analyzing peptides by FAB/MS is that the $(M+H+n)^+$ ($n \geq 2$) peak intensities are higher than theoretically calculated. It is believed that reduction reactions occurring in the matrix are responsible for the distortion observed in the isotopic cluster which can lead to the misidentification of the peptide. The positive deviation noted in the isotopic peak ratios appears to be particularly exaggerated with the presence of a disulfide bridge in the molecule.

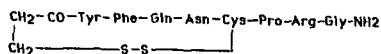
In order to study the effect of instrumental parameters on this phenomenon, deamino arginine vasopressin (DAVP) and arginine vasopressin (AVP), which are both neurohormonal peptides, have been chosen. The molecular structures of these peptides are shown in figure 1. In preliminary experiments conducted in thioglycerol, it was noticed that DAVP and AVP stabilized with acetic acid produced parent ions at m/z 1071 and 1086 respectively. The protonated molecular ions for these compounds should occur at m/z 1069 and 1084. The same peptides stabilized with trifluoroacetic acid, however, showed the parent ion at the correct mass. The results obtained for DAVP are illustrated in Figure 2 which shows a portion of the mass spectra of both types of acetates. Using "extended resolution multiple peak monitoring" and signal averaging techniques, later experiments were conducted to accurately measure the isotopic peak ratios. The results obtained in these experiments demonstrated that the $(A+2)/A$ isotopic peak ratio increased with time. Both the trifluoroacetate and acetate salts showed a time dependence of the $(A+2)/A$ ratio, but the rate of reduction for the acetate salt was found to be approximately 30 times faster than that of the trifluoroacetate. In other experiments, trifluoroacetic acid was added to the acetate sample prior to bombardment and it was found to decrease the amount of reduction substantially so that the parent ions for DAVP and AVP now occurred at m/z 1069 and 1084 respectively. Even though spectra obtained under these conditions provided parent ions at the correct mass, the reduction process was not completely eliminated. If the addition of trifluoroacetic acid was made after the sample had been bombarded, the reduction could not be inhibited. This observation indicates that the reduction process is irreversible under the experimental conditions used.

In order to assess the importance of solution parameters on the reduction process, the peptide DAVP stabilized with acetic acid was analyzed in different matrices. The results of these experiments are shown in Table I where the measured $A+2/A$ ratios are given as a function of the nature of the matrix. It can be seen from the data that reduction as measured by the $(A+2)/A$ ratio is most prominent when a 5:1 mixture of dithiothreitol/dithioerythritol (magic bullet) is utilized as matrix. This is not surprising since these molecules contain two thiol groups which are known to reduce disulfide bridges in solution. As would be expected, the observed reduction process is lesser with thioglycerol in which only one thiol group is found. Furthermore, samples analyzed in glycerol show reduction, but to a much smaller extent, while samples analyzed in 2-hydroxyethyl disulfide and 3-nitrobenzylalcohol exhibit the least amount of reduction. The disulfide bridge in hydroxyethyl disulfide and the nitrophenyl moiety in nitrobenzylalcohol are electron scavengers and can therefore be expected to decrease the possibility of reduction.

The results obtained in this work are useful in determining the types of reduction reactions that can occur when analyzing peptides by FAB/MS. It can be concluded that the choice of a proper matrix and addition of trifluoroacetic acid can be useful in inhibiting the possibility of reduction processes and therefore misidentification. Both of these parameters have determining effects on the accuracy of the results, since the $A+2/A$ is found to vary from 8.5, under the worst conditions (acetate salt in magic bullet), to approximately 0.28 in experiments where either TFA is added or a proper matrix is used. Thus the results obtained indicate that not only can accurate values be obtained for the isotopic ratios when analyzing peptides containing disulfide bridges but that the factors influencing reduction have drastic effects since they can change the reduction rate by a factor of 30.

Another finding in this study that is worthy of mention concerns the thiolglycerol adducts of the reduced form of the peptides. Close examination of the spectra of DAVP and AVP revealed that, in thiolglycerol, adduct ions $(M + \text{Thiolglycerol} + H)^+$ corresponding to the non-reduced form of the peptides were observed at m/z 1177 and 1192 respectively. However, thiolglycerol adducts of the reduced forms expected to be observed at m/z 1179 and 1194 were essentially absent from the spectra. This implies that either the adduct is actually a reaction product of the peptide and thiolglycerol or that thiolglycerol adducts "protect" the molecule from being reduced. Studies are presently being conducted to explain this peculiar observation and to elucidate other factors involved in the more general phenomenon of reduction of different types of peptides under FAB conditions.

DEAMINO ARGININE VASOPRESSIN (DAVP)



ARGININE VASOPRESSIN (AVP)

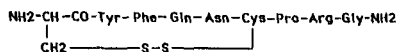


Figure 1: Molecular structures of DAVP and AVP

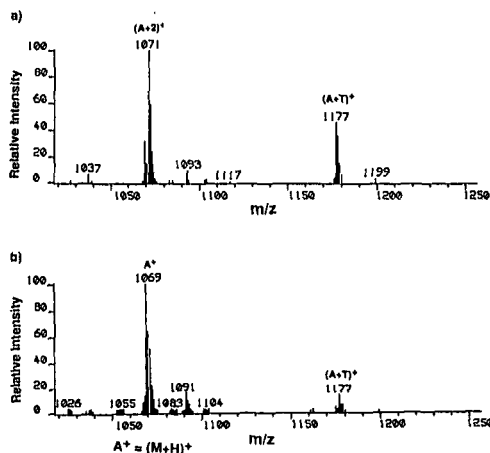


Figure 2: FAB Mass Spectra of DAVP as a) acetate salt and b) trifluoroacetate salt

TABLE I: MATRIX DEPENDENCE

COMPOUND USED: DAVP-AcOH

THEORETICAL $(A+2)/A$: 0.274

MATRIX	MAGIC BULLET	THIOGLYCEROL	GLYCEROL	2-HYDROXYETHYL DISULFIDE	3-NITROBENZYL ALCOHOL
$(A+2)/A$	8.6	4.3	0.684	0.382	0.282

A MASS SPECTROMETRIC TECHNIQUE FOR DETECTING AND LOCATING
ERRORS IN SYNTHETIC PEPTIDES

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The utility of a new mass spectrometric technique¹ for detecting and identifying peptide by-products produced in the synthesis of peptides is demonstrated. The technique involves three sequential steps: (1) Practically nondestructive ²⁵²Cf plasma desorption mass spectrometric analysis of monolayer amounts of peptide(s) of interest bound to a thin layer of nitrocellulose. (2) Enzyme catalyzed microscale chemical reaction of the surface bound peptide(s) to produce structurally informative hydrolysis products. (3) Plasma desorption mass spectrometric analysis of these hydrolysis products. The first step determines the presence and molecular weights of unwanted by-products resulting from errors or incomplete reactions during synthesis. The subsequent two steps provide information on the precise location in the peptides where errors have occurred.

In the present paper, the technique is applied to an investigation of unwanted peptide by-products associated with the use of tryptophan, protected on the indole nitrogen with the formyl group. Results are presented on two tryptophan containing synthetic peptides in which large formyl group containing impurities are observed². The sequence of the first peptide, melittin is G I G A V L K V L T T G L P A L I S W I K R K R Q Q-NH₂. The PD mass spectrum of a 1nmol HPLC purified melittin sample, (Fig.1(a)) showed the presence of an impurity ion ($M_1^1 + H$)⁺, with mass 28u higher than that of the desired melittin ion ($M_1 + H$)⁺. The 28u mass difference indicated the retention of the formyl group on the impurity and was thought to be the result of either an incomplete deprotection of Trp(For) or a transformylation modification during deprotection. In order to locate the position of this formyl group, the sample used to obtain the spectrum shown in Fig.1(a) was removed from the mass spectrometer and treated with 10⁻¹¹ mol of trypsin for 10 minutes at 37°C. The mass spectrum of this trypsin treated sample (Fig.1(b)) exhibits a series of new peaks corresponding to the products of hydrolysis of melittin and the melittin impurity. Since all the fragments are observed as doublets (28u apart), we conclude that the formyl group in the impurity, M_1^1 , is located between residues 8-21 inclusive. To obtain more information regarding the location of the formyl group, 1 nmol of freshly deposited sample on NC was treated with 10⁻¹⁰ mol of proteinase K for 10 min. The mass spectrum of the resulting hydrolysis products is shown in Fig.1(c). The ion (20-26)⁺ clearly lacks the companion peak (20-26)⁺₁, which indicates the absence of the formyl group in the impurity melittin in residues 20-26. However, the presence of the pair (19-26)⁺ and (19-26)⁺₁ indicates that the formyl group is present in residues 19-26. Therefore the formyl group is located on Trp-19 of the impurity melittin. Supporting evidence for the location of the formyl group on Trp-19 was similarly deduced from the mass spectrum (Fig.1(d)) obtained after α -chymotrypsin treatment of the melittin sample. A summary of the observed cleavages from all three enzymatic hydrolysis is shown in Fig. 2.

A similar investigation was carried out on a sample of synthetic [Bpa-8]dynorphin A (1-17), with sequence Y G G F L R R Bpa R P K L K W D N Q, where Bpa denotes p-benzoyl-phenylalanine. The PD mass spectrum of this sample, (Fig.3) also showed the presence of an impurity ion ($M_2^1 + H$)⁺ 28u higher than the Dynorphin ion ($M_2 + H$)⁺. An investigation of the mass spectrometric fragmentation in Fig.3 and the mass spectra following digestion with α -chymotrypsin, proteinase K, and carboxypeptidase B (data not shown) demonstrated² that the formyl group is no longer present on Trp-14, but in this case has migrated to the neighboring lys-11 or lys-13.

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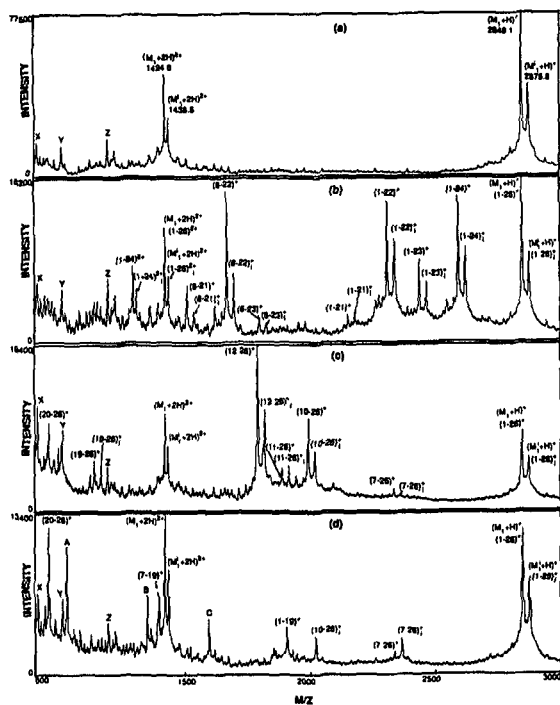


Fig. 1

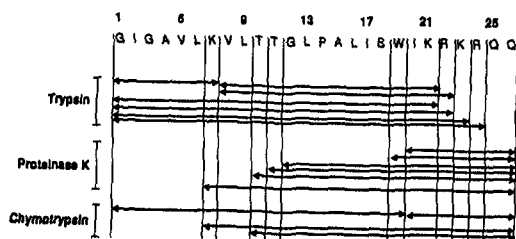


Fig. 2

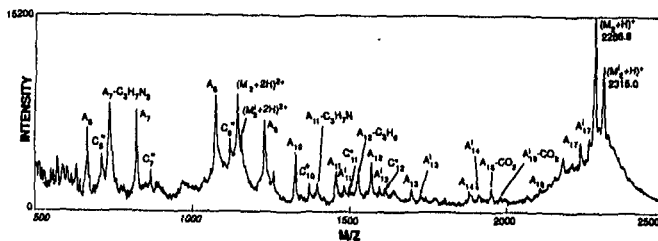


Fig. 3

**PULSED ION BOMBARDMENT TIME-OF-FLIGHT MASS SPECTROMETER
WITH OPTIMUM SENSITIVITY FOR PEPTIDES IN 1-2 PICOMOLE RANGE**

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Ion bombardment time-of-flight (TOF) mass spectrometry is an extremely sensitive technique. In order to obtain the best response from a sample, it is desirable that all the available sample be efficiently utilized in producing the mass spectrum. This can be achieved by concentrating the sample on a small area; bombarding it with a sufficiently intense, tightly focussed ion beam to desorb the entire sample; and analysing the secondary ion masses with an efficient TOF mass analyzer. We have designed and constructed a pulsed ion bombardment TOF mass spectrometer (PIB-MS) that utilizes this principle (1,2) and have achieved a sensitivity with this instrument such that the highest yield occurs for the peptide amounts of the order of 1 picomole. The PIB-MS is schematically shown in Figure 1. 30 keV indium ion beam from an electrohydrodynamic liquid metal ion source is focussed to a 100 micron diameter spot on the sample probe (2mm diameter) and is caused to sweep across it when pulsed. The beam is pulsed at a rate of 2 kHz and each pulse of 5-10 nanoseconds duration contains 500-1000 indium ions.

In the PIB-MS the sample utilization is quite high in the area of bombardment (100 micron x 2mm = 0.2 mm²). This is evident from Figure 2 which shows the decay of the (M+H)⁺ ion signal from arginine vasopressin (MW=1083) as a function of the duration of bombardment by indium ion beam. Here 1 picomole of the peptide was absorbed to a thin film of nitrocellulose deposited on the 'whole area' of the probe tip. The signal decreased nearly exponentially with a half life of about 23 minutes. The data shown in Figure 2 also shows that by steering the beam to a different spot on the probe tip, after a 90 minute bombardment, the signal returns to its original level. This indicates that higher sensitivities could be obtained if all the sample were concentrated in the area swept by the bombarding beam. To achieve this further increase in sensitivity the peptides were restricted to remain on a small area nitrocellulose (NC) stripe (250 um x 2mm) on the probe tip. Figure 3 compares the spectra obtained from 100 femtomoles of arginine vasopressin deposited on the 'whole area' and on the NC 'stripe'. A three fold increase in signal for the peptide on the stripe can be seen.

Figure 4 compares the (M+H)⁺ ion yield as a function of the amount of arginine vasopressin for the case of peptide deposited on the whole probe tip area versus that of peptide deposited on the NC stripe. In the former case the signal increased linearly for amounts up to 10 picomoles. For amounts in excess of 10 picomoles, the yield first levels out and then actually begins to decrease. For the sample deposited on the stripe on the other hand, the signal reached a saturation value for an amount of about 1 picomole.

Such yield maximization at the 1-2 picomoles level was also observed with other peptides in the mass range 1000-6000u (e.g. a synthetic peptide analogue of calcitonin (MW=3576) and bovine insulin (MW=5733)).

Thus the design goal of attaining 1 picomole optimum sensitivity for peptides was accomplished by efficient utilization of the available sample with the described pulsed ion bombardment time-of-flight mass spectrometer.

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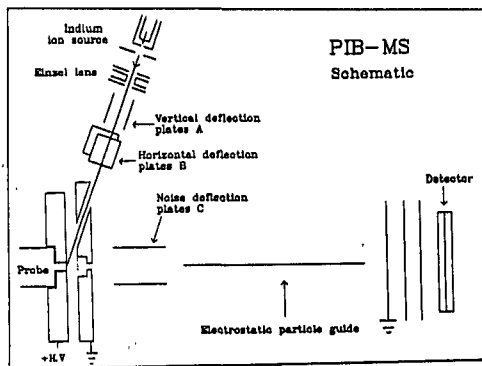


Figure 1

Arginine vasopressin decay on continuous bombardment (1 pmol on 'whole area')

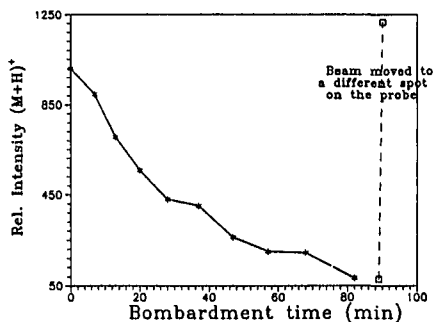


figure 2

ARGININE VASOPRESSIN 100 fmol on 3 mm² Run time = 3 min

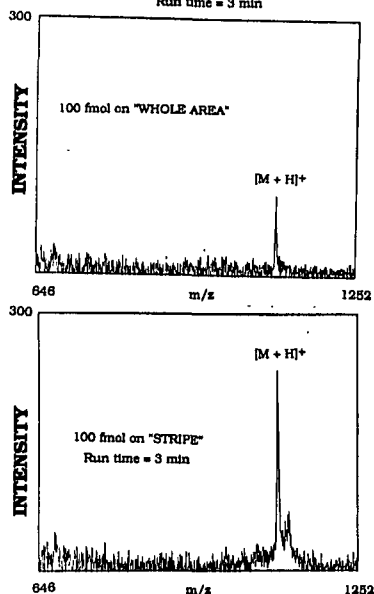


Figure 3

ARGININE VASOPRESSIN

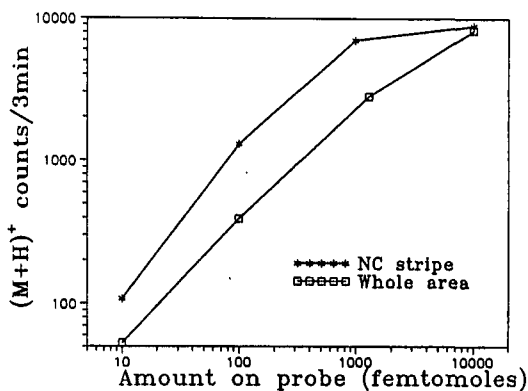


Figure 4

The Effect of Primary Ionization on the MS/MS Spectra
of Small Peptides Using a Triple Quadrupole MS

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Recent advances in MS/MS instrumentation have fostered interest in the Collisionally Induced Dissociation (CID) daughter spectra of peptides. Structurally significant fragmentation of biomolecules induced by Collisional Activation (CA) has been shown to provide both primary sequence and modification site information. The technique has been used for peptide structural studies not applicable to classical Edman degradation.

A common perception is that MS/MS of peptides can be best performed using FAB primary ionization followed by high energy CA in multisection instruments. We have examined the utility of simpler and less expensive triple quadrupole instrumentation for this purpose.

The inherent inefficiency of CA, the low collision energies attainable (<200 eV) and poor high mass sensitivity of the quad relative to sector instruments makes acquisition of high mass CID spectra challenging. Quads, however, are uniquely suited for detection of Unimolecular Decomposition (UMD) products of the parent ion.

In both sector and quad instruments, MS/MS fragment ions are observed only if decomposition (CID or UMD) occurs in the collision cell. Consideration of ion flight times for both instruments reveals that selected parent ions reach the collision cell at nearly the same time, but reside in the quad cell ca. 500 times longer. Quad UMD fragments are readily observable due to this statistical advantage. Parent ions of sufficient internal energy to undergo UMD produce useful daughter spectra in the absence of CA. UMD must be exploited for successful MS/MS of massive parent ions using quadrupoles of limited CA capability.

The UMD and CID MS/MS daughter spectra of Pertirelin (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-NH-C₆H₅ MW=1153), a nonylpeptide, using FAB and Thermospray (TSP) primary ionization is shown. The FAB UMD spectrum (A) shows significant fragmentation in the absence of CA suggesting FAB produces parent ions of high internal energy. Collision energies less than 2 eV were employed with no collision gas. Primary structure information is readily attainable as highlighted by the figure. The CID spectrum (B) shows improved low mass fragment sensitivity, but provides little additional structural information. This spectrum was recorded at a collision energy of 15 eV with 0.4 torr Argon collision gas.

Conversely, the TSP UMD spectrum (C) shows very little fragmentation suggesting parent ions of much lower internal energy are produced by this ionization method. A collision energy of 120 eV or greater was required to produce TSP CID fragmentation (D) similar to the FAB UMD data. Similar results have been noted for many small peptides ranging in molecular weight from 400 to 2000 daltons using a Finnigan TSQ-70 triple quadrupole spectrometer.

It is concluded that, triple quadrupole MS/MS systems are relatively sensitive to UMD fragmentation due to the statistically long sampling times. The FAB primary ionization process produces parent ions of small peptides of sufficient internal energy to undergo UMD. Below 1000 Da., CA adds little additional information to the daughter spectrum. Above 1000 Da., CA is required to obtain optimal daughter spectra. In triple quads, most of the energy required for peptide fragmentation must be provided by the primary ionization method due to the limited capabilities of low energy CA.

THREE DIMENSIONAL CELLULAR MAPPING OF ANTIBODY-ANTIGEN INTERACTIONS BY SECONDARY ION MASS SPECTROMETRY

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INTRODUCTION

Colloidal gold probes are widely used for light and electron microscopic immunocytochemistry, for example, in cell surface labeling and as markers in histological samples.^{1,2} One approach for labeling fixed histological sections involves a sequential reaction, first with a primary antibody having specificity for an antigen of interest, followed by treatment with a secondary antibody linked to colloidal gold particles. The signal produced by the gold particles using backscattered electron or light microscopic imaging can be greatly enhanced using a physical silver developer to enlarge the staining particle size. This approach was utilized in this study to label antigenic sites along the basement membranes (basal laminae) of glomeruli in human kidney tissue. It provides the first demonstrated application of elemental tags with high ionization efficiency in SIMS to image sites of antigen-antibody interactions in fixed biological tissue.

EXPERIMENTAL

Human kidney tissue sections were treated with a two stage immunogold silver staining (IGSS) process. Anti-human collagen Type IV monoclonal antibodies (Chemicon International, Inc. Catalog no. MAB1910) were used to bind to antigenic sites on glomerular basement membranes. The secondary antibody was goat anti-mouse IgG conjugated with 5nm gold particles (Janssen Life Sciences Products AuroProbe EM # 23.685.17-GAM1G). The silver enhancement reaction was then used to enlarge the gold particle size by deposition of silver (Janssen Intense M kit). Control sections treated in the above manner, except for the omission of the primary antibody, did not exhibit any detectable silver incorporation. Sections which did not utilize silver enhancement provided inadequate labeling for direct imaging of the gold tags using ion microscopy. The SIMS measurements were performed using a Cameca IMS-3f ion microscope equipped with a magnetically filtered primary Cs⁺ beam and a custom digital secondary ion imaging system.³

RESULTS AND DISCUSSION

Figure 1 illustrates SIMS results for glomeruli from the IGSS labeled kidney tissue. In addition to elements characteristic of the organic constituents (H, C, N, O, S), the negative ion spectrum (Fig. 1) shows high concentrations of the Ag label and halogens with very high secondary ion yields (Cl, Br, I). The lack of Au⁻ intensity is readily explained by the difference in the relative volumes of the Au cores and Ag overlayers of about 10⁵. The Ag⁻ and Br⁻ signals are several orders of magnitude more intense in the tissue treated with the Ag. The enhanced Br⁻ may result from binding to Ag particles during H and E staining for light microscopy.

Results of digital ion imaging studies are summarized in Figure 2 for a selected portion of a single glomerulus with its associated basement membrane (Bowman's Capsule). The SIMS analytical area was confined to a circle 150 μ m in diameter centered in the ion beam sputtered area.

The tissue morphology is shown in the backscattered electron micrograph (Fig. 2A) and is correlated with the surface images of the same feature obtained by SIMS (Figs. 2B). The CN⁻ image identifies the regions containing tissue, the dark channel representing the urinary (Bowman's) space. The binary SIMS image overlay of Ag⁻ and CN⁻ in Figure 2B illustrates the predominance of Ag in surface regions associated with the basement membrane (Bowman's Capsule).

Results of SIMS 3-D or image depth profiles are shown in Figures 2C and 2D for CN⁻ and Ag⁻, respectively. Vertical slicing of the digital image stack was selected to go through the basement membrane located in the bottom right quadrant of micrographs shown in Figures 2A-2B. About 0.4 μ m were sputtered in increments between each successive set of image acquisitions. The result confirms that the Ag is confined to the outer surface of the section. That is, sputtering of the outer 0.4 μ m before acquiring the second Ag⁻ image was sufficient to virtually eliminate all detectable Ag (Fig. 2D). In contrast, the CN⁻ signal actually increases somewhat for the second and third data sets (Fig. 2C). This may reflect attenuation by remnants of a carbon overlayer in the first data set, and progressive ion beam damage effects which may influence the CN⁻ signal. It should be noted that SIMS sensitivity is such that less than 15nm depth normally is consumed to acquire a single Ag⁻ image, and about 0.1 nm for each CN⁻ image.

CONCLUSION

The immunogold silver staining process is shown to be useful for the localization of antigen-antibody interactions in biological tissues using SIMS. The 3-D image depth profiling mode provides information on the specificity and penetration of the antibodies used to label antigenic sites. Further improvements are expected in SIMS lateral resolution using focused liquid metal ion sources, in methods to display 3-D information, and in the development of chemical tags for biomolecules tailored to mass spectral detection. These are important early steps in the evolution of imaging mass spectrometries as molecular microprobes with applications to biology and medicine.

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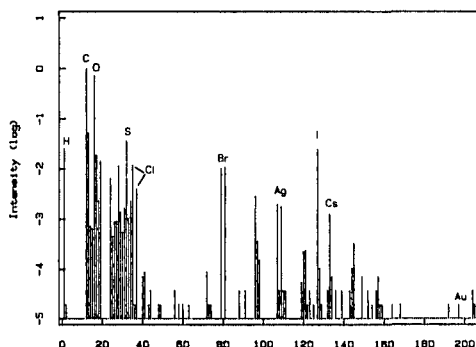


Figure 1.--Negative ion mass spectrum of kidney glomerulus region labeled with Ag enhancement step (log intensity scale normalized to carbon intensity at mass 12).

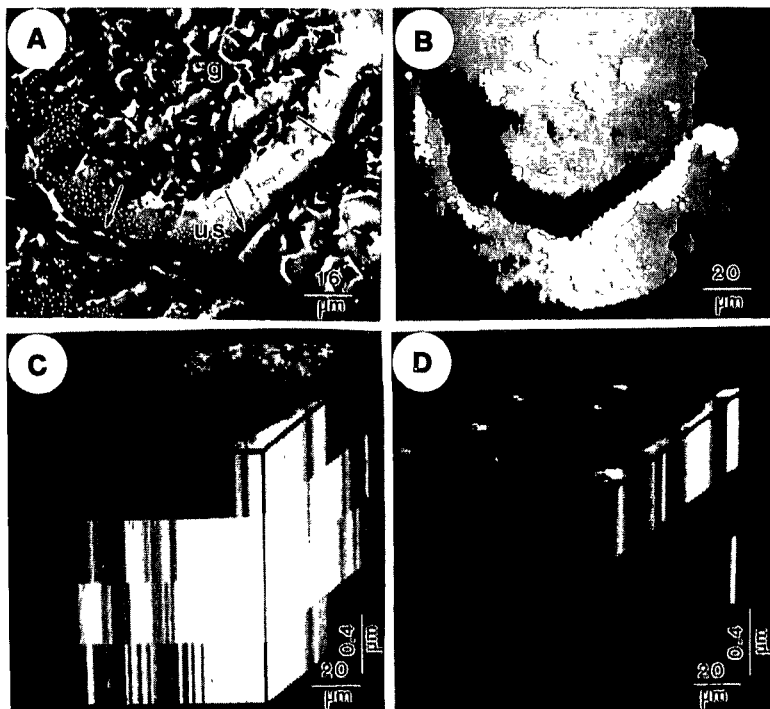


Figure 2.--SIMS imaging of labeled kidney glomerulus: A. Backscattered electron micrograph (after ion sputtering during SIMS measurements) -- g = glomerulus, us = urinary space, arrows = basement membrane (Bowman's Capsule). B. Binary overlay of Ag⁺ and CN⁻ SIMS images -- black = no Ag⁺ or CN⁻ above background; grey = CN⁻ above background, but no Ag⁺; white = CN⁻ and Ag⁺ above background. C. 3-D SIMS image of CN⁻. D. 3-D SIMS image of Ag⁺.

TOPOGRAPHY OF MEMBRANE-BOUND PROTEINS: PHOTOAFFINITY STUDIES OF COLICIN Ia.

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Ion channels are membrane proteins that permit and control the transport of selected small ionic species across cell membranes. They are found in numerous cell types, playing key roles in nervous system signaling, muscle contraction, and hormone release. Molecular level studies of ion channels have focused on molecules such as the acetylcholine receptor, a large (270kD) transmembrane glycoprotein. Colicin Ia is a smaller (67kD, 626 residues) monomeric bacterial ion channel, which is an excellent model for higher eukaryotic ion channel function.

In order to completely understand the mechanism by which this bacterial ion channel forms and the details of the ion channel structure, we have undertaken to map the topography of its transmembrane form, using photoaffinity labeling followed by a combination of proteolytic digestion, HPLC, and mass spectrometric techniques. The essential feature of our experiments is that they are an attempt to specifically identify that portion of the colicin molecule that is imbedded in the membrane when colicin is functioning as a transmembrane ion channel.

Experimental

A. Preparation of Mass Map

Colicin Ia was prepared and purified according to the method of Mel and Wolberger (1). *Staphylococcus aureus* V8 enzyme was used to digest the protein. The resulting mixture of peptides was separated by reverse phase HPLC (C-4 column, water/acetonitrile/0.1% TFA solvent system). Liquid secondary ion mass spectrometry was used to identify the peptides by their molecular masses. For many of the earlier-eluting peptides, it was necessary to prepare the hexyl esters (2) in order to obtain mass spectra. In cases where the molecular masses of two different peptides coincided, MS/MS (Kratos Concept 4-sector (3)) was used to distinguish between the two possibilities. Using these methods, approximately 77% of the protein could be successfully mapped from a single digest of less than 1 nmol of protein. The remaining unmapped portions of the molecule were difficult to map due to the lack of suitable cleavage sites for the V8 enzyme, so that other enzyme digests may be required to complete the map.

B. Photoaffinity labeling

Lipid vesicles (liposomes) were prepared according to the method of Szoka and Papahadjopoulos (4). The photoaffinity label we used was 3-(trifluoromethyl)-3-(*m*-iodophenyl) diazirene (TID) (5). Upon uv irradiation, TID loses molecular nitrogen to form a carbene, which rapidly inserts into adjacent C-C and C-H bonds.

TID is lipid-soluble, and when added to a suspension of liposomes, it dissolves in and remains almost exclusively in the lipid bilayer. If a membrane-spanning protein, such as colicin, is also present in the bilayer, then the carbene formed upon uv irradiation will immediately react with adjacent portions of the protein, thus selectively tagging those residues that reside in the membrane. Isolation of the protein followed by proteolytic digestion and mass mapping should allow identification of the modified residues. In our experiments, TID was labeled with ¹²⁵I to aid in rapid identification of the modified peptides.

Results and Discussion

A 2 nmol sample of colicin Ia was labeled with ^{125}I TID as described. The labeled protein was digested with *S. aureus* V8 enzyme (1:10, 4 hrs at 37°C) and the resulting peptide mixture was separated by HPLC. Fractions were collected at 1 minute intervals using an automated fraction collector. The ^{125}I content of each fraction was assayed by gamma-ray counting. The resulting plot of counts per minute vs. fraction number is shown in Fig. 1.

It is clear from Fig. 1 that our first major goal of specific labeling of peptides was achieved. The total amount of labeling was approximately 1%, i.e., 1 TID molecule per 100 colicin molecules. This proportion is similar to other reported experiments using TID. Remaining problems include the fact that the quantities of TID-labeled peptides so far generated are too small for direct identification by mass spectrometry, and the fact that the addition of TID to a peptide affects its HPLC retention time, so that direct identification by means of this parameter is not straightforward.

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Acknowledgements

This work was supported by the National Institutes of Health (Grants RR01614 to ALB and GM24485 to RMS), the National Science Foundation Biological Instrumentation Program and the University of California, San Francisco (Claypole Fellowship and Graduate Division Mentorship to SFM).

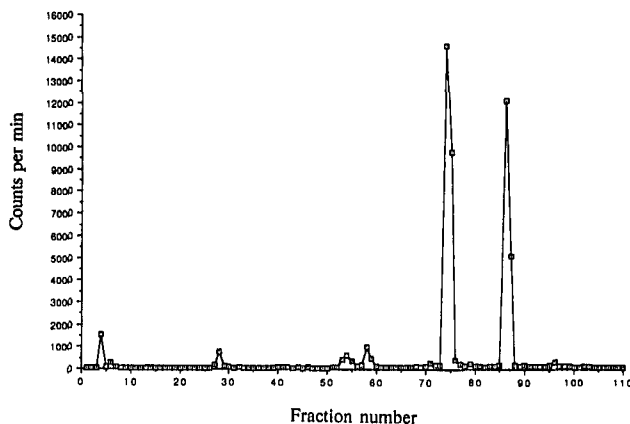


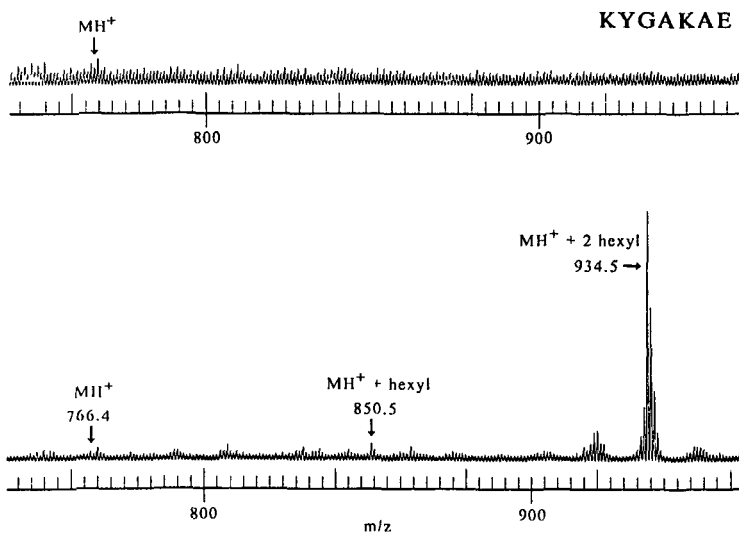
Fig. 1 Radioactive labeling of HPLC fractions from *S. aureus* V8 digest of colicin Ia after photoaffinity labeling with ^{125}I TID.

DERIVATIZATION AS AN AID TO PEPTIDE MAPPING BY LSIMS.

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Peptide mapping by LSIMS is often complicated by the poor ionization efficiencies of some peptides, particularly small, hydrophilic ones, making them effectively invisible to LSIMS analysis. Derivatization has been shown to be useful in the analysis of such peptides (1). We have developed an improved method for the derivatization of difficult peptides which increases their hydrophobicities, thereby improving the signal-to-noise level in their LSIMS spectra. In our procedure, samples are collected in tubes, then dried and the hexyl esters formed by treatment with a hexanol/HCl mixture. The derivatized samples can be analyzed directly or they can be dried and resuspended in 0.1% TFA for analysis. The figure below shows the effect of hexyl ester formation on a heptapeptide obtained from a 500 picomole *S. Aureus* V8 digest of colicin Ia. The HPLC fraction containing this peptide was collected and divided into two equal aliquots (approximately 50 picomoles each); one was run directly on the mass spectrometer, while the second was derivatized with hexanol. This illustrates the improvement in S:N ratio and absolute signal intensity commonly found in this procedure. The utility of this method was further demonstrated when we derivatized one-half of the eluent collected from each of the first ten peaks detected on the HPLC of the colicin digest. No peptide ions were discernable in 8 out of 10 of the underivatized fractions, whereas 9 out of 10 of the derivatized fractions gave easily measured signals whose masses corresponded to hexyl esters of predicted peptides from this digest. The hexylation reaction has also been useful in the MS/MS analysis of these peptides, as C-terminus daughter ions show an 84 amu shift relative to the underivatized peptide ions. The procedure is simple, rapid and easily applied to large numbers of samples and has proven useful in the analysis of difficult peptides in our laboratory.

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TANDEM MASS SPECTROMETRY (HIGH ENERGY CID) OF HUMAN HEMOGLOBIN FROM ERYTHROCYTES MODIFIED IN VITRO BY STYRENE-7,8-OXIDE.

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Introduction

Alkylation of DNA by electrophilic metabolites of xenobiotics is believed to be the major initiating process that may result in carcinogenesis. The study of hemoglobin alkylated *in vivo* by chemical carcinogens has previously been proposed as an indicator for DNA alkylation (1). Modified proteins are not readily amenable to conventional methods for amino acid sequencing. Tandem mass spectrometry, however, allows structural elucidation of chemically modified proteins (2). Styrene is widely used in the plastics industry and the major metabolite, styrene-7,8-oxide, is both mutagenic and carcinogenic in rodents (3). Human hemoglobin was modified *in vitro* with styrene-7,8-oxide and digested with trypsin. LSIMS mass mapping of unmodified hemoglobin confirmed the sequence of the protein and provided a library for the identification of modified peptides. Tandem MS of modified peptides allowed assignment of specific residues modified.

Results and Discussion

LSIMS confirmed 95% of the amino acid sequence for human hemoglobin. Hemoglobin isolated from human erythrocytes was alkylated by incubating with [¹⁴C-8] styrene oxide. Tryptic peptides were fractionated by HPLC and the radiogram indicated a number of peptides containing covalently bound styrene oxide (Fig. 1). LSIMS of fraction 13 showed molecular ion MH⁺ m/z 1269.6 as the major component and this is consistent with peptide 133-144 in the β -chain containing one hydroxyphenylethyl moiety (covalent binding of one styrene oxide molecule). The corresponding unmodified peptide eluted in fraction 9. The specific site of modification was established by tandem MS experiments. The high energy CID spectrum of fraction 13 (20 pmole) shows the of the histidine immonium ion m/z 110 (Fig. 2). The ion m/z 230 arises from the immonium ion for histidine containing a hydroxyphenylethyl moiety. This is confirmed by ions of the W, X, Y and Z series containing the histidine residue which show an analogous shift in 120u. As expected, ions of the A, B, C and D series (charge retention at the N-terminus) do not show a mass shift except for fragments containing the histidine residue, e.g. m/z 1095 (A11) compared with m/z 975 (A11) in the unmodified peptide. Other sites of covalent modification were similarly established and are described elsewhere (4). The application of high performance tandem MS to identify aralkylation of hemoglobin with styrene oxide illustrates the potential of this approach for *in vivo* biological monitoring. At present, however, the strategy outlined is not readily amenable for monitoring occupational exposure in humans, largely due to the high levels of unmodified peptides which co-elute during the HPLC fractionation and render difficult identification of modified peptides. This problem may be significantly reduced by the use of immunoaffinity chromatography in the isolation of the modified protein. This approach will be pursued for *in vivo* studies.

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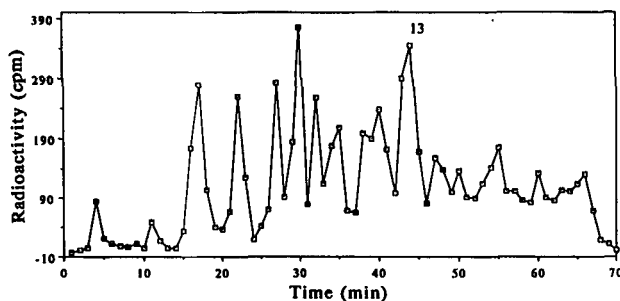


Fig. 1 HPLC radiogram of tryptic digest of human globin from hemoglobin treated *in vitro* with [8-¹⁴C] styrene-7,8-oxide.

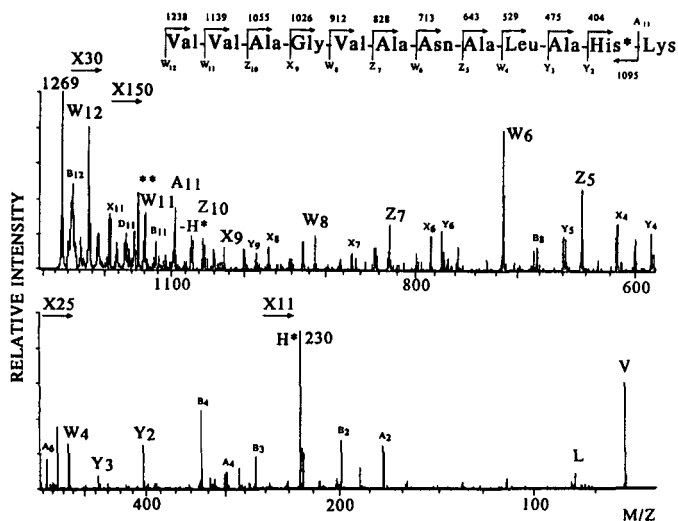
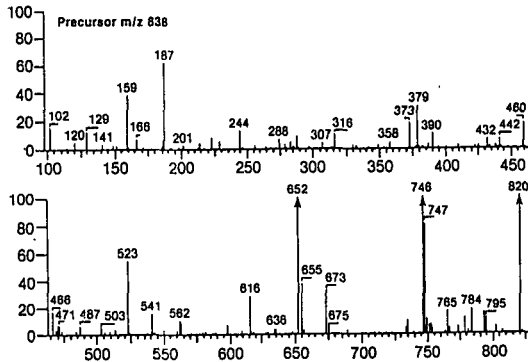
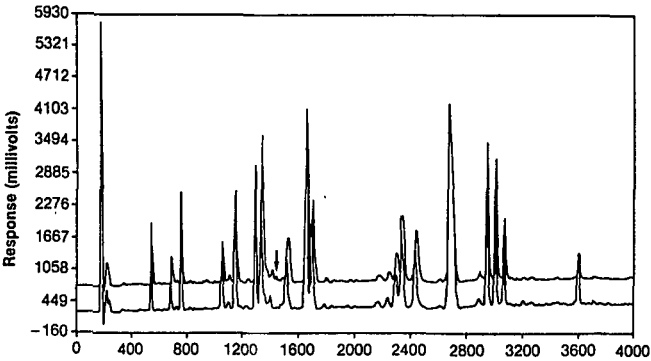
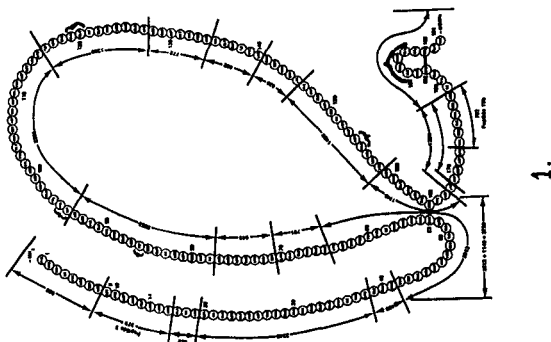


Fig. 2 CID mass spectrum for molecular ion MH^+ m/z 1269 in fraction 13 corresponding to tryptic peptide 133-144 from the β -chain containing one hydroxyphenylethyl modification. Fragments containing histidine residue show 120 Da shift compared to spectrum for corresponding unmodified peptide. His* Histidine modified with $C_2H_3OHC_6H_5$.

** Loss of $C_2H_3OHC_6H_5$ from the molecular ion MH^+ .



Oligopeptide Fragmentation Viewed as Constant Neutral Loss

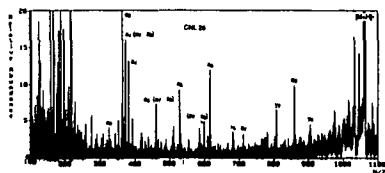
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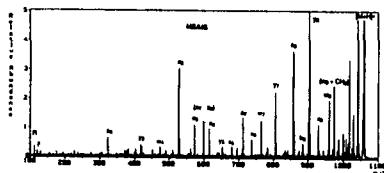
*Johns Hopkins University, Baltimore, MD 21205

The observation of preselected decompositions defined by constancy in the mass of the neutral fragment ¹ has allowed mechanism-based enhancement of signal to noise sensitivity for probing mixtures, and of specificity for characterizing target analytes in complex matrices. Working with tri- and tetra- peptides dePauw ² has suggested that the CNL scans can extend the same advantages to spectra of peptides, that contain multiple series of sequence ions.³ We have evaluated the usefulness of CNL for sequencing peptides in the mass range of 1000-3000amu. We have looked at the elimination of neutral fragments of 17, 18, 28, and 48 amu in the unimolecular and collisionally induced decomposition of bradykinin, LHRH, somatostatin, renin substrate, melittin, and glucagon. These experiments were carried out on a JEOL HX110/HX110 tandem mass spectrometer. CNL scans of anion and cation processes were compared to quadratic scans and B/E scans obtained in MS/MS experiments. The FAB gun was operated at 6keV with Xenon as the FAB gas. In the CID experiments, helium was the collision gas and the parent ion was attenuated by 80%. Among the several candidates, observation of the elimination of 28 amu provided the most information about the amino acid sequence, i.e., the most complete series. Most of the losses correspond to the process "b -> a", and their observation in the complimentary analysis permits the "a" and "b" series ions to be distinguished in the quadratic and MS/MS experiments. Some side chain cleavage processes were also detected, e.g., "d->c", "w-> x", and "z->w". The "z->w" loss is only possible with isoleucine. The CNL window for the loss of 28 amu was found to be 2-4 amu wide, with skewing of the mass window to the lower mass side. The ratio of signal to noise was improved relative to the quadratic scan. The collision gas pressure and matrix solvent are important variables in successfully obtaining sequence information from CNL scans. The width of the CNL window must also be determined for each experimental set up. Overall, the CNL of 28 provides one with a reading frame which is useful in the interpretation of quadratic and MS/MS spectra of peptides.

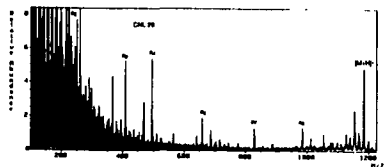
CNL 28 Bradykinin



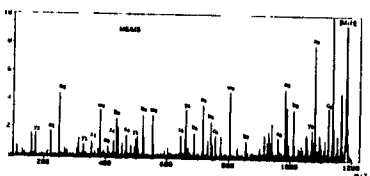
MS/MS Bradykinin



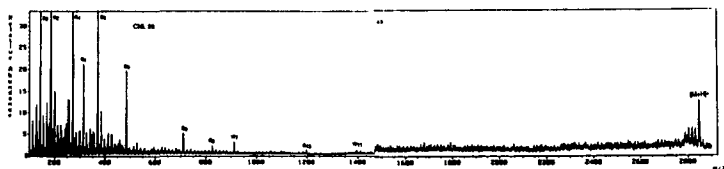
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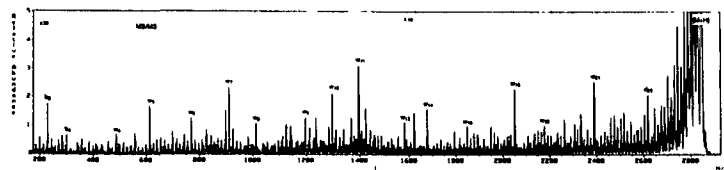
MS/MS LHRH



CNL 28 Melittin



MS/MS Melittin



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INVESTIGATION OF ARTIFACTS FORMED DURING CYANOGEN BROMIDE DIGESTION OF PROTEINS.

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Shively and coworkers (1) reported the formation of artifacts due to exposure of proteins to 70% formic acid during cyanogen bromide digestion, which might block the amino terminus of cyanogen bromide peptides and thereby prevent sequencing by Edman degradation. The use of positive ion fast atom bombardment (FAB) mass spectrometry in this and other laboratories (2) has confirmed this observation and has shown that the artifact corresponds to the addition of 28 mass units to the protonated molecular ion of a cyanogen bromide peptide fragment, $[M+H+28]^+$. We examined the site of peptide formylation using mass spectrometry, and measured the stability of adduct in aqueous solution as a function of pH. The effect of formylation on sequencing by Edman degradation was also investigated. Finally, conditions for the prevention of formylation during cyanogen bromide digestion, which had been suggested by Shively and coworkers (1), were confirmed.

Cyanogen bromide digestion of horse heart cytochrome *c*, bovine adrenal medulla dodecapeptide, bovine adrenal peptide E, and bacteriorhodopsin was carried out as previously described (4). Protein samples were digested for 24 hours in 70% aqueous formic acid at room temperature under argon. The formic acid solution was diluted 20-fold with water and then freeze dried. Cyanogen bromide peptide fragments were purified by high performance liquid chromatography (HPLC) using a Vydac C18 column and a gradient solvent system consisting of water and acetonitrile (each containing 0.1% trifluoroacetic acid). HPLC-purified peptides were analyzed using a JEOL HX110HF double-focussing mass spectrometer, equipped with fast atom bombardment, DA5000 data system, B/E linked scanning, and a collision cell in the first field-free region. The pressure of the helium collision gas was adjusted so that the signal was attenuated 70% during B/E linked scans. Peptide sequencing using Edman degradation was carried out using a Applied Biosystems Model 475A.

Positive ion FAB mass spectrometric analysis of cyanogen bromide peptide fragments of cytochrome *c* and bacteriorhodopsin showed formylated adducts corresponding to $[M+H+28]^+$. Bovine adrenal medulla dodecapeptide and bovine adrenal peptide E were not formylated during digestion. The molecular ion region of the mass spectrum for one of the three cyanogen bromide peptides from cytochrome *c* (CNBr-2) is shown in Figure 1. Both formylated and nonformylated ions are present in this mass spectrum. Formylated peptides could be resolved from the corresponding nonformylated peaks using reversed phase HPLC. When sequenced by Edman degradation, formylated CNBr-2 produced a sequence identical to the nonformylated peptide. This indicated that the formyl group was labile under the conditions of Edman degradation. Saponification, incubation in an aqueous buffer at pH 6.00, 37°C, for 30 minutes, or storage at pH 2.4, -20°C, for one week all resulted in conversion of formylated to nonformylated CNBr-2 from cytochrome *c*.

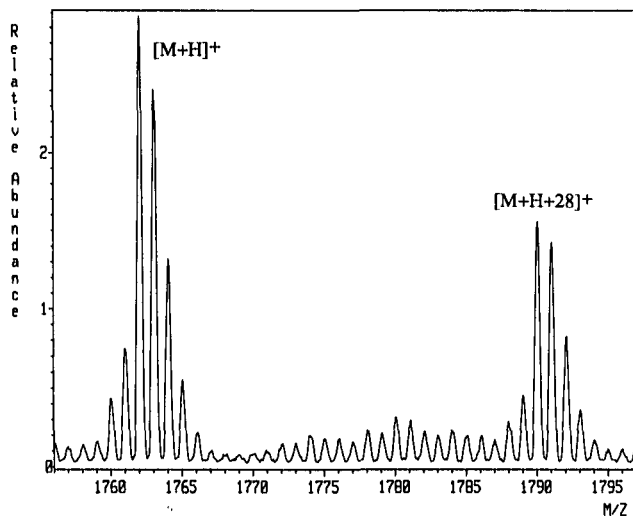


Figure 1. Positive ion FAB mass spectrum of the molecular ion region of CNBr-2 from cytochrome *c*.

B/E linked scanning with collisional activation was used to obtain daughter ion mass spectra of the $[M+H]^+$ and $[M+H+28]^+$ molecular ions of CNBr-2 (See Figure 2.) The most abundant daughter ions, which localized the formyl adduct, were detected at m/z 513 in the formylated CNBr-2 mass spectrum and m/z 485 in the mass spectrum of the non-formylated peptide. These fragments corresponded to x_5 carboxyl terminus sequence ions and resulted from fragmentation between isoleucine and proline. Formylation occurred in the sequence Pro-Gly-Thr-Lys-Hsl (where Hsl is homoserine lactone). Because Shively and coworkers (1) reported that lysine modified at the ϵ -amino group was misidentified during sequencing by Edman degradation, lysine was probably not formylated in this peptide.

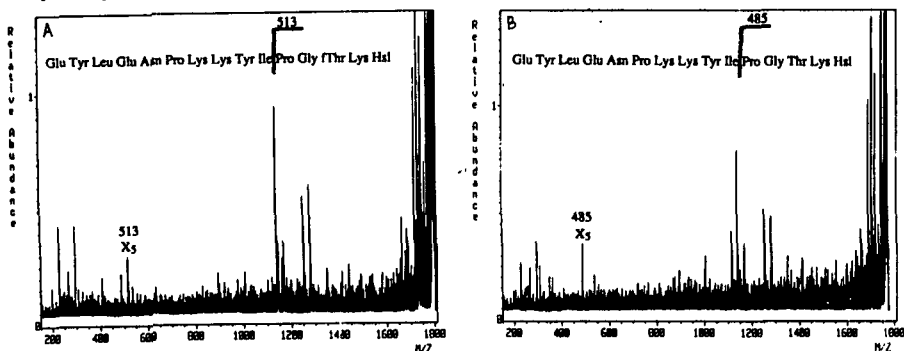


Figure 2. B/E linked scan of protonated molecular ions of A) formylated and B) non-formylated CNBr-2 from cytochrome *c*.

These data and the instability of the adduct suggest that the hydroxyl group of threonine was the site of formylation. Furthermore, we have observed that only proteins containing serine or threonine were formylated during cyanogen bromide digestion in 70% formic acid. The use of 70% trifluoroacetic acid as a solvent for cyanogen bromide digestion eliminated formylation and generated little or no trifluoroacetylation.

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NITRO-PHENYLISOTHIOCYANATE AS A REAGENT FOR PEPTIDE SEQUENCING BY NEGATIVE ION MASS SPECTROMETRY,

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The ability to qualitatively and quantitatively analyze the amino acid residues from a peptide sequencing experiment is important for the study of protein. Protein microsequencing using the Edman degradation is currently possible in the picomolar range using high performance liquid chromatography with UV absorbance to detect and identify the amino acid phenylthiohydantoin released at each cycle.

We are developing modified Edman reagents that form good electron-capturing derivatives for the detection by negative ion mass spectrometry. The use of 4-nitro-phenylisothiocyanate (4-NPITC) (1) in peptide sequencing is being explored. Amino acid 4-nitro phenylthiohydantoin (4-NPTH) derivatives have been prepared for all twenty amino acids. Coupling of the amino acid with 4-NPITC was achieved by reaction at 45°C for 40 min in a buffer system of pyridine:water(1:1) and triethyl amine, the pH of which was maintained between 8-9. Conversion of ATZ to NPTH amino acids was carried out at 55°C in 25% trifluoroacetic acid for 45 min. The resulting 4-NPTH derivatives were analyzed on an HP 5985 B mass spectrometer equipped with Vacumetrics DCI probe. EI and CI spectra were recorded at source temperatures 200°C and 100°C respectively.

For most of the derivatives NI CI mass spectra show only the molecular anion with no fragmentation (TABLE 1). Hence the amino acid residue can be identified by the diagnostic mass. PCI mass spectra show M+1, M+29, ..., and fragment ions in some cases. EI mass spectra show characteristic fragmentation including fragment ions that allow the differentiation of leucine and isoleucine (data not shown).

The use of 4-NPITC in peptide sequencing is demonstrated by sequencing the first five residues from a nonapeptide of known sequence (FIG.1). Except for arginine in the first cycle which gives some unidentified ions, 4-NPTH derivatives from other cycles were easily identified by the molecular anion.

The combination of 4-NPITC with NI CI mass spectrometry would offer the advantages of speed, since no chromatography is required and selectivity, since only electron-capturing molecules are detected. Moreover, it would also enhance the sensitivity because for most of the derivatives, ion current is concentrated on the molecular anion. Experiments to find out the detection limits and coupling yields are underway.

References;

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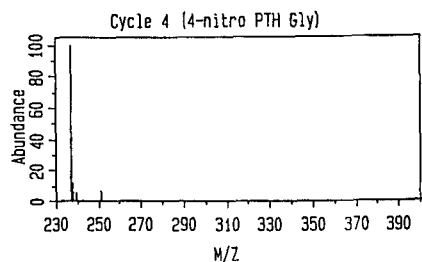
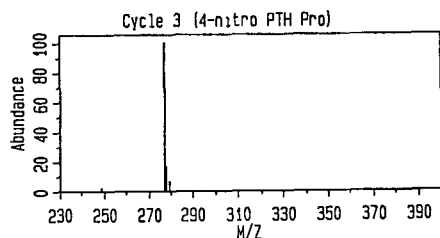
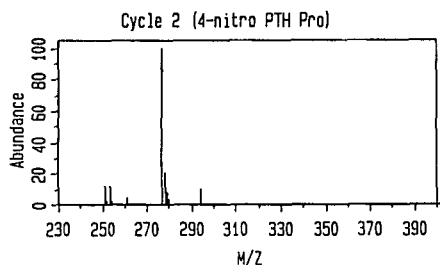
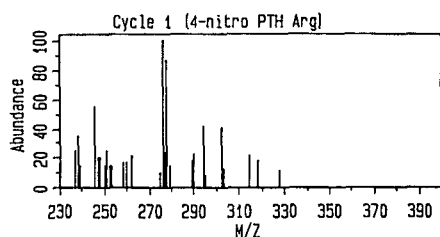


TABLE 1. Ions observed in NCI spectra of amino acid 4-Nitro phenylthiohydantoins

Amino acid 4-NPTH	M ⁺ (abundance)	Other ions (relative abundance to base peak)
Gly (G)	237 (100)	-
Ala (A)	251 (100)	-
Val (V)	279 (100)	-
Leu (L)	293 (100)	250 (5.4%)
Ileu (I)	293 (100)	250 (5.8%)
Pro (P)	277 (100)	-
Phe (F)	327 (100)	-
Trp (W)	366 (100)	-
Met (M)	311 (100)	-
Ser (S)	267 (100)	249 (4.5%)
Thr (T)	281 (100)	263 (14.2%), 237 (16.9%)
CM cys(C)	341 (100)	-
Tyr (y)	343 (100)	-
His (H)	317 (100)	-
Asn (N)	294 (100)	-
Asp (D)	295 (100)	-
Gln (Q)	308 (100)	-
Glu (E)	309 (100)	-
Lys (K)	308 (95)	247 (100%), 237 (42.5%)
Arg (R)	336 (?)	278 (86.5%), 237 (25.3%) 294 (41.6%), 276 (100%)

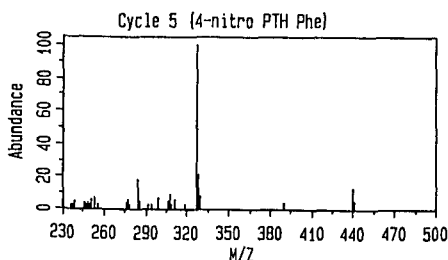


FIG.1 NCI mass spectra of amino acid 4-NPTH derivatives obtained for the first five cycles by consecutive Edman degradation of Bradykinin.

QUANTIFICATION OF SUBSTANCE P BY FAB-MS

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INTRODUCTION

Endogenous SP in human CSF¹ tooth pulp² was quantified in our laboratory by radioimmunoassay. Also, different RRA and RIA patterns of endogenous opioid peptides in human postmortem pituitaries³ were found. RIA provides very good sensitivity but does not provide specificity. These results prompted us to develop a method for simultaneous determination of SP and ME in human tissues and fluids by mass spectrometry, which provides almost equal sensitivity but greater specificity than RIA.

SAMPLE PREPARATION

Post-mortem human pituitaries, of known weight were homogenized in 10 ml of trifluoroacetic acid (TFA) solution (pH 1.5), and one μg of d5-ME and of d7-SPOH (free acid) as internal standard were added. The mixture was homogenized, and an aliquot (100 μl) was taken for protein determination. The homogenate was centrifuged, and the resulting supernatant was prepurified and desalted with SEP-PAK C-18 disposable cartridge. Peptides were eluted with 50% acetonitrile and 0.1% TFA solution. The extraction recovery for this procedure, established with ³H-SP, was 60%. Eluent was concentrated to 400 μl under a stream of nitrogen, and subjected to RP-HPLC for peptide separation. A C-18 $\mu\text{Bondapak}$ column (4 x 250 mm) was used. Mobile phase was 21% acetonitrile and triethylamine-formic acid (TEAF) buffer. Collected fractions were lyophilized, and reconstituted in 50 μl of 0.1% heptafluorobutyric acid (HFBA), and stored until FAB-MS analysis.

MASS-SPECTROMETRY

The FAB-MS measurements were obtained with a Finnigan MAT 731 instrument equipped with a FAB gun, which was operated at 8 keV with xenon as bombardment gas. The protonated molecular ion of SP and ME were monitored in the single ion monitoring mode. Quantitative data were acquired by an Apple II microcomputer. To optimize MH⁺ production of peptides, we examined two matrices, glycerol and 3-nitrobenzyl alcohol. A perfluorinated carboxylic acid was added to manipulate the relative hydrophobicity of the peptide in the matrix. Total ion current from glycerol increased six times using HFBA as an additive compared to TFA; in 3-nitrobenzyl alcohol, the inverse was found. The biological sample measurement was performed using 0.2 μl of glycerol and 0.1% HFBA as FAB matrix.

RESULTS

The standard curve for SP was generated from the ratio of molecular ion current SP/d7-SPOH plotted versus ng SP (Figure 1). The limit of detection of the SP MH⁺ was 0.5 ng (0.37 pmol). We always want to achieve optimal molecular specificity, so we monitored the metastable transition for SP MH⁺ (1348.7) to 1002. The detection limit is 20 ng, which is much poorer related to the molecular ion sensitivity. We rationalize that the poor SP B/E data (Figure 2) are due to the low relative intensity of the transition ion 1002, which is 0.1%. For example, the ME sensitivity for MH⁺, and for B/E (measured for the 425 fragment ion) equals 125 pg and 250 pg respectively. The relative intensity of ion 425 is 1.3%.

The measured amount of SP in human pituitaries is shown in Table 1. We also report the amount of ME in the same pituitaries (Table 2). For ME, we are able to acquire data using the molecular ion and N-terminus (m/z 425) product ions. The last data were acquired in linked-field scan mode. The amount of ME in human pituitary as determined in the linked-field scan mode, is lower, relative to the amount measured for the molecular ion. We rationalize that the B/E mode is more specific and enables us to decrease any biological matrix interference during the quantification of endogenous peptides.

CONCLUSIONS

1. In this work, endogenous SP and ME were quantified by FAB-MS./2. ME was quantified in human pituitaries by using the MH^+ and 574 → 425 transition. Both measurements are in good agreement with each other. 3. Our research on human samples requires increases in sensitivity, fragmentation, mass range, and specificity. Therefore, quantification of SP by the linked-field scan method is possible with a four sector instrument, equipped with an array photo-diode detector. 4. In the next step, we will use deuterated SP instead of d_7 -SP free acid as internal standard. This work was supported by NIH GM 26666.

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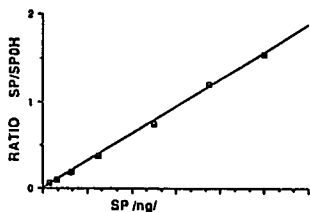


FIGURE 1
STANDARD CURVE FOR SP (MOLECULAR ION)

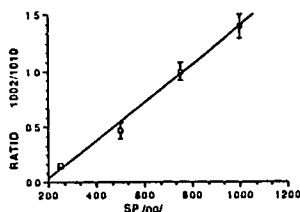


FIGURE 2
STANDARD CURVE FOR SP (B/E MODE)

TABLE 1
SP level in human pituitary
(ng/g of wet tissue)

PITUITARY	WEIGHT (g)	SP (ng/g)
1	0.432	664
2	0.175	750
3	0.185	1450

TABLE 2

ME level in human pituitary (ng/g of wet tissue)

PITUITARY	WEIGHT (g)	ME (ng/g) *	ME (ng/g) **
1	0.432	1720	1303
2	0.175	2783	2400
3	0.185	2838	2286

* Level of ME measured by MH^+

** Level of ME measured by transition $MH^+ \rightarrow 425$
 $YGGFM \rightarrow YGGF-$

QUANTIFICATION OF β -ENDORPHIN IN HUMAN PITUITARY BY FAB

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β -Endorphin is an endogenous opioid peptide consisting of 31 amino acid residues with molecular weight of 3463 Da. β -Endorphin and other related peptides form one of the three known major groups of endogenous peptides; the other two being the enkephalins and the dynorphin-related peptides. β -Endorphin is synthesized biochemically by proteolytic cleavage of the large gene product precursor proopiomelanocortin (POMC). β -Endorphin forms the C-terminal segment of POMC, and is the only component of precursor peptide with agonist activity at an opioid receptor.

β -Endorphin has been implicated in several neurochemical and pharmacological functions of the body. To understand the physiological significance of endorphins, it is critical to determine their amounts in various body tissues. Highly structure-specific and sensitive methods are needed to identify and quantify endogenous peptides so that the data reflect with high fidelity the structure of the target compound.

The objective of the present study is to develop a MS method for quantification of β -endorphin in extracts of human tissues. FAB-MS is the method of choice for the analysis of highly polar and non-volatile compounds such as peptides. Three different approaches were attempted. In the first method (Method 1), the doubly-charged $[(M+2H)^{++}]$ ion of intact β -endorphin which falls into the highest sensitive mass range of VG 7070 E-HF mass spectrometer was monitored using narrow mass (10 u wide) magnet scans acquired in the multichannel analysis (MCA) mode. A variety of FAB matrices (glycerol, a 3:1 mixture of dithiothreitol=DTT and dithioerythritol=DTE, α -thioglycerol, 3-nitrobenzyl alcohol=NBA) and chemical modifiers were tested to determine their effect on the sensitivity of the method. NBA with the addition of 1 μ l of 0.1% TFA and 2.5 μ g of glutathione was the most appropriate FAB matrix. The calibration curve (Curve A) is linear in the concentration range of 2.9-100 pmol of β -endorphin. The lowest detection limit of this method is 2.9 pmol of β -endorphin.

In Methods 2 and 3, β -endorphin was digested with trypsin to provide smaller fragment 20-24 (NAIK) (see Scheme 1). The rationale of using this protocol is based on the fact that low MW compounds are more efficiently ionized. Thus, this approach will improve detection sensitivity (see reference). In Method 2, the $(M+H)^+$ ion of NAIK (at m/z 558) was monitored using narrow mass (10 u wide) MCA scans. DTT/DTE with the addition of 1 μ l of 0.1% TFA and 0.4 μ g of glutathione was found to yield the most intense ion current. A linear calibration curve (Curve B) was obtained in the concentration range 86 fmol-3 pmol of β -endorphin. The curve deviated towards the x-axis at a concentration above 3 pmol. The lowest detection limit of this method is 86 fmol of β -endorphin.

Although monitoring of the $(M+H)^+$ provides high detection sensitivity, it nonetheless lacks the molecular specificity needed for measurements of endogenous neuropeptides. A high level of molecular specificity can be achieved by use of the MS/MS technique. In MS/MS, a product ion can be correlated incontrovertibly to the mass-selected precursor ion. Method B is based on measurement of the area under the peak due to the transition $NAIK \rightarrow NAI$ (i.e., 558 $\rightarrow$ 299; see Scheme 2) acquired using multiple reaction monitoring (MRM) mode. The same combination of FAB matrix as used for Method 2 provided highest sensitivity. The calibration curve (Curve C) is linear over the concentration range 0.5-30 pmol of β -endorphin. The detection limit of this method is 580 fmol of β -endorphin.

These methods were applied to the analysis of β -endorphin in human pituitary extracts. Five human pituitaries were combined, extracted, purified by gradient RP-HPLC and then divided in two portions. Half of the amount was analyzed by Method 1. Although high ion current was obtained for $(M+2H)^{++}$ of β -endorphin, that ion could not be identified unambiguously from the background, indicating that some other biological compounds are still eluting from RP-HPLC at the retention time (73 min) of β -endorphin. The remainder of the pituitary sample was digested with trypsin, and the NAIK was separated by isocratic RP-HPLC. Amounts of 36 and 31 pmol/mg of protein were estimated by Methods 2 and 3, respectively.

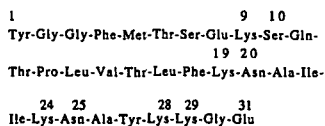
In conclusion, it can be said that Method 2 is very simple and straightforward, but is less sensitive and also lacks in specificity. Method 2 provides the highest detection sensitivity,

but involves additional experimental steps such as trypsinolysis and isocratic HPLC. These two steps increase specificity and also provide cleaner sample. The most important attribute of Method 3 is high molecular specificity, which results from the combination of trypsinolysis, isocratic HPLC, and MRM techniques. The detection sensitivity of the method is also quite good.

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HUMAN β -ENDORPHIN 1-31 MW = 3463



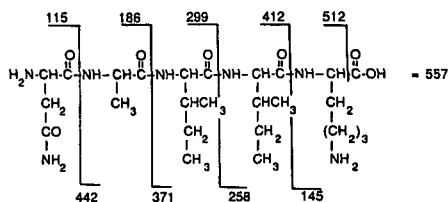
Residue 1-9 MW = 1018, Residue 10-19 MW = 1133,

Residue 20-24 MW = 557, Residue 25-28 MW = 494,

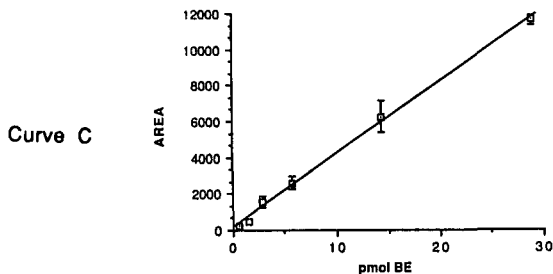
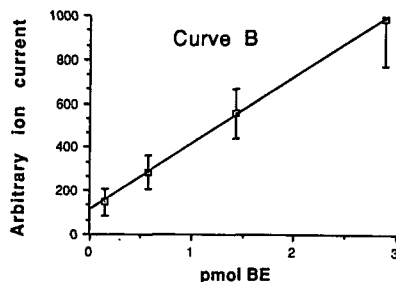
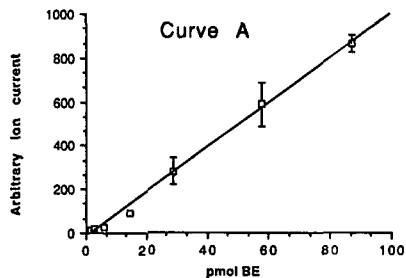
Residue 29 MW = 146, Residue 30-31 MW = 204

Scheme 1

HUMAN β -ENDORPHIN₂₀₋₂₄



Scheme 2



MATRIX REACTIONS AND THEIR EFFECTS ON THE ISOTOPIC PEAK RATIOS OF PEPTIDES MEASURED BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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In recent years, fast atom bombardment mass spectrometry (FABMS) has been used extensively in the characterization of peptides. For the higher molecular weight compounds, the molecular ion cluster in the mass spectrum can provide information regarding the molecular weight as well as the elemental composition of the peptide. Unfortunately, the relative intensities of isotopic peaks obtained from the FAB spectra of organic compounds are often found to be larger than theoretically expected. The $(A+1)/A$ peak ratios, where $A=(M+H)^+$, are usually close to the predicted theoretical values within experimental error. However, subsequent isotopic peak ratios, i.e. $(A+n)/A$ ($n \geq 2$) often display substantial positive deviations. In addition to peptides, the distortion of isotopic peak ratios has been reported in the analysis of other classes of organic compounds including dyes, quinones and nucleotides^{1,2,3}. It has been suggested that reduction processes occurring either in the matrix or intramolecularly are responsible for the discrepancy between theory and practice.

Since the deviations observed in the isotopic peak ratios for peptides can vary substantially for different compounds studied under similar experimental conditions, a study was performed in the hopes of determining the factors responsible for the reduction occurring during analysis. In order to determine if the concentration of the analyte had an influence on the reduction, preliminary experiments were conducted using narrow voltage scanning and signal averaging techniques. Typical results obtained for methionine-enkephalin in a glycerol/water (2:1) matrix are illustrated in Figure 1. The $(A+2)/A$ ratio appears to be constant for concentrations above 0.03 M whereas it varies below that concentration and this is attributed to signal interferences at low S/N.

In attempts at quantifying the extent of reduction occurring in different peptides, experiments were conducted using narrow scans over the molecular ion cluster and signal averaging techniques. The results obtained in these experiments indicated that a relation existed between the extent of reduction and bombardment time. Since acids are commonly added to the matrix when studying peptides in order to increase the intensities of parent molecular ions, the effect of pH and type of acid added to the sample was investigated using methionine-enkephalin in a glycerol matrix. The results from these experiments indicated that in pure glycerol, the reduction effect was seen immediately and increased slightly with time. The addition of hydrochloric acid, a common matrix additive in peptide analysis, indicated that the initial $(A+2)/A$ ratio was higher than for glycerol alone and that the reduction proceeded at a slightly greater rate as well. Although the addition of hydrochloric acid may produce more intense spectra, it poses a limitation in the determination of isotopic peak ratios. Since earlier work with disulfide-containing peptides analyzed in thio glycerol had demonstrated that the addition of trifluoroacetic acid added to the sample prior to bombardment could inhibit reduction considerably, the effect of this acid was also evaluated. Samples containing trifluoroacetic acid tended to show a deviation in the $(A+2)/A$ ratio similar to that of hydrochloric acid, but yielded initial values lower than those obtained with hydrochloric acid.

The other factors that can influence the reduction process are structural elements contained in the peptides and in the matrix and these were studied by varying the nature of the peptide and the composition of the matrix. There are several functional groups, such as carbonyl, aromatic rings, and disulfide bridges, present in peptides which can act as reduction sites and the effect of these groups on the extent of reduction can be evaluated from the data given in Table I. The table illustrates the extent of isotopic peak enhancement for five different peptides in a thio glycerol matrix. As expected, the peptides containing disulfide bridges show the greatest reduction effect. Furthermore, pentaphenylalanine which contains more aromatic rings than either methionine-enkephalin or bradykinin exhibits more reduction than the latter. Thus the contribution of disulfide bridges to reduction is more significant than that of aromatic rings which is more important than that of carbonyl groups. In a similar way, the presence of functional groups such as NO_2 , S-S, and aromatic groups in the matrix inhibit the reduction of peptides whereas the presence of thiol or hydroxyl groups accelerate the process.

In order to determine whether the matrix composition affects the extent of reduction significantly in peptides which do not contain disulfide bridges, five different peptides were studied in

matrices composed of glycerol/water (2:1), thioglycerol, and water. The experiments conducted in water were done using continuous-flow FAB (CFFAB) in order to verify if the constant refurbishing of the sample could have an effect on the reduction process. As can be seen in Table II, there is no apparent trend to the reduction process occurring in the different matrices. However, the values measured in glycerol/water, thioglycerol or CFFAB are always higher than the theoretical values indicating that reduction is present in all cases and that it is not avoided even if a dynamic system is used. Thus this observation suggests that the reduction of peptides not containing disulfide bridges is FAB-induced. The reduction in disulfide-containing peptides, however, is accelerated during the FAB process but occurs in solution as well. Furthermore, experiments were conducted to compare the effect of the probe tip material on the reduction process. The results for a brass and gold tip demonstrated that these support materials have no significant effect on the reduction of peptides.

In conclusion, experimental results obtained in this study indicate that there are, in fact several mechanisms involved in the reduction of peptides in the analysis by FABMS and that the use of proper experimental conditions can minimize these effects which are dependent on several factors including the nature of the sample and of the matrix, the concentration of the analyte, the pH, the nature of the acids used as additives and also the time of analysis and atom beam energy.

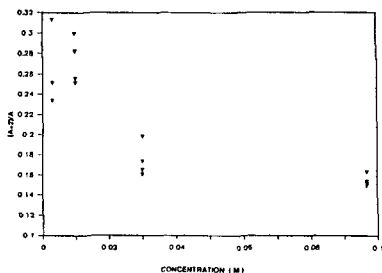


Figure 1. Effect of concentration on isotopic peak ratio measurements

TABLE I: SAMPLE DEPENDENCE

SAMPLE	MATRIX THIOLGLYCEROL		
	THEORETICAL	EXPERIMENTAL	% VARIATION
MET-ENKEPHALIN	0.114	0.123	+ 8
BRADYKININ	0.219	0.227	+ 4
PENTAPHENYLALANINE	0.154	0.225	+ 45
DAVP-TFA	0.274	0.514	+ 88
DAVP-AcOH	0.274	4.3	+1489

% VARIATION = (observed - theoretical) / (theoretical) * 100

TABLE II: ISOTOPIC RATIO MEASUREMENTS IN DIFFERENT MATRICES FOR PEPTIDES WITHOUT DISULFIDE BRIDGES

COMPOUND	(A+1) +				(A+2) +			
	THEORETICAL	GLY/H ₂ O	THIOGLYCEROL	CFFAB	THEORETICAL	GLY/H ₂ O	THIOGLYCEROL	CFFAB
Met-enkephalin YGGFM-OH	33.8	31.3 ± 0.8	33.9 ± 0.4	-----	11.4	13.3 ± 0.2	12.3 ± 0.3	-----
Bradykinin RPPGFSPR-OH	61.5	64 ± 2	61.4 ± 0.3	61.6 ± 0.9	20.7	24 ± 1	22.7 ± 0.3	22.9 ± 0.7
WRVFWHP-OH	69.2	68.0 ± 0.7	67.1 ± 0.4	67 ± 1	24.5	29 ± 3	28.5 ± 0.5	34 ± 1
Substance P RPPKQQFFGLM-NH ₂	78.9	78 ± 2	76.0 ± 0.4	74.8 ± 0.6	37.4	41 ± 2	38 ± 2	40 ± 2
Pentaphenylalanine FFFFF-OH	52.4	55 ± 2	57.3 ± 0.6	-----	15.5	18 ± 1	22.5 ± 0.8	-----

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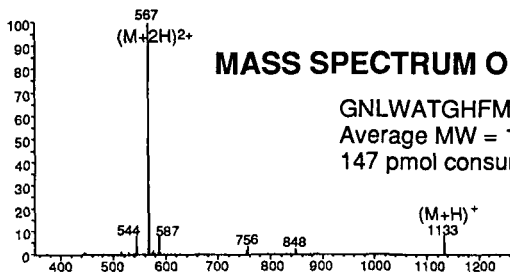
ANALYSIS OF CANCER CELL LINES FOR THE PRESENCE OF PEPTIDE GROWTH HORMONES AND OTHER PEPTIDES BY ION EVAPORATION IONIZATION / TANDEM MASS SPECTROMETRY.

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Outline : Small cell lung cancers stimulate their own growth by producing both peptide hormones and receptors for those peptides. The mRNAs of several prohormones, for example insulin-like growth factor-I (IGF-I) and gastrin releasing peptide (GRP), have been sequenced, and appear to code for multiple potentially-bioactive peptides. We are attempting to determine whether peptides predicted from these mRNAs are synthesized by the cells, and whether they have activities which may be used as targets for novel therapies. We also have identified novel C-terminal α -amidated peptides by a thermospray LC/MS technique (presented at ASMS 1988; in press *J. Chromatogr.* 1989) and are purifying them to determine whether they represent novel peptide hormones. These peptides are being analyzed using ion-evaporation ionization/ tandem mass spectrometry.

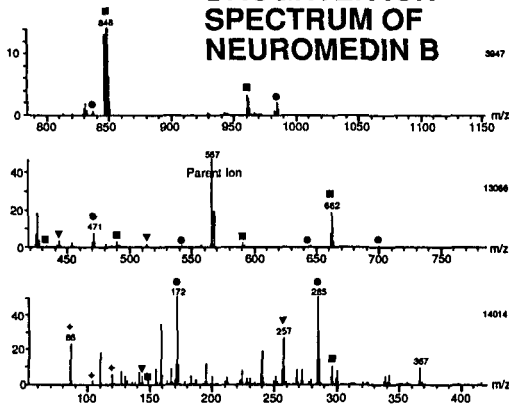
Methods : A Sciex triple quadrupole TAGA 6000 E was used for all studies. Ion evaporation mass spectra were obtained using a Sciex TAGA 6000 E triple-quadrupole mass spectrometer equipped with an Ionspray interface. Samples were infused at a flow rate of 2 μ L/min. Spectra were acquired for 1-2 min. MS/MS was carried out with an argon collision gas thickness of 1.6×10^{14} atoms/cm² and an energy of 60-70 eV. Experimental samples were obtained from the spent media of cancer cell lines (NCI-N417 and NCI-H716). The media was fractionated on C₁₈ HPLC columns using acetonitrile or methanol gradients. Fractions of interest were identified by either 1) immuno-cross-reactivity to peptides predicted from the known prohormone sequences of IGF-I or GRP, or 2) a thermospray LC/MS technique for identifying C-terminal α -amidated peptides. Lyophilized samples and standards were dissolved in CH₃CN/water with 0.5% formic acid. Samples in solution were acidified with 0.5% formic acid, or were diluted 1:1 with CH₃CN/aqueous formic acid.

Results and Discussion : Peptides tend to form multiply-charged ion species under the conditions used here. This process permits accurate mass determinations on peptides with masses beyond the stated capacity of the mass filter : 1400 da. for the Sciex quadrupole (see spectra GRP₁₄₋₂₇). The peptide fragmentation patterns induced by CID MS/MS are similar to those which have been reported for EI and FAB. Ions of the B, A and Y series are formed (figures show MS/MS spectra for the (M+2H)²⁺ ion of GRP₁₄₋₂₇, and the (M+2H)²⁺ ion of bombesin). For ions with >2 charges some of the rules regarding fragmentation seem more complex, or are complicated by the formation of multiply-charged daughter ions. However, it appears that fragmentation is driven by instabilities in the peptide structure due to the presence of multiple charges. Peptides isolated from the conditioned media of cancer cell lines have been analyzed, but as yet no firm sequence assignments have been made.

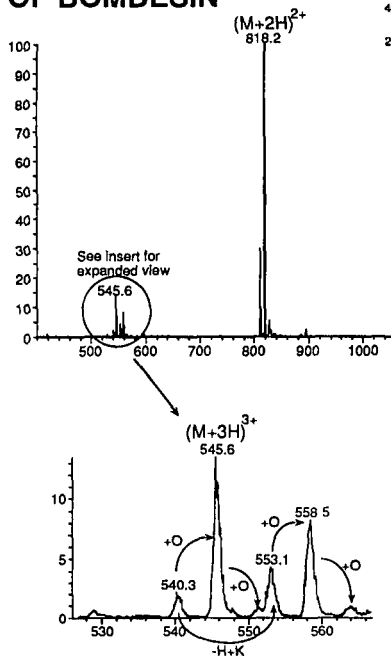


Legend :
Identified ions of the
Y (■), A (▼) and B (●)
series and composition
ions (♦) diagnostic of
particular amino acids.

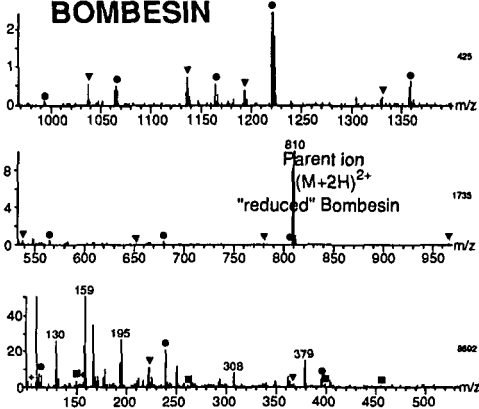
**DAUGHTER ION
SPECTRUM OF
NEUROMEDIN B**



MASS SPECTRUM OF BOMBESIN



**DAUGHTER ION
SPECTRUM OF
BOMBESIN**



ANALYSIS OF PHOSPHORYLATED AND SULFATED PEPTIDES BY
FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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The characterization of phosphorylated and sulfated peptides can be difficult, especially when both qualitative (location of modified sites) and quantitative (extent of phosphorylation and sulfation at each site) information is required. We have examined the behavior of a number of phosphorylated and sulfated peptides by FAB MS. The location of phosphate groups in peptides can usually be determined from the fragmentation information available in the positive ion FAB mass spectrum (Figure 1) (1,2). Quantitative information on the extent of phosphorylation can also be obtained by FAB by determining the molecular protonated ion intensity ratio for the phosphorylated and non-phosphorylated peptide and plotting it versus mole ratio. Although the ion intensity ratio versus the molar ratio plot is linear for all peptides examined, as illustrated in Figure 2, the slope is significantly less than one and a standard curve should be prepared. Table 1 gives the slopes for the peptides studied. The method is useful for protein digests since the presence of other peptides in the sample does not appear to affect the relationship. When less than 10% phosphorylation is present, no peak may be observed for the phosphorylated peptide so one must be careful about drawing conclusions at those levels. Work published by Poulter, *et al.* indicated that a relationship is evident between hydrophobicity and ionization efficiency and that the difference in ion intensities for protonated molecular ions of phosphorylated and the corresponding non-phosphorylated peptide could be corrected for by modifying the matrix (3). In our experiments, however, addition of strong acids only complicated the spectra and decreased the signal-to-noise ratios. The differences may be due to a different matrix selection.

In contrast, sulfated peptides can show a major fragment ion in both the positive and negative ion modes for the loss of the sulfate group (Table 2). The fragment appears at the same mass as the molecular ion of the non-sulfated analog. Thus, it is difficult to determine whether or not there is a mixture of sulfated and non-sulfated peptides. FAB MS can generally indicate the presence of a sulfate group but not the amount of sulfation. Matrix additives were examined to see what effect they had on the appearance of the mass spectrum of the sulfated peptides. Preliminary results seem to indicate that the addition of tetramethyl ammonium hydroxide increases the molecular ion intensity in the negative ion mode. We have also attempted to stabilize the sulfate group by preparing the methyl ester derivative, but the sulfate group is either cleaved or unmodified.

CONCLUSION

FAB MS can differentiate between phosphorylated peptides and the corresponding non-phosphorylated analogs; and the location of phosphorylation can be determined from the fragmentation pattern. Because no fragment ion for the loss of H_2PO_3 is observed at M-80, the ratio of the molecular ions of the phosphorylated and non-phosphorylated peptides can give quantitative information. The ratio of the molecular ion intensities do not directly correspond to the molar ratios but by plotting the intensity ratio versus the molar ratio, a linear relationship is observed. A standard curve must be prepared. Sulfated peptides, however, are not so easily distinguished from their non-sulfated analogs. The molecular ion is usually seen, but a fragment ion for the loss of the sulfate is observed at the same mass as the molecular protonated ion for the non-sulfated analog. The sulfated peptides do not always give sequence information because the sulfate group is so labile. It may be possible to modify the FAB matrix to increase to molecular ion intensity relative to the M-80 ion.

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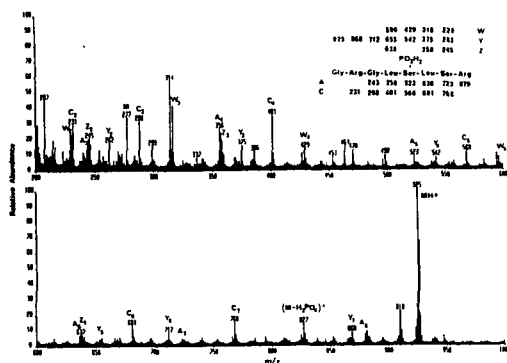


Figure 1

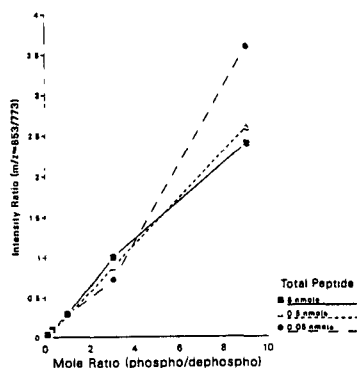


Figure 2

Table 1. Hydrophobicity indices and slopes of molecular ion intensity ratio versus mole ratio plots for peptides and corresponding phosphopeptides.

Peptide	Hydrophobicity Index	Matrix	Slope		
			5 nmol	0.5 nmol	0.05 nmol
GRGLSLR	67	DTT / DTE	0.4	0.5	0.3
GRGLS(PO ₃ H ₂)LSR	148	DTT / DTE	0.4	0.5	0.3
RRKASGP	501	DTT / DTE	0.2	0.5	-
RRKAS(PO ₃ H ₂)GP	594	DTT / DTE	0.2	0.5	-
LRRASVA	88	DTT / DTE	0.2	0.4	0.4
LRRAS(PO ₃ H ₂)VA	181	DTT / DTE	0.2	0.4	0.4
LRRASLG	-11	DTT / DTE	0.3	0.2	0.4
LRRAS(PO ₃ H ₂)LG	81	DTT / DTE	0.3	0.2	0.4
LRRASLG ^a		DTT / DTE	0.3	0.2	0.4
LRRAS(PO ₃ H ₂)LG		DTT / DTE	0.3	0.2	0.4
GRGLSLR ^b		DTT / DTE	0.4	0.4	-
GRGLS(PO ₃ H ₂)LSR		DTT / DTE	0.4	0.4	-
LRRASLG		DTT / DTE	-	0.9	-
LRRAS(PO ₃ H ₂)LG		DTT / DTE	-	0.9	-
LRRASLG		DTT / DTE	-	1.2	0.4
LRRAS(PO ₃ H ₂)LG		DTT / DTE	-	1.2	0.4

^a in a mixture containing an equimolar quantity of VESK and an equimolar quantity of GRGLSLR.

^b in a mixture containing an equimolar quantity of VESK and an equimolar quantity of LRRASVA.

Table 2

(A) Positive ion FAB MS results for Ac-Asp-Tyr(SO₃H)-Nle-Gly-His-Nle-Arg-Phe-NH₂ using various matrices

Matrix	(MH 80) ⁺	(MH 80 + Na) ⁺	MH ⁺	(M + Na) ⁺	(M + 2Na) ⁺	Other
2-HED	32	21	25	100	87	
Glycerol	100	-	27	15	-	
Glycerol + BaCl ₂	100	-	33	-	-	(M + Ba) ⁺ 40
Thioglycerol	95	25	42	100	39	
DTT / DTE (5:1)	100	30	30	82	20	

(B) Negative ion FAB MS results for various sulfated peptides

Peptide	Matrix	(M H) ⁻	(M 80) ⁻	Other
Ac-Asp-Tyr(SO ₃ H)-Nle-Gly-His-Nle-Arg-Phe-NH ₂	DTT / DTE	100	24	
Ac-Asp-Tyr(SO ₃ H)-Nle-Gly-Trp-Nle-Arg-Phe-NH ₂	DTT / DTE	100	5	
Ac-Asp-Tyr(SO ₃ H)-Nle-Gly-His-Nle-Arg-Phe-NH ₂	DTT / DTE	100	28	
Ac-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-NH ₂	DTT / DTE	100	-	
Ac-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-NH ₂	DTT / DTE	-	100	(M-H + K) ⁺ 73
Ac-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-NH ₂	DTT / DTE	-	100	
Ac-Asp-Tyr(SO ₃ H)-Met-Gly-Trp-Met-NH ₂	DTT / DTE	54	-	M-H + N(CH ₃) ₃ ⁺ 100
Ac-Asp-Tyr(SO ₃ H)-Nle-Gly-His-Nle-Arg-Phe-NH ₂	DTT / DTE	100	6	

CALIFORNIUM-252 PLASMA DESORPTION AND CESIUM ION LIQUID
SECONDARY ION MASS SPECTROMETRY STUDIES OF SOME NATURAL AND
RECOMBINANT PROTEINS.

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Californium-252 plasma desorption time-of-flight mass spectrometry (PDMS) has demonstrated the ability to analyze peptides and small proteins, whereas magnetic sector instruments using 10 keV xenon atoms or cesium ions as the primary beam, have also been employed in the analysis of small peptides up to 10 kDa. Recently, the use of higher energy ion guns has not only improved the liquid secondary ion mass spectrometry (LSIMS) sensitivity in the analyses below 10 kDa, but also expanded the useful analytical mass range of the technique. We have compared ^{252}Cf -PDMS and 35 keV Cs^+ -LSIMS in the analysis of a number of recombinant and naturally occurring proteins in order to determine their respective strengths and limitations as well as to assess their analytical utility.

In the PDMS analysis, a protein solution in 0.1% aq. TFA was deposited onto a nitrocellulose-coated aluminum foil as a 2:1 (v/v) mixture in ethanol, rinsed with 0.1-1.0 ml of a 0.1% TFA solution and spin-dried before its insertion into the BIOION 20 plasma desorption mass spectrometer. In the LSIMS analysis the protein sample, preferably in lyophilized form, was dissolved in 2 μl of m-nitrobenzyl alcohol or a glycerol/thioglycerol mixture directly on the probe tip followed by addition of 2-3 μl of neat TFA. The sample mixture was thoroughly mixed with a pasteur pipet prior to its insertion into the source of a VG ZAB-SE double sector mass spectrometer operating at 5 kV and equipped with a cesium ion gun generating a primary beam of 5-10 μA of Cs^+ at 35 kV. Two types of scans were generated, a wide range scan and a narrow scan of 3000 Da centered on the expected molecular weight. The protein samples that have been successfully analyzed were recombinant human α -2 interferon (rh-IFN α -2) and recombinant human granulocyte-macrophage colony stimulating factor (rh-GM-CSF; Schering-Plough/Sandoz), porcine trypsin, porcine and bovine somatotropin and a cyanogen bromide fragment of GM-CSF. The PDMS and LSIMS spectra presented similarities in the shape of the quasi-molecular ion peaks and the background continua. All these spectra were characterized by the presence of singly and multiply charged protonated molecule ions as shown in Figures 1 & 2. The mass measurement accuracy for the observed ions as determined by PDMS and narrow scan LSIMS experiments was better than 0.2% and 0.4% respectively, whereas the wide scan LSIMS experiments provided lower accuracy mass measurements.

Two factors were found to be important in obtaining LSIMS spectra: the amount of salt present in the protein sample and the liquid matrix used in the analysis. The presence of salt decreased the intensity of the molecule ion in the LSIMS spectrum of porcine trypsin in either m-NBA and thioglycerol/glycerol matrix. Similar effect on the intensity of the PDMS signal can be readily minimized by washing the nitrocellulose-adsorbed protein sample to remove salt contaminants. Among the several matrices attempted, m-NBA with neat TFA provided enhanced sensitivity and longer signal lifetimes. Sensitivity studies using porcine trypsin showed that the yield of the singly charged LSIMS molecule ion increased with increasing concentration from 100 pmol and 2 nmol, where it reached a saturation point. In the PDMS analysis the singly charged ion signal was observed from the 10 pmol sample, whereas those of the multiply charged species were observed from the 1 pmol sample.

In conclusion, 35 keV Cs⁺-LSIMS has comparable or slightly lower sensitivity and mass measurement accuracy compared to the PDMS method, even though both these mass spectrometric techniques have much better mass accuracy compared to that obtained by the most commonly used methods of gel-chromatography and SDS-electrophoresis.

FIGURE 1

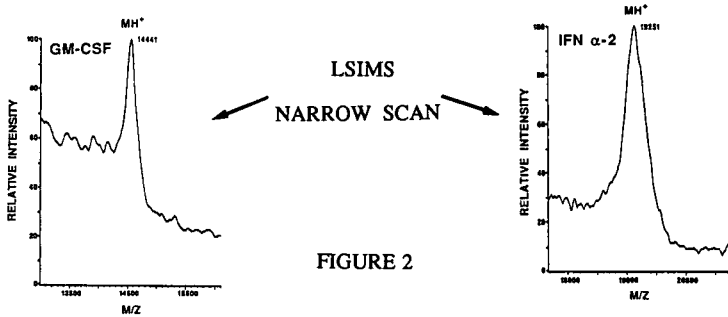
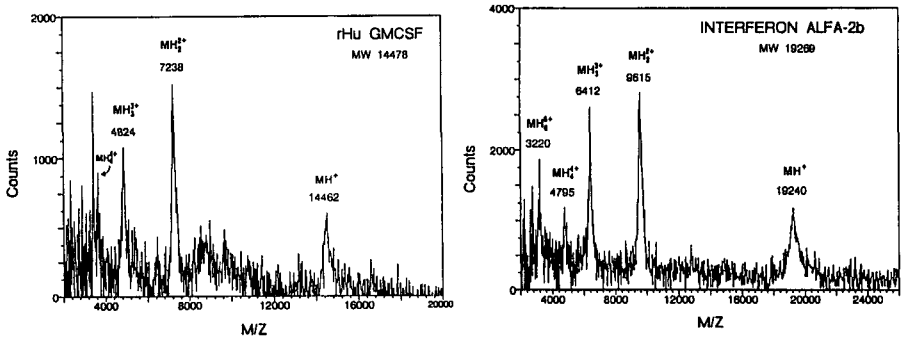
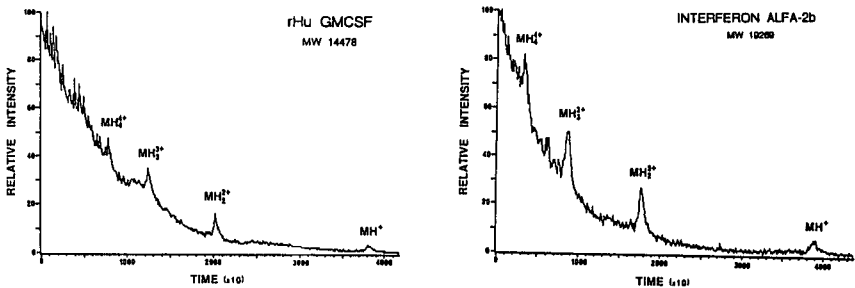


FIGURE 2



SOLVING DIFFICULT PEPTIDE STRUCTURAL PROBLEMS BY TANDEM MASS SPECTROMETRY

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That MS/MS with a tandem double-focusing instrument (e.g., a VG ZAB-SE 4F) yields much enhanced spectral information from peptides is by now well-established. Here we illustrate that MS/MS with high energy collisions is able, even in complex sample mixtures, to distinguish isomeric amino acids, sequence N-terminally blocked peptides, sequence through cystine bridges, handle unusual peptide modifications, and resolve complex amino acid insertion/deletion problems. Clarification of these problems would have been difficult by any other method. It should be evident that deducing chemical structures of modified peptides based solely on arithmetic differences between the expected and the observed molecular ion masses is potentially fraught with error.

SEQUENCING OF CYSTINE-BRIDGED PEPTIDES: Premature termination of peptide syntheses can, upon oxidation of cysteine thiols, result in mixtures of homogeneous and heterogeneous disulfide dimers, the exact chemical nature of which is difficult to determine. If one maintains the cystine bridges intact, however, it is possible to sequence through the disulfide bond. Fragmentation of the cystine yields a characteristic spectral pattern consisting of a very intense triplet of signals separated by one Da derived from disulfide cleavage bracketed on either side by peaks representing $\pm$ sulfur. This is illustrated in Figure 1 by the tandem mass spectrum of a simple, N-terminally blocked homodimer. Note the intensity of the disulfide cleavage ions (m/z 604 is 7 times more intense than m/z 545). In general we treat the smaller of the linked peptide sequences as a modification of the cysteine of the larger peptide and use the traditional ABCD/VWXYZ nomenclature. We then reverse the peptides and treat the larger sequence as a modified cysteine of the smaller and employ 'A'B'C'D'/V'W'X'Y'Z'. 'Y' indicates internal fragments equivalent to Y-type cleavages from a single peptide chain formed by collisional reduction of the dimer.

DISULFIDE MAPPING OF PROTEINS: Tandem MS also promises to be useful in disulfide bond mapping of proteins. This technique can be applied to situations in which the parent ion signal intensity is too weak to allow tandem mass spectrometric sequencing through a cystine bridge as described above. For example, it is still often possible to perform disulfide mapping of higher mass compounds such as insulin or disulfide-bridged peptides in enzymatic digestion mixtures of proteins, even in cases where the parent ion signal would normally be considered insufficient for tandem MS. In these cases virtually the only fragments observed correspond to cystine bridge cleavage which yields the molecular weights of the linked peptides.

DISULFIDE SULFUR DELETION: A series of synthetic somatostatin analogues containing L- and D-amino acids and a putative disulfide were found to be 32 Da mass deficient by FAB. Exact mass measurements strongly suggested deletion of a sulfur rather than amino acid substitution. The compound did not display a mass shift when treated with DTT/DTE/ NH_4OH to reduce the disulfide thought to be present. By first opening the cyclic peptide by prolonged chymotryptic digestion (24 hrs, 35% yield) and acquiring a tandem mass spectrum of the hydrolyzed peptide ($M + \text{H}_2\text{O}$)⁺, it was possible to demonstrate that the two cysteines were still bridged but that a sulfur had been split out. The tandem mass spectrum of the ring-opened peptide is rich in sequence information (Figure 2). Fragment ions not explained in the scheme correspond to internal double cleavages.

SIMULTANEOUS AMINO ACID INSERTIONS AND DELETIONS: Unexpected insertions or deletions of amino acids are a common problem in peptide synthesis. In one case, a synthetic peptide purified as a single peak by RP-HPLC, and which had the expected amino acid composition, was shown by FABMS to be a mixture of five components differing by insertions and deletions of glycine. The tandem mass spectrum of the first glycine deletion peak revealed further heterogeneity in that the glycine deletion could be demonstrated in either one of two positions. Proof of deletion in the third position would require internal double cleavages which are often weak or not observed. Apparently the number of glycine deletions balanced the number of insertions so that the amino acid composition was fortuitously correct.

ISOMERIC ASPARTIC ACIDS: One batch of recombinant tumor necrosis factor (TNF) had proven inactive. FAB mapping of the inactive protein revealed a tryptic fragment 1 Da more massive than expected (1). The deamidation site was localized to a specific asparagine residue by tandem mass spectrometry; however, the deamidation process can result in isomeric forms of aspartic acid. By comparing the tandem mass spectrum of the tryptic peptide with tandem spectra of synthetic model compounds containing α - and β - aspartic acid, it was determined that the inactive protein contained a mixture of the aspartic acid isomers in place of the asparagine. D, V, and W ion fragments enable aspartic acid isomers to be distinguished much like the leucine/isoleucine method (2).

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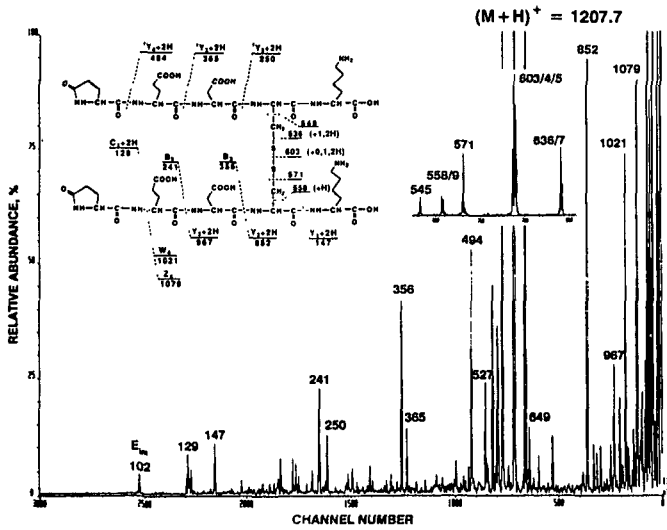


FIGURE 1

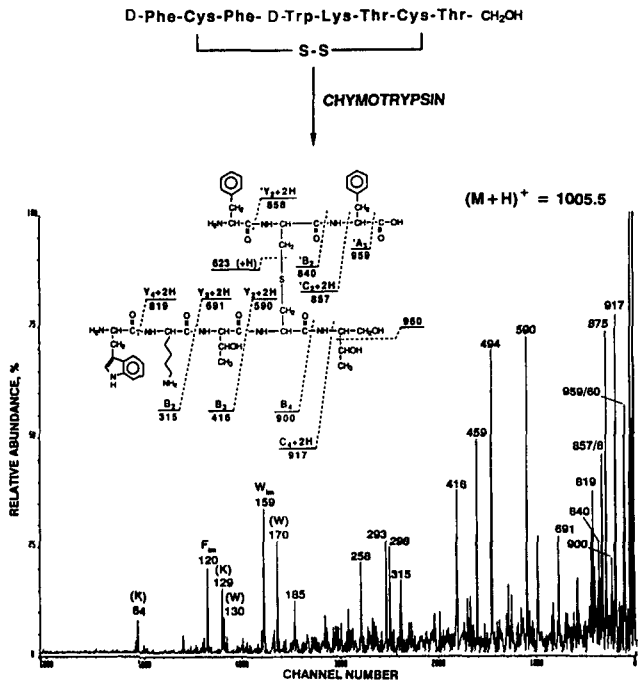


FIGURE 2

NEW TECHNIQUES IN THE HYBRID TANDEM MASS SPECTROMETRY OF PEPTIDES

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Recent studies in this and other laboratories have established the utility of hybrid tandem MS in the characterization of peptides, while recognizing the distinct advantages of four sector instruments for certain applications. We describe here several new developments which enhance the value of hybrid tandem MS in the detection and structural analysis of peptides. All analyses were performed on a VG ZAB SEQ instrument of BEqQ configuration. Acquisition was via a VG 11-250 data system. Ionization was by fast atom bombardment with a primary atom beam of xenon (8 keV). Two means of sample introduction were used: (i) conventional FAB probe introduction using 2,2'-dithiodiethanol/thioglycerol (1/1) as the matrix; (ii) continuous flow introduction using the following solvent composition - glycerol/acetonitrile/water (1/2/7) plus 0.1% trichloroacetic acid. The solvent was delivered at 4.5 μ l/min using a Waters high pressure syringe pump under control of a microliter-adapted Waters Automatic Gradient Controller. Injections were made through a Rheodyne 7520 injector with a 0.5 μ l fixed internal loop. Silanized fused silica tubing (1m x 0.05 mm, id) was swaged into the injector and the rear of the continuous flow probe with graphitized vespel ferrules. The space between the capillary and the "bullet" shaped probe tip was sealed by wrapping a small piece of teflon tape around the capillary and screwing the tip firmly in position. Capillary placement was adjusted to be slightly recessed in the probe tip. The probe tip was heated to 70 C for all injections.

1) Structural analysis.

Low energy CAD in the quadrupole collision cell of a hybrid instrument has been shown to provide useful structural information for small peptides (with M_r to above 1000). The observed fragmentations generally correspond to cleavages of the peptide chain. CAD of peptide MH^+ ions at keV collision energies have been shown (1) to promote additional fragmentation of amino acid side chains, permitting, for example, the differentiation of leucine and isoleucine residues. When collisional activation of peptide MH^+ ions is performed in a quadrupole collision cell, $E_{lab} > 200$ eV is required for detection of these fragment types and signal intensities are poor (2). Instruments of BEqQ geometry provide the facility for both low energy CAD (in q) and high energy CAD (between B and E). In the latter case, simultaneous scanning of E and Q yields daughter ion spectra with good mass resolution.

An appropriate strategy for structural characterization of peptides using a BEqQ instrument therefore involves (a) low energy collisional activation in q with scanning of Q to provide sequence information, and (b) high energy collisional activation in the cell between B and E, with linked E/Q scanning over selected narrow mass ranges to permit the differentiation of isobaric amino acid residues. Figure 1 illustrates the second stage of this strategy applied to Substance P. The E/Q linked scan recorded following high energy CAD of MH^+ ions includes the A_{10} fragment of m/z 1171 and an ion of m/z 1129 corresponding to additional loss of 42u, characteristic of a leucine (rather than isoleucine) residue.

2) Trace analysis.

Continuous flow sample introduction for FAB MS facilitates applications to trace detection of peptides. When analyses are performed on a tandem instrument, improved selectivity of detection may be achieved using selected reaction, rather than selected ion, monitoring. Figure 2 illustrates this approach with the analysis of replicate aliquots (500 pg) of Substance P, fragment 2-11. The fragmentation of MH^+ ions (m/z 1152) to m/z 966 (Y_g) was monitored.

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FIGURE 1

Substance P: E/Q linked scan.

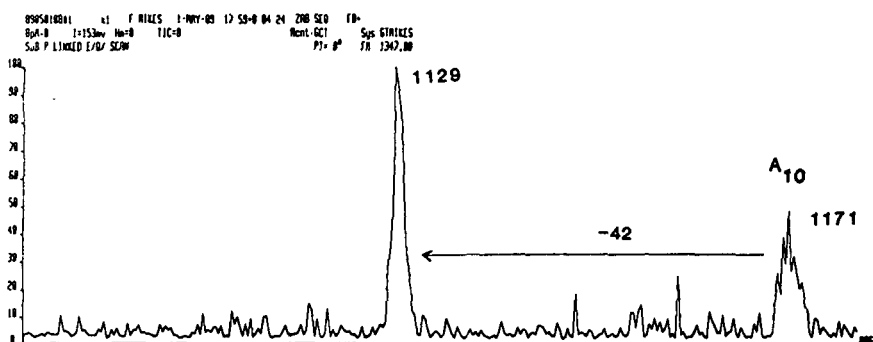
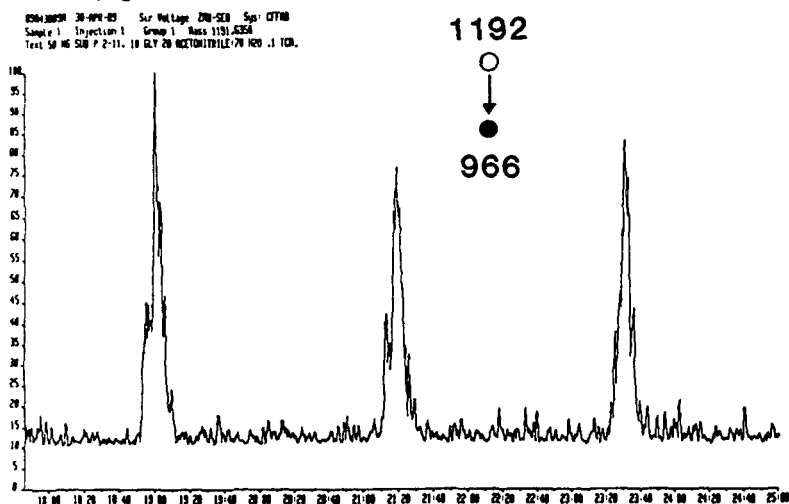


FIGURE 2

**Selected Reaction Monitoring
500 pg Substance P (fragment 2-11)**



STUDIES ON STRUCTURES OF BRAIN CALBINDINS:
AMINO ACID SEQUENCE ANALYSIS ON THE TSQ 70

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Calcium binding proteins, calbindins, whose synthesis is regulated by vitamin-D, have a widespread distribution in vertebrate tissue. Recently, two new calbindins, CaBP-28k and CaBP-29k, have been isolated from the Purkinje cells of rat cerebellum (1). Both proteins exhibit cross-reactivity with antibodies raised against chick intestinal calbindin-D_{28k}. It has been suggested that in the nervous system CaBP-28k plays a role in cell differentiation and in calcium mediated enzyme activities; the role of CaBP-29k is unknown. Recently, a 29 kDa calcium-binding protein, calretinin, was isolated from chick retina and brain (2). However, the neuronal distribution of calretinin does not coincide with the distribution of chick brain calbindin, as calretinin is absent in cerebellar tissue. Our initial objective was to determine if the two brain calbindins were derived from a single precursor. Most recently our objectives have been to characterize the complete amino acid sequence of both proteins and to determine the extent of homology between the two proteins and chick calretinin.

Both calcium binding proteins were isolated from the Purkinje cells in rat cerebellum using gel permeation chromatography and preparative gel electrophoresis. Each protein was purified further by reverse phase HPLC. Typically, proteolytic digestion of the reduced and S-carboxymethylated protein (3-5 nmoles) was accomplished using trypsin, endo-Glu-C, or endo-Asp-N at 37°C for 8 to 24 hours. In addition, approximately one nmole of the native protein was digested by either trypsin or endo-Lys-C at 37°C for 24 hours. Peptides were fractionated by reverse phase HPLC. Peptides in the resulting fractions were characterized by tandem mass spectrometry using either a triple quadrupole or a quadrupole Fourier transform instrument.

In an effort to determine if the two calbindins were derived from the same precursor, both proteins were enzymatically digested under identical conditions with trypsin, endo-Asp-N, and endo-Glu-C. The resulting peptides from each digest were fractionated and the molecular weights were recorded. Each of the three digests produced only a few peptides which were found in both proteins, determined by similarities in molecular weight and HPLC retention time. Partial sequence analysis indicates that the two proteins are only 67% homologous, which further suggests that these two calbindins are different proteins.

Sequence data indicates that CaBP-28k is rat spot 35 protein, a Purkinje cell-specific calcium-binding protein, which has been sequenced using oligodeoxynucleotide probes (3). An endo-Lys-C digest of 800 pmoles native CaBP-28k yielded 75 percent of the sequence. In addition, metastable decomposition and photodissociation QFTMS spectra strongly suggest that the amino terminus is acetyl-alanine.

Interestingly, several peptides isolated from different digests of CaBP-29k only exhibit homology to chick calretinin, whose partial sequence has been determined from the chromosomal gene clone. This suggests that CaBP-29k may be rat calretinin or a closely related calcium binding protein. Research will continue to determine the primary structure of both cerebellar calbindins.

Shown below in Figures 1-3 are spectra that illustrate the current status of peptide sequence analysis on the TSQ-70 mass spectrometer in our laboratory. Collision experiments are conducted in the energy range of 25-40 eV on a 2/1 mixture of argon and xenon at 3 mtorr. Sample quantities are typically 1-10 pmol below m/z 1,000; 5-50 pmol below m/z 2,000 and 30-300 pmol below m/z 2600.

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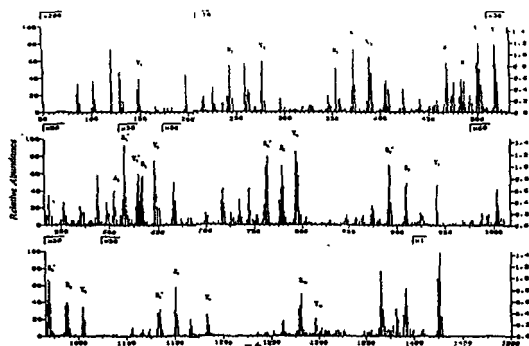


Figure 1. Collision activated dissociation mass spectrum of the oligopeptide Glu-Lys-Glu-Asn-Phe-Phe-Gln-Glu-Lys from 29 kDa calbindin.

29 KDA CALBINDIN PEPTIDE

130	243	372	486	633	780	908	1037	1150	1229	1425	R _n
Glu	Leu	Glu	Asn	Phe	Phe	Gln	Glu	Leu	Glu	Lys	
1425	1226	1183	1054	940	793	646	518	389	276	162	Y _n

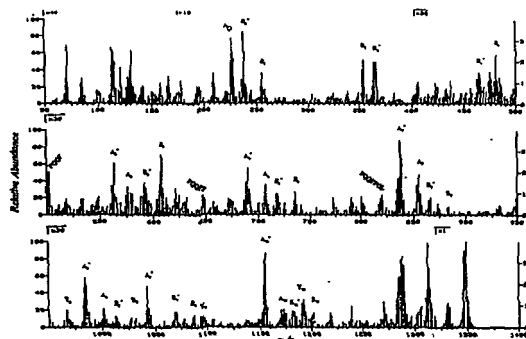


Figure 2. Collision activated dissociation mass spectrum of 1 picomole Substance-P.

1 PMOL SUBSTANCE-P

157	254	382	478	607	735	853	1030	1087	1200	1348	R _n
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met-NH ₂	
1348	1192	1095	956	849	741	613	466	319	262	149	Y _n

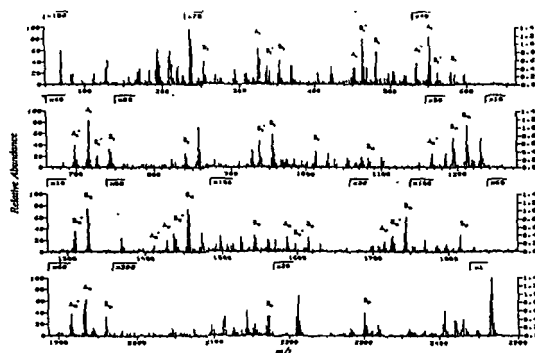


Figure 3. Collision activated dissociation mass spectrum of 50 picomoles ACTH Clip.

157	254	382	478	607	735	853	1030	1087	1200	1348	R _n
Arg	Pro	Lys	Pro	Gln	Gln	Phe	Phe	Gly	Leu	Met-NH ₂	
1348	1192	1095	956	849	741	613	466	319	262	149	Y _n

TOF-SIMS and FAB of Modified Peptides: A Comparison of Sensitivities

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The last years have shown the necessity of ultimate sensitivity in the analysis of peptides in protein and/or DNA analysis. The sample amount directly available or available after preseparation techniques such as high pressure liquid chromatographies is in or even below the pmol- range.

We investigated a number of modified, pharmaceutically relevant peptides in the mass range between 500 and 900 u (see figure 1) both with a high resolution TOF-SIMS built at the University of Münster ($I_p = 1$ pA, spot diameter 50 μ m, $m/\Delta m_{50\%} = 4000$ [1]) and with a Kratos MS 80 FAB instrument (acceleration voltage 4 kV, $I_p = 10$ μ A, $m/\Delta m_{10\%} = 1000$). Both instruments were operated in the same laboratory of the Bayer AG pharmaanalytical research center.

For SIMS measurements 1 μ l of diluted solutions ($10^{-3}, \dots, 10^{-7}$ mol/l) in methanol was deposited onto an area of about 40 mm² of an etched silver substrate. In the case of FAB 0.5 μ l of the corresponding solutions was mixed with 0.5 μ l m-nitrobenzyl alcohol (MNBA) as matrix.

Table 1 shows the results obtained for the different peptides.

The FAB detection limit is mainly determined by background peaks originating from the matrix material and the transmission of the double focusing magnetic spectrometer. The lowest detectable sample amount was 50 pmol, in one case 5 pmol.

The TOF-SIMS sensitivity is limited by chemical background from the solutions and the fact that the analyzed area is about 10^4 times smaller than the prepared sample area. The lowest deposited sample amount was 1 pmol, in one case 0.1 pmol, corresponding to 0.1 fmol and 0.01 fmol in the analyzed area respectively. By an improvement of the sample deposition technique it should be possible to reduce the required sample amount.

Future investigations will concentrate on this point in the case of TOF-SIMS and will include a Finnigan MAT 90 instrument for the FAB investigations.

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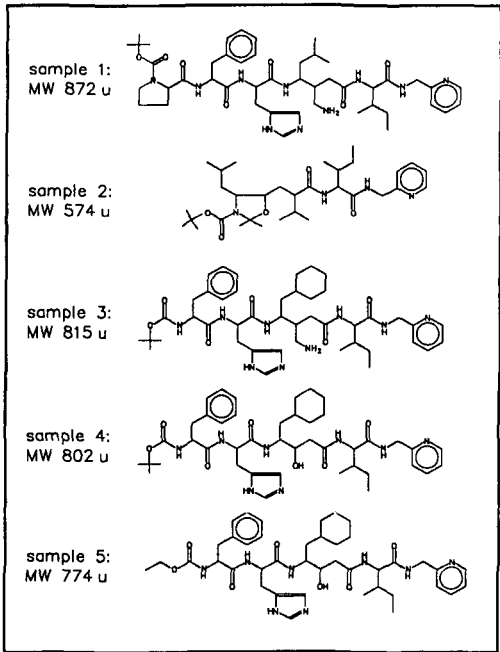


Figure 1: Investigated peptides

Sample	TOF-SIMS		FAB
	pmol deposited	fmol in analyzed area	pmol
1	1	0.1	50
2	1	0.1	5
3	0.1	0.01	50
4	1	0.1	50
5	1	0.1	50

Table 1: Obtained sensitivities

Structural Characterization of Lens Proteins by FABMS

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Cataract, which is the most common cause of blindness throughout the world, is due to the failure of the lens to transmit light. Of the several different factors which contribute to opacification of the lens, aggregation of proteins located within the lens is believed to be the most important. Although cataract is most often treated by extraction of the lens, it is desirable to understand the causes of cataract at the molecular level so that preventive treatments may be developed. A complete understanding of cataractogenesis will be possible only when the structures and reactivities of the lens proteins have been well characterized.

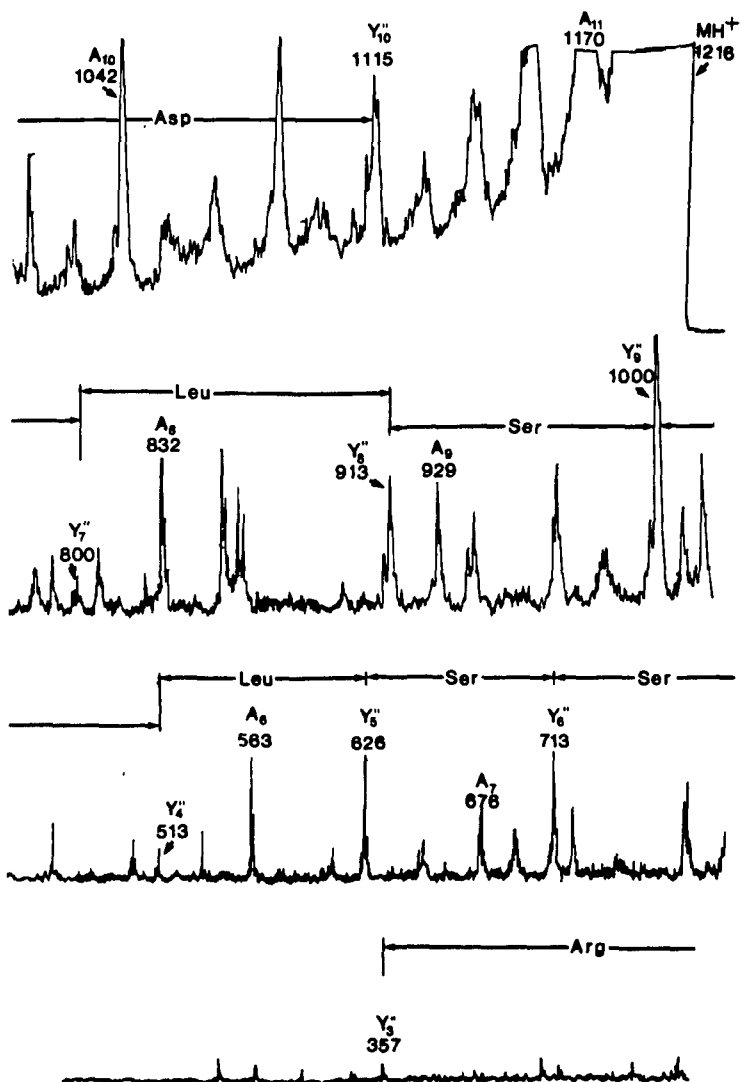
Approximately 30% of the wet weight of the lens comprises a group of structural proteins often referred to as crystallins. These proteins are classified as α , β , and γ , according to their tendency to form high molecular weight aggregates. Each of these general types of crystallin consists of five to ten forms which differ in amino acid sequence. For some forms, the complete sequence is known. Other forms have been characterized primarily by their chromatographic properties or by their peptide maps. This is the best characterized of the lens proteins. All of the lens proteins contain cysteinyl residues which may participate in intermolecular disulfide crosslinking, and hence formation of covalently bonded aggregates.

The goal of this investigation is to characterize at the molecular level the structures of lens proteins so that future investigations of protein aggregation, as it relates to cataract, will be possible. This will require isolating the different forms of the lens proteins and confirming or determining their amino acid sequences. FABMS will be used to make molecular weight maps of peptides produced by proteolytic fragmentation of homogeneous lens proteins. This information will be used to confirm or correct sequences which have been predicted or determined by other methods. For those crystallins for which complete sequences have not reported, this information will be used as a measure of the homology of lens proteins.

In this preliminary investigation, highly purified forms of two of the best characterized forms of lens proteins, β Bp and γ II, have been isolated from bovine lenses. These native proteins were fragmented with trypsin and *Staphylococcus aureus* V8 protease and fractionated by reversed phase HPLC. Molecular weights of the peptides, determined by FABMS, were used to verify the sequences of these proteins. These results indicate that sequences predicted from the corresponding cDNA are correct, where as those based on conventional sequencing methods contain several errors.

To achieve the ultimate goals of this investigation, we must be able to identify as well as locate modifications to the lens proteins. Linked scan daughter ion mass spectra, as illustrated in the accompanying figure, will undoubtedly play a major role in this investigation. This spectrum, which was obtained for the 90-100 segment of β Bp, contains a nearly complete set of peaks for both the A and Y" series of fragments.

Results of this preliminary investigation of the crystallins suggest that FABMS will be very useful in identifying modifications which ultimately lead to cataractogenesis. Peptide mapping results of two of the crystallins, including linked-scan daughter ion spectra, have been used to confirm their primary structures. From these results, it is apparent that FABMS can be used to determine precisely which portions of the lens proteins participate in intermolecular crosslinkages.



Linked Scan Daughter Ion Spectrum

COLLISION-INDUCED DISSOCIATION OF MULTIPLY CHARGED PEPTIDES

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In order to fragment ions in MS/MS, a variety of methods have been proposed to deposit sufficient internal energy into an ion to effect decomposition. These include collision induced dissociation (CID), photodissociation, surface induced dissociation (SID), and electron induced dissociation. CID has proven to be the most popular because of the instrumental simplicity, large cross section for excitation, and ease of changing parameters such as collision gas thickness and collision energy which can effect fragmentation efficiency. One draw back is that CID efficiency has been observed to decrease with increasing mass (ca. 800 d or higher) because of a dissipation of the collision energy into many vibrational modes with a resulting small fractional increase in the internal energy of the molecule.

With Ion Evaporation ionization, intense multiply charged molecular ions are produced from peptides and proteins effectively extending the mass range by a factor of 10 - 100 fold. A Sciex API III triple quadrupole mass spectrometer with Atmospheric Pressure Ionization and an Ion Spray interface was used to produce Ion Evaporation spectra and conduct MS/MS studies of multiply charged ions.

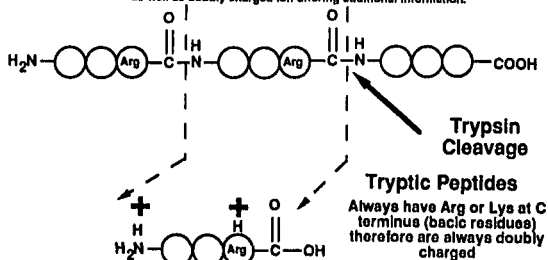
The majority of the work on the MS/MS of peptides has concentrated on the CID of the singly charged pseudo-molecular ion. Studies were undertaken to see if CID could be performed on molecules in the 2 - 20 kdalton range on molecules with as many as 20 charges. Because of their highly charged state internal repulsive forces put these molecules into a "strained" configuration allowing for a very efficient CID process at low collision voltages. Daughter ion spectra of the $(M+2H)^{2+}$, $(M+3H)^{3+}$, and $(M+4H)^{4+}$ of bovine insulin (mw = 5732) are shown.

It has been determined that the charges are localized at the basic amino acid residue sites in a peptide (arginines, lysines, and histidines) as well as at the amino terminus. Systematic studies correlate the type of fragmentation observed with the position of the charged site in the molecule. With doubly or triply charged parent ions with the charges localized toward the amino terminus sequence information is primarily of the "A and B" type. Ions of the "D" type are also observed. Substance P was used as one of the model compounds for this study. If the charges are located at either end of the molecule the fragmentation is dominated by "Y and B" ions with valuable sequence information being observed.

Proteins treated with trypsin yield peptides that are all doubly charged because trypsin cleaves at the carboxy terminus side of arginines and lysines. On-line LC/MS separation of the tryptic fragments of hemoglobin are shown. Daughter ion spectra of the doubly charged tryptic fragments yield the desired "Y+B" fragmentation providing extensive sequence information.

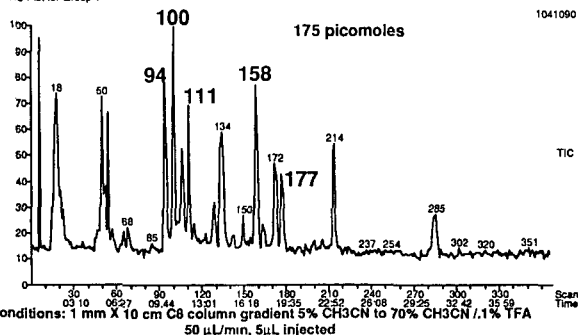
Tryptic Peptides From Protein Digests are Always Doubly Charged with Charge Localized at Ends

- Exceptions:
1. Carboxy terminus peptide is likely to be singly charged because it will not have an arginine or lysine. It can therefore be identified.
 2. Fragments containing an internal histidine will have a triply charged as well as doubly charged ion offering additional information.

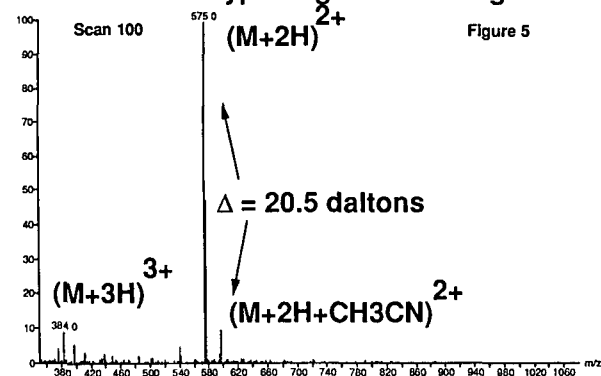


TIC (300-1400 daltons) of a Tryptic Digest of Human Hemoglobin

hemoms.TDT Mar 26, 1989 15 10:57. 366 scans in 1 group 40:04
TIC Plot for Group 1

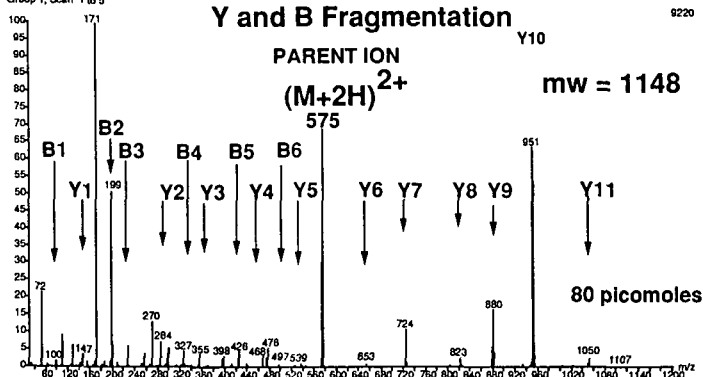


Scan 100 From Tryptic Digest of Hemoglobin



Daughter Ion Spectrum of Tryptic Peptide T-14 from Hemoglobin

hemoms.TDT May 1, 1989 17:58 43 6 scans in 1 group: 05:16
Group 1, Scan 1 to 5



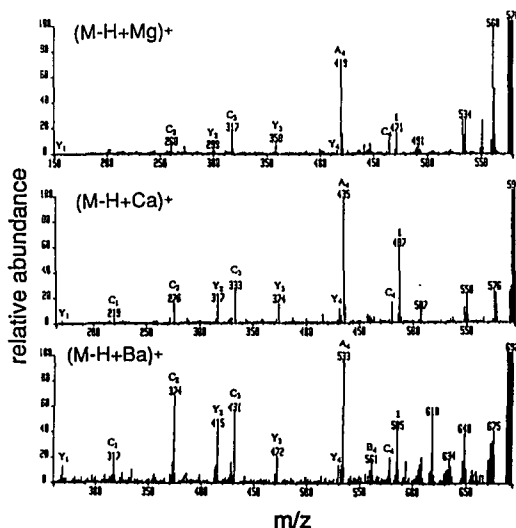
COLLISION-INDUCED DECOMPOSITIONS OF PEPTIDES CATIONIZED WITH ALKALINE EARTH AND ALKALI METAL IONS

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A few research groups recently have investigated the complexation of peptides and alkali metal ions, mainly Li^+ and Na^+ , by collisionally activating the $(\text{M}+\text{Cat})^+$ ions (1-3). The predominant fragmentation observed in the collision-induced decomposition (CID) spectra was proposed to be a rearrangement involving the cleavage of the C-terminal amino acid to produce $(\text{B}_{n-1}+\text{OH}+\text{Cat})^+$ ions (1,2).

In work presented here, CID spectra of $(\text{M}+\text{Cat})^+$ and $(\text{M}-\text{H}+\text{Cat})^+$ ions, where Cat=alkali and alkaline earth metal ions, respectively, of 13 model peptides were investigated. The parent ions were desorptively ionized by fast atom bombardment (FAB), then B/E linked scanning was used to obtain the CID spectra. Trends in relative ion abundances with changing metal ion are observed.

The $(\text{M}-\text{H}+\text{Cat})^+$ series, in which Cat=an alkaline earth metal ion, for leucine enkephalin is shown in Fig. 1 and is a representative example displaying the characteristic fragmentations. Collisional activation (CA) of



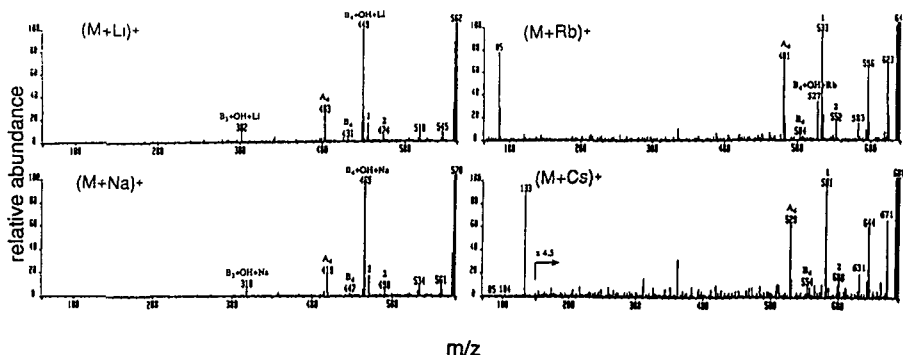
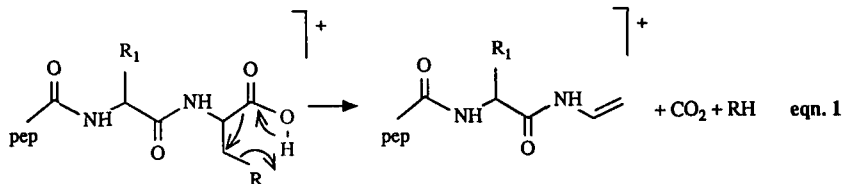


Fig. 2 CID spectra of Tyr-Gly-Gly-Phe-Leu (leu-enkephalin) cationized with alkali metal ions. The A and B series follow the nomenclature in ref. 4. The 1 ions arise from the loss of the tyrosine side chain. The 2 ions arise from the mechanism in eqn. 1, discussed below. To conserve space, (M+K)⁺ is not included.

Because fragment ions containing both the N- and C-terminal amino acids are observed in CID spectra of (M-H+Cat)⁺ ions, CIDs of these ions may be used to complement structural assignments derived from CIDs of (M+H)⁺ ions. Within the alkali-ion series of (M+Cat)⁺ ions, CID spectra show an increase in the abundance of (A_{n-1}-H+Cat)⁺ ions as the size of the metal ion increases, which suggests that the larger cations prefer a site other than the carboxylate terminus.

We also find that (M+Cat)⁺ parent ions that have an appropriate R group on the C-terminal amino acid decompose via a rearrangement reaction that involves the C-terminal carboxylate and the C-terminal R group (eqn. 1). This fragmentation is useful for delineating leucine from isoleucine and from other C-terminal amino



acids. In cases in which the C-terminal amino acid is leucine, the identifying fragment corresponds to loss of 88 u (labeled as 2 in Fig. 2). For isoleucine, the fragment ion arises from loss of 74 u; for methionine, the fragment ion arises from loss of 106 u.

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PD/FAB-MAPPING OF BOVINE NEUROPHYSINS.

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We have previously reported on the feasibility of determining the pattern of bovine pituitary peptides by mass spectrometry [1]. The largest molecules observed were oxytocin-neurophysin (OT-NP) and vasopressin-neurophysin (VP-NP) with molecular weights of almost 10,000 u. We have since studied these small proteins in more detail for the following two reasons: i) The molecular weights determined for equivalent samples from duplicate chromatographic separations differed considerably. We suspected that this variation was largely due to sample heterogeneity (multiple cationization, methionine oxidation, cystine reduction, amino acid substitutions, methylations, truncations, etc.) rather than to technical difficulties of the mass measurement in this mass range. We therefore initiated this study to prove the heterogeneity of our neurophysin samples. ii) Many neuronal peptides coexist in multiple forms due to only partial completion of proteolytic and other post-translational modification reactions. As these modifications proceed, the chemical message being conveyed by the corresponding secretory neurons may change. The neurophysins are interesting model compounds to study these processes because of their microheterogeneity and because they are believed to be subject to methylation and C- and N-terminal truncations. Neurophysin variants with only single amino acid differences may be resolved and quantified with RP-HPLC, but the common practice [2] of identifying the variants by amino acid analysis, carboxypeptidase digestion, gel electrophoresis, and Edman degradation is time-consuming. We were interested in exploring the possibility of more rapidly identifying the variants by mass spectrometry.

We have resolved our crude neurophysin fraction by RP-HPLC (Fig. 1) and analyzed the fractions that correspond to single HPLC peaks by FAB-MS and by PD- and FAB-mapping. Following purification, at least some of the individual neurophysin variants can be mass measured with reasonable accuracy although multiple cationization still leads to broad molecular ion peaks. Molecular weight information may thus lead to tentative identification of particular neurophysin variants including their methylated forms. Fig. 2 shows the FAB spectrum of fraction 5 (Fig. 1), which, in combination with N-terminal sequence information from Edman degradation, identifies this variant as OT-NP(1-91). The proteins in fractions 4 and 3 have been tentatively identified as OT-NP(1-92) and OT-NP(1-93), respectively.

Tentative identifications can be confirmed by peptide mapping. The neurophysins in fractions 1 and 2 have been characterized by PD- and FAB-mapping as Val-89-VP-NP and Ile-89-VP-NP, respectively. Fig. 3 compares the expected tryptic fragments for the known bovine VP-NP and OT-NP variants. Using the crude tryptic digest, not all tryptic peptides were observed but, unless methylation patterns are to be deduced, complete maps are not necessary. Since the protein cores of the neurophysins are conserved and the amino acid variation is only in the C- and N-termini, the latter unambiguously characterize the neurophysins. Fortunately, they give rise to prominent ions in the PD- and FAB-maps (Figs. 4 & 5). The N-terminus allows differentiation between VP-NP and OT-NP ($[M+H]^+$ 934 vs. 900) and the truncated C-terminus VP-NP(87-93) allows differentiation between Val- and Ile-VP-NP ($[M+H]^+$ 761 vs. 775). The full length of the VP-NP variants was deduced from analysis of proteolytic digests with *S. aureus* V8 protease (for example, $[M+H]^+$ 887 for Val-89-VP-NP(88-95)), Fig. 5).

Multiple molecular ions due to cationization with protons and sodium and potassium ions were seen throughout the study. With FAB-MS this can only be avoided/reduced by careful choice of reagents and matrices, whereas PD-MS offers the possibility of removing the interfering ions by washing of the sample on the target. Unfortunately, however, the small peptides may also be removed during this procedure (Fig. 4) and this option therefore has to be used with caution.

In conclusion, the mass variations previously observed for the neurophysins are largely due to sample heterogeneity. Individual neurophysin variants can, after chromatographic resolution, rapidly be identified by PD- and FAB-MS in conjunction with enzymatic digestions.

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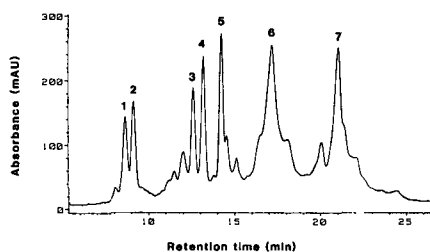


Fig. 1 RP-HPLC of crude NP fraction (C18, 250 x 4 mm; 27-45% CH₃CN in 0.1% TFA) (1) Val-VP-NP, (2) Ile-VP-NP, (3) OT-NP(1-93), (4) OT-NP(1-92), (5) OT-NP(1-91), (6) B-End(1-27), (7) N-terminus POMC.

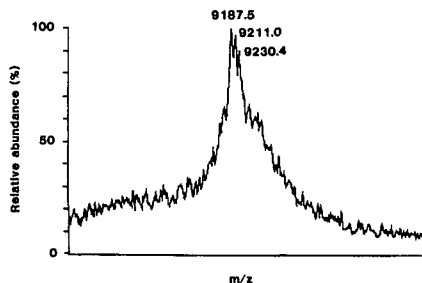


Fig. 2 FAB-MS of molecular ion region of putative OT-NP(1-91); calculated [M+H]⁺: 9188.3 u.

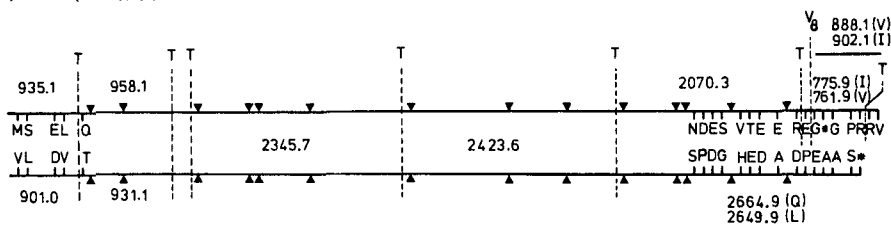


Fig. 3 Expected tryptic and V₈ peptides for bovine neurophysin variants. VP-NP on top, OT-NP on bottom; * indicates microheterogeneity; ▲ indicates position of cysteines; numbers refer to [M+H]⁺.

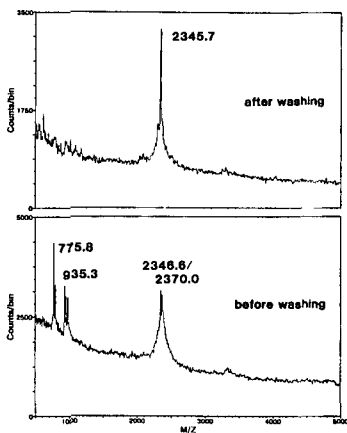


Fig. 4 PD-MS map of tryptic digest of Ile-VP-NP with and without washing of the loaded target.

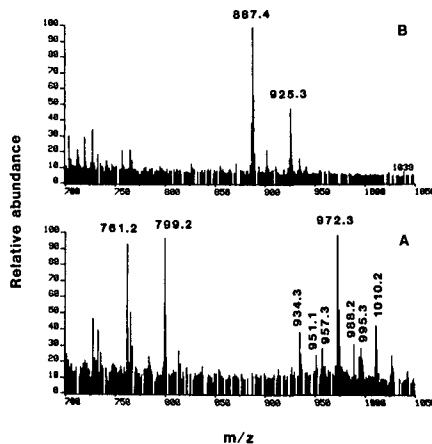


Fig. 5 FAB-MS map of A) a tryptic and B) a V₈ digest of Val-VP-NP.

RESOLUTION AND IDENTIFICATION OF DERIVATIZED ENANTIOMERS OF α -AMINO ACIDS
ON A CHIRAL DIAMIDE PHASE BY GC/MS:

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The Fourth Department, Chung-Shan Institute of Science and Technology
Lung-Tan, Tau-Yuan, TAIWAN, R. O. C.

INTRODUCTION:

Much of the research carried out in recent years on the resolution of optical isomers has centered around systems in which the solute-solvent association necessary for chiral recognition is effected through hydrogen bonding. In particular, the highly selective diamide phase derived from L-phenylalanine, N-Lauroyl-L-Phe t-butylamide, permits the resolution of different classes of compounds. However, their most important application lies in the area of α -amino acids, both N-trifluoroacetyl (N-TFA) isopropyl esters and N-TFA t-butylamides have been resolved on L-Phe diamide phase^{1,2}. Generally, mass spectrometers provide more sensitivity and selectivity compared with other GC detectors. A extension of this resolution system of enantiomers of α -amino acids from GC to GC/MS is clearly useful. The enantiomers of derivatized α -amino acids (e.g., Ala, Val, Leu, Pro, Phe and Phg) were resolved on L-Phe phase by GC. Subsequently, the eluted enantiomers were identified by EI or CI (CH_4 , NH_3 and D_2O as reagent gases) mass spectrometry.

MECHANISM OF RESOLUTION:

Diamides can interact through hydrogen bonding in a number of ways to form structures of the pleated sheet type. The diamide molecules can, for instance, be aligned parallel or antiparallel to each other, as illustrated in Fig. 1. As in the chromatographic process the solute is always present in a very low concentration in the liquid phase, its potential for hydrogen bonding will be fully implemented by interaction with surrounding solvent molecules, resulting in intercalation.

In the separation of derivatized of α -amino acids on diamide phase, it is assumed that the interaction between pertinent solute and solvent molecules occurs through hydrogen-bonded association of the " $\text{C}_5\text{-C}_5$ " or " $\text{C}_5\text{-C}_7$ " type, as shown in Fig. 1. N-TFA- α -amino acid esters have only a C_5 conformation. N-TFA- α -amino acid t-butylamides have both C_5 and C_7 sides. The stereoselectivity observed should result from the differences in interaction between the substituents at the asymmetric centers in the diastereomeric interaction complexes.

EXPERIMENTAL:

A stainless-steel capillary column (25 m x 50 mm) coated with N-Lauroyl-L-phenylalanine t-butylamide was mounted on a Finnigan 4023 GC/MS. The temperature of the injector was 200 C; column temperature was programmed from 120 to 150 C at 7 C per minute. The helium flow-rate was 1 ml/min. The order of elution of the peaks was established for Ala, Val, Leu and Pro by operating with mixtures enriched in the L enantiomer, and for Phenylglycine (Phg) enriched in the D enantiomer. This result was extrapolated to Phe.

The pressure of reagent gas in the ion source was 0.4, 0.7, and 1.0 torr for CH_4 , NH_3 and D_2O , respectively.

RESULTS:

A mixture of α -amino acid enantiomers were injected into the GC/MS, separated using a chiral column and identified by the selected ion monitoring mode of the mass spectrometer. The D-enantiomeric derivatives eluted prior to the L-enantiomeric derivatives on L-Phe diamide phase in all cases at the operating temperatures used (Fig. 2).

The mass spectra (EI or CI) of enantiomers were almost identical with respect to fragmentation patterns and peak intensities for both derivatives of α -amino acid of N-TFA isopropyl esters and N-TFA t-butylamides. The EI-MS base peak in most cases resulted from fragmentation of the C-terminal bond adjoining the asymmetric carbon of α -amino acids with retention of the charge on the N-terminal group.

The NH_3 CI spectra of the N-TFA isopropyl esters of phenylalanine and phenylglycine are dominated by $(\text{M}+4)^+$ ions which are proposed to be $(\text{M}+\text{NH}_4\text{-CH}_2)^+$ ions (Fig. 3). Further evidence for this structure is the formation of $(\text{M}+\text{D-CH}_2)^+$ ions with D_2O CI (Fig. 4).

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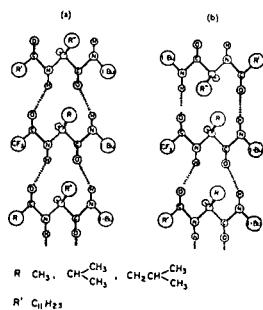


Fig. 1: Schematic representation of the intercalation of a diamide solute molecule in the diamide solvent aligned parallel (a) or antiparallel (b).

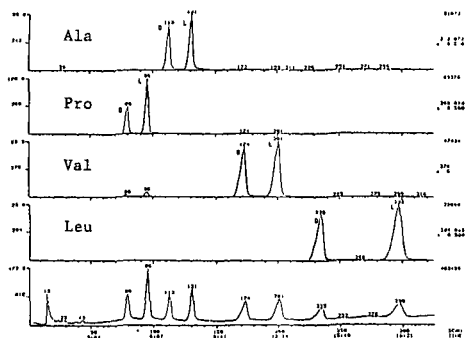


Fig. 2 GC/DPCI MASS CHROMATOGRAM OF N-TFA-1-BUTYLAMIDE (PRO, ALA, VAL, LEU) RESOLVED ON L-PHE PHASE.

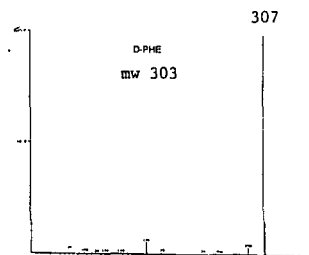


Fig. 3 MASS SPECTRA OF N-TFA ISOPROPYL ESTER OF D-PHE AND D-PHG ON GC/NH₄PCIMS.

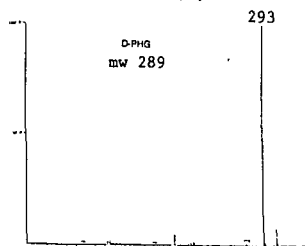
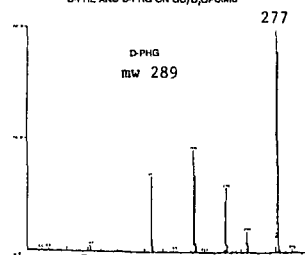
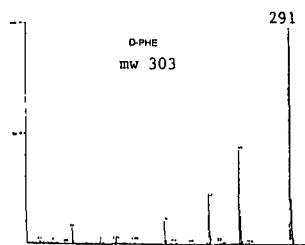


Fig. 4 MASS SPECTRA OF N-TFA ISOPROPYL ESTER OF D-PHE AND D-PHG ON GC/D₄PCIMS.



IDENTIFICATION OF AN AMINO ACID REPLACEMENT IN A MUTANT PHYCOCYANIN BY MS/MS WITH A HYBRID INSTRUMENT

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INTRODUCTION

C-Phycocyanin (PC) is a light-harvesting protein in the photosynthetic apparatus of cyanobacteria and red algae. The visible chromophores on PC are open-chain tetrapyrroles, phycocyanobilins, attached to cysteinyl residues through thioether bonds (1). The posttranslational attachment of phycocyanobilin to apophycocyanin may be an enzyme-catalyzed process (2). In *Synechococcus* PCC7002, insertional inactivation of one of the genes in the PC operon, *cpcE*, leads to loss of phycocyanobilin from the PC α subunit. A phenotypic revertant of a *Synechococcus* PCC7002 *cpcE*⁻ mutant produced an altered PC. The structural difference between wild type PC and that produced by this revertant (designated PCR) was determined by a combination of peptide mapping and mass spectrometry.

EXPERIMENTAL

Purified *Synechococcus* PCC7002 and *cpcE*⁻ PCs (3) were reduced and carboxymethylated with iodoacetate. Peptides were obtained by tryptic digestion followed by subdigestion of purified tryptic peptides with chymotrypsin or staphylococcal V8, as required. All peptide purification was performed by reverse phase HPLC. All samples were desalted on a reverse phase C-18 column prior to MS analysis.

Mass spectra were obtained on a VG ZAB2-EQ mass spectrometer of BEqQ geometry. FAB experiments were performed using a cesium ion gun operated at 20 kV and 1.5 emission current. For CAD experiments the parent ion was selected with 1:1000 resolution and transmitted into the collision quadrupole at 20 eV (laboratory frame-of-reference) energy. Argon was used as the collision gas at a pressure which optimized daughter ion current. Daughter ion spectra were obtained by scanning from 50-950 in 20s and acquiring 2-3 scans. The mass scale of the quadrupole was calibrated with the m/z 69 and m/z 866 from CAD of the m/z 866 ion derived from tris-(perfluoroheptyl)-S-triazine. Accurate mass measurement of the chymotryptic peptide from PCR was made by peak matching against met,lys-bradykinin (MH⁺ 1319) at 1:10,000 resolution.

RESULTS AND DISCUSSION

The revertant grew more slowly than wild type and produced a spectroscopically altered PC. Comparison of the tryptic peptide profiles of wild type and mutant PC showed that an altered peptide was present in PCR. FAB MS and sequential Edman degradation in a gas-phase sequenator identified the tryptic peptide as TFDLSPSWXVEALK (I), where X is tyrosine in the wild type PC. Sequence analysis showed X was altered in PCR. Peptides TFDLSPSWXVE (II) and DLSPSWXVEALK (III) were prepared from I by subdigestion with staphylococcal protease V8 and chymotrypsin, respectively.

FAB-MS of I determined that the mass of the peptide from PCR was 2 AMU lower than that of the corresponding PC peptide. Sequence indicative ions in the FAB spectra of I and II (Figure 1) localized the changed amino acid to residues 8-11. Low energy CAD of the Y₆⁺ ion (PSWXVE) from II (Figure 2) confirmed that the changed residue was at position 7 in peptide II, and that this residue had a nominal mass of 161 (MW-H₂O). Exact mass measurement of peptide III matched the inclusion of

S-carboxymethylcysteine in the empirical formula of the modified peptide. The revertant compensated for the absence of the *cpcE* gene product by substitution of a cysteine for a specific tyrosine in the PC α subunit.

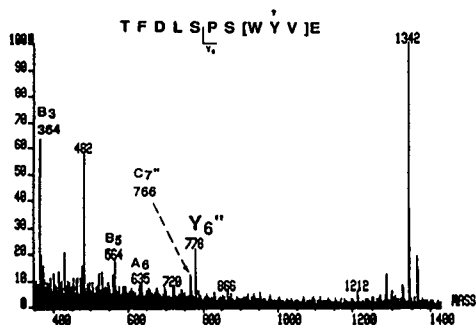


Figure 1. FAB MS of staphylococcal protease V8 peptide II from PCR.

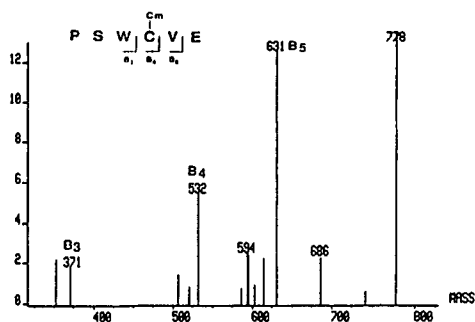


Figure 2. Low energy CAD (20eV) of the Y₆'' fragment ion (Figure 1) from PCR peptide II.

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CHARACTERIZATION OF RECOMBINANT HUMAN CYCLOPHILIN

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Cyclophilin is a soluble, highly conserved cytosolic protein present in high concentrations (0.1-0.4% of total cytosolic proteins) in many mammalian systems and nearly all organisms. It forms a tight stoichiometric complex with cyclosporin A (CsA), a cyclic 11 residue peptide that is a powerful suppressor of the immune response. CsA is used to prevent organ transplant rejection. Recent efforts to characterize peptidyl-prolyl cis-trans isomerase (PPI) showed the n-terminal sequence was identical to cyclophilin, suggesting the two proteins are identical. Therefore, it is possible that cyclophilin might not be just a high affinity binding site for CsA but also the site of the drugs primary biological effect - inhibition of the enzymatic activity of PPI.

Studies to characterize the cyclophilin binding site of cyclosporin A are underway for the express purpose of understanding this interaction and to design new immuno-suppressant drugs that are more potent and less toxic. The CSA activity of cyclosporin is sulphhydryl dependent which suggests that one of the 4 cysteines is involved in binding CsA. To determine which cysteine binds to CsA, each of the 4 cysteine residues were individually changed to serines by recombinant DNA technology. The conventional analytical techniques for identifying single point mutations in a protein like cyclophilin are quite time consuming. Fast Atom Bombardment (FAB) mass spectrometry is a fast and sensitive method for characterizing peptides and protein digests. A tryptic map was used to characterize the modified cyclophilins (Figure 1). The molecular ions (M+H)⁺ of the tryptic peptides can uniquely be assigned to discrete portions of the protein sequence (Figure 2). In the spectrum of the modified proteins, the molecular ion containing the serine change is reduced in mass by 16 Daltons (Table 1). The assignments from a FAB Map can be further confirmed by a single step of Edman Degradation where the molecular ion is reduced by the loss of the n-terminal residue. In a short period of time, the protein sequence was analyzed and the modifications to the protein sequence confirmed.

TABLE 1: Comparison of molecular ions from human cyclophilin to the modified proteins containing single residue change of cysteine to serine.

Residue	Cleavage	Human Cyclophilin (M+H) ⁺	Modified Human Cyclophilin (M+H) ⁺	Edman Degradation on Modified Human Cyclophilin (M+H) ⁺
62	56-69	1542	1526	1414
115	114-118	535	519	423
161	156-165	1062	1046	933

Figure 1 : FAB map on 1 nmole of tryptic digest of human Cyclophilin

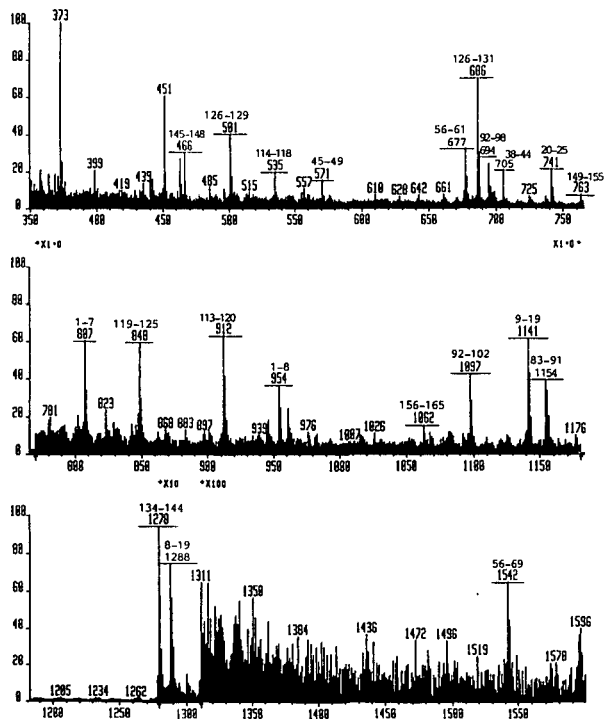
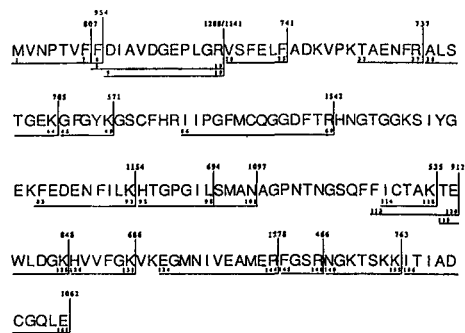


Figure 2 : Sequence of human Cyclophilin



MODIFIED PHENYLISOTHIOCYANATES FOR PEPTIDE SEQUENCING BY
NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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Protein microsequencing using phenylisothiocyanate (PITC) is currently possible in the low picomole range using HPLC with UV absorbance to identify the phenylthiohydantoin (PTH) derived from the amino acid released at each cycle. For many interesting proteins that can be isolated in only small quantities, the sensitivity of detection is insufficient to permit sequencing. Recently, several techniques have been put forward for high sensitivity sequencing, centering around the direct identification of PTH-amino acids. We are investigating the use of negative ion mass spectrometry as a detection method. PITC can be modified in a number of ways to make it electron capturing. We have compared meta-trifluoromethyl phenylisothiocyanate (mCF₃PITC) and pentafluoro phenylisothiocyanate (PF₅PITC) (1,2) to the normal unsubstituted PITC to assess their suitability as Edman reagents.

One important aspect of the comparison is the speed and completeness of coupling reaction. Our results (Figure 1) show that PF₅PITC has the fastest speed and highest yield of the three in the coupling reaction with amino acids. On this basis, it was selected for further study. There is little or no by-product formation with any derivative. Almost all PF₅PTH-amino acids undergo fragmentation (2) in negative ion chemical ionization (electron capture) mass spectrometry (NICI-MS). Amino acids can be grouped into four categories based on fragmentation: (a) the largest group has a base peak at M - 28 (presumably loss of CO), (b) the second largest group has a base peak at M - 48 (presumably loss of CO and HF), (c) the third group has a base peak at M - 18 (loss of H₂O) and (d) the fourth group shows more extensive fragmentation. In spite of this fragmentation, one or more diagnostic ions are produced for each amino acid.

Using a model peptide (Phe-Gly-Gly), manual sequencing with PF₅PITC was attempted. The cycle time was 60 minutes, although this may improve with optimization. In each cycle, the expected PF₅PTH-amino acid was indicated by a diagnostic ion (Figure 2). There was no observed overlap between cycles or by-product formation. When scanning the full mass range (m/z 245 - 610), five picomoles applied to the DCI probe was easily observed. To enhance sensitivity still further, selected ion monitoring can be used (Figure 3). In this mode, 155 femtomoles of PF₅PTH-Asn was observed with a signal-to-noise ratio of over 80.

CONCLUSION:

Pentafluoro PITC is a promising reagent for peptide sequencing using NICI-MS for detection of the modified PTH-amino acids. There is no need for chromatography since the negative ions can be detected selectively and since the mass (or combination of masses) is unique to each derivative.

Pentafluoro PITC couples to amino acids faster and to greater extent than either meta-trifluoromethyl PITC or unsubstituted PITC. The excess PF₅PITC and amino acid derivatives are nonpolar and easily extracted into the organic solvents.

The major drawback of the pentafluoro PTH-amino acids is that almost all of them undergo fragmentation in NICI-MS, with a few of them having more than three major ions. This causes some loss of sensitivity, but allows the PF₅PTHs to be identified with greater confidence, since the presence of multiple ions in the proper ratio is unlikely to occur by chance. Work is continuing to demonstrate sequencing with longer peptides and to understand the mechanism of fragmentation.

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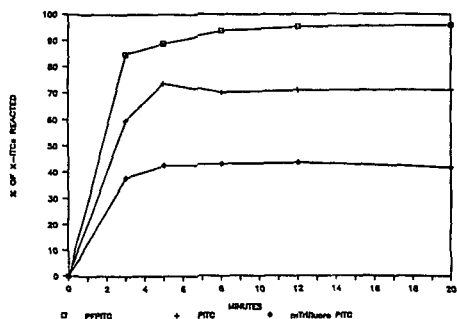


Fig 1. Coupling yields of PFPITC, mTrifluoropITC and PITC with Phenylalanine.

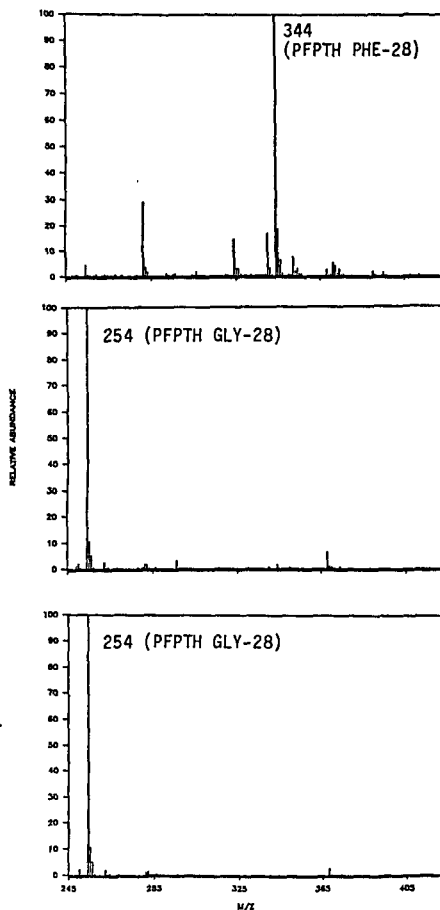


Fig 2. NICI-MS of 1, 2 & 3 cycle of a tripeptide.

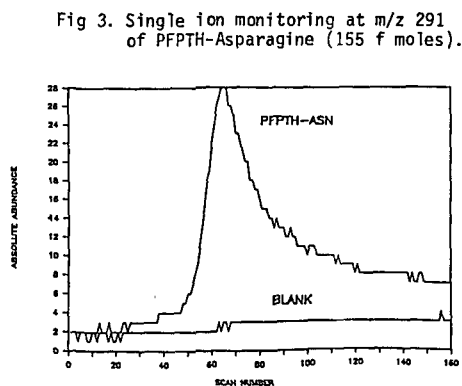


Fig 3. Single ion monitoring at m/z 291 of PFPTH-Asparagine (155 f moles).

LSIMS ON MULTIPLE CYSTINE-LINKED PEPTIDES FROM
RECOMBINANT HIRUDIN VARIANT 2-Lys 47 (rHV2-Lys 47) DIGESTION.

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Natural hirudin isolated from the salivary glands of the medicinal leech *Hirudo medicinalis* is a highly potent anti-thrombotic agent destined for pharmaceutical use in venous and arterial thrombosis. Production of recombinant hirudin variant 2-Lys 47 (rHV2-Lys 47) (65 amino acids, 3 cystine bridges) has been undertaken through recombinant DNA technology using a *Saccharomyces cerevisiae* host and a secretion vector based on the alpha-pheromone export signals (1, 2). Hirudin for pharmaceutical purpose requires periodic routine characterization on production lots. To this end, average mass measurements from Liquid Secondary Ion Mass Spectrometry (LSIMS) on different hirudin variants and improperly processed hirudin have been described (3, 4). The primary sequences of various hirudins have been previously obtained by Edman degradation of tryptic peptides after cysteine modifications (5, 6), and cystine bridge determination was performed after thermolysin digestion which generated numerous peptides. In this paper, both primary and secondary structure derived from the combination of LSIMS and Edman degradation of successive tryptic and V8 protease digestions is described.

From tryptic digestion of rHV2-Lys 47, four peaks are separated from RP-HPLC (Figure 1). Complete sequencing of rHV2-Lys 47 could be easily done with three steps of Edman degradation obtained on the tripeptide T1, and 30 steps on peptide T3 where the three sequences are read simultaneously, thus 33 steps in total.

LSIMS was performed on peptides chains linked by cystine bridges. In addition to the mono and doubly protonated molecular ions, other peaks are observed and are generated by fragmentation of disulfide bonds. They appeared at m/z values corresponding to the protonated masses of the peptide which a chemical reduction would have produced.

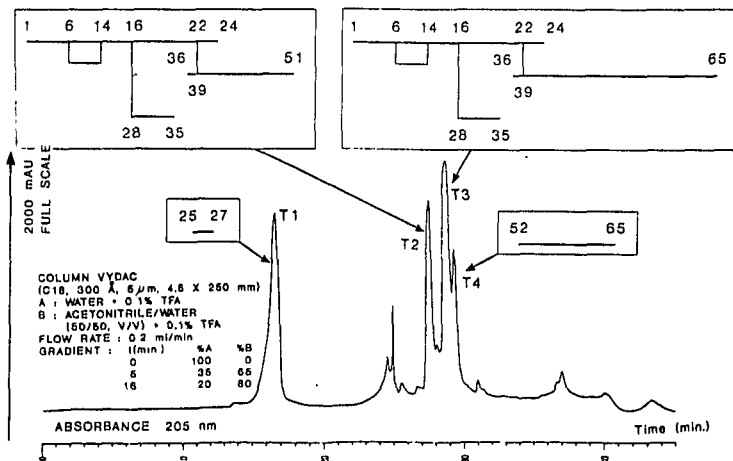


FIGURE 1 : RP-HPLC RUN OF rHV2-Lys 47 TRYPTIC DIGEST

Cystine bridge identification was performed after V8 protease digestion of tryptic fragment T3. By RP-HPLC three fractions containing cystines were isolated (Figure 2). LSIMS on V8 subfragments showed mono and doubly protonated molecular ions, as well as other peaks generated by disulfide bond fragmentations. Results from Edman degradation and LSIMS performed on the RP-HPLC purified peptides retain a maximum of 2 indistinguishable combinations of cystine bridges (S-S 6-14, 16-28, 22-39 or S-S 6-16, 14-28, 22-39) from the 15 theoretical arrangements possible.

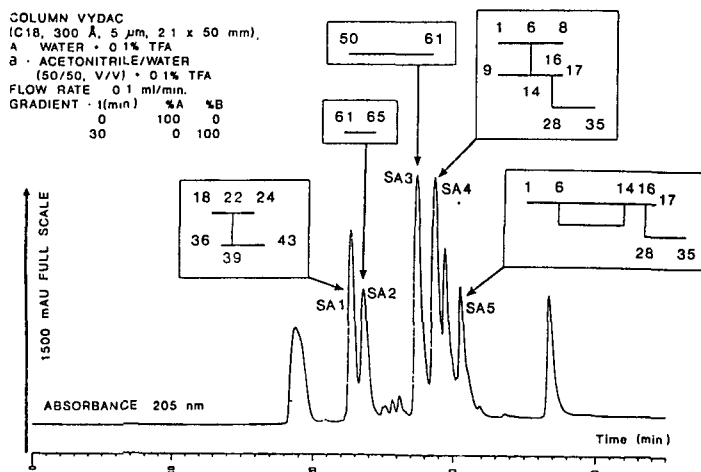


FIGURE 2 RP-HPLC RUN OF V8 DIGEST OF TRYPTIC FRAGMENT-T3

In this method, the enzymatic digestions generate few peptides and only two RP-HPLC runs are required. In conclusion, the combination of tryptic and V8 protease digestion, LSIMS and Edman degradation (33 steps) performed on rHV2-Lys 47 allowed a very efficient characterization of the primary sequence and cystine bridge identification in 3 days. This simple and relatively rapid method could be used as a routine technique for quality control of a recombinant hirudin production.

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CONFIRMATION OF INDOLE-3-ACETIC ACID BOUND TO ZEIN

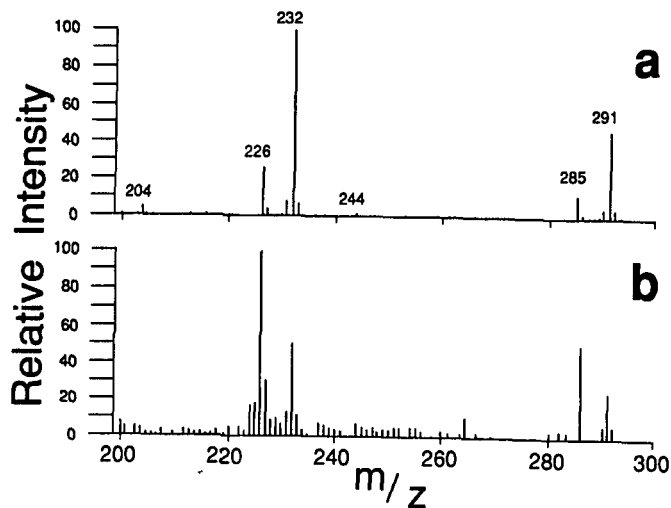
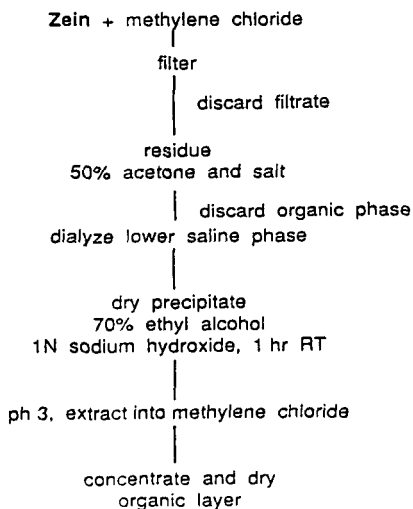
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Indole-3-acetic acid (IAA), a plant hormone, was reported earlier by other workers to be released by alkaline hydrolysis from zein, the dominant storage protein in the corn kernel. Bioassays were used in the analysis. Since then studies have primarily addressed the metabolic fate of lower molecular weight conjugates of IAA, the primary auxin of plants. The present work uses combined gas chromatography-mass spectrometry (GC/MS) to confirm the presence of IAA in zein. Protein bodies from mature corn kernels were isolated by isopycnic centrifugation. Protein extracted from these organelles and subjected to SDS gel electrophoresis revealed major bands corresponding to 22 and 19 kD, which are in agreement with known zein monomers. Zein (lot 0833 EL) was also purchased from Aldrich Chemical Company, Inc. The commercially obtained zein and the protein bodies prepared in our laboratory were purified, as follows. Samples (200 mg) were washed in methylene chloride, filtered and the dry residue was solubilized in 50% acetone. The addition of salt gave an upper organic phase and a lower saline phase which was dialyzed against water overnight at 4°C. The protein precipitate was dried, solubilized in a small volume of 70% ethyl alcohol and subjected to alkaline hydrolysis in 1N NaOH for 1 hour at room temperature, which releases esterified IAA. Following adjustment of the mixture to pH 3 with the addition of HCl, the free IAA was extracted into methylene chloride. Dried samples were methylated with ethereal diazomethane and trifluoroacetylated with trifluoroacetic anhydride. The derivatized samples were analyzed using a Kratos MS-80 high performance mass spectrometer interfaced with a Carlo Erba Fractovap 4160 series gas chromatograph fitted with a SPB-5 capillary column (0.32 mm I.D., 20 m long, coated with 0.25 µm 5% phenyl methyl siloxane). The samples were dissolved in 5 µl of methylene chloride and were injected on-column, maintaining the column temperature at 38°C. The temperature was then raised to 200°C and maintained for 2 minutes before the eluent was introduced into the mass spectrometer. The temperature of the GC was programmed from 200°C to 260°C at 5°/minute during the time of data acquisition. Data were acquired using the selected ion monitoring (SIM) technique.

When quantification of IAA was desired, an internal standard (¹³C₆-IAA) was added after the methylene chloride rinse early in the purification procedure. Using molecular ions 291 and 285 and base peaks 232 and 226, for the analog and endogenous IAA, respectively, several SIM analyses revealed an average of 50 µg IAA/g zein, dry weight. Partial mass spectra for the internal standard and authentic IAA as well as a purified extracted sample are shown in the Figure (A: standards ¹³C₆-IAA and IAA; B: purified, hydrolyzed extract of zein to which a known amount of ¹³C₆-IAA had been added).

IAA HYDROLYSIS FROM COMMERCIAL ZEIN



CHARACTERISATION OF PHENYLGLYCIDYL ETHER NUCLEOSIDE ADDUCTS USING FAB/MS/MS.

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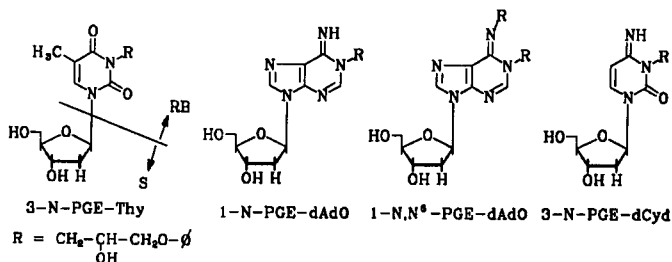
Epoxides are of vast industrial importance because of their application in plastic and resin industry.¹ Their properties are generally believed to be induced by covalent reactions with cellular DNA. The aim of this study was the structure elucidation of phenylglycidyl ether (PGE)-adducts of 2'-deoxynucleosides with the aid of spectroscopic techniques. The PGE-adducts were synthesised by reacting PGE with Thymidine (Thy), 2'-deoxyadenosine (dAdo) and 2'-deoxycytidine (dCyd) at 37°C in methanol. The corresponding PGE-adducts were isolated by centrifugal circular thin layer chromatography (Kieselgel 60 PF 254, eluant: CH₂Cl₂/THF) and reverse phase HPLC (10RP18, eluant: 0.01M HCOONH₄/CH₃OH). The adducts were collected and analysed by U.V.- and ¹H-NMR spectroscopy. In this work we wish to present the mass spectral data.

FAB/MS, using glycerol as the matrix, yielded intense [MH]⁺ and [M-H]⁻ ions. These ions were selected in CAD/MS/MS experiments (VG70SEQ, EBQQ) using Argon as collision gas in the 3rd FFR. The following ions could be detected: [RBH₂]⁺, [RBH₂-O-OH]⁺, [RBH₂-O-OH-H₂O]⁺, [S]⁺ or [RB]⁻, [RB-O-OH]⁻, [(M-H)-C₃H₆O₃]⁻.

In the case of 2'-deoxyadenosine a prolonged reaction time (48 hrs instead of 24 hrs) led to the formation of both mono- and bis-alkylated PGE-adducts (MW=401 and 551). Both [MH]⁺=402 and [MH]⁺=552 were selected for a CAD/MS/MS experiment.

FAB/MS analysis of the 2'-deoxycytidine/PGE-adduct gave the expected protonated molecular ion [MH]⁺ at m/z=378. However it was noticed that the abundance of m/z= 379 was too high taken into account the isotope contribution of m/z=378. Slightly heating of the glycerol matrix, containing the adduct, followed by consecutive FAB/MS recordings showed

the ion at $m/z=379$ gradually to increase in intensity. Both $m/z=378$ and $m/z=379$ were selected for a CAD/MS/MS experiment giving diagnostic ions at $m/z=262$ and $m/z=263$ respectively. They were assigned to $[\text{RBH}_2]^+$ -ions. DCI(NH_3) mass spectrometry of the same sample gave ions at $m/z=379, 280, 263, 245, 169, 134$ and 116 . The aforementioned results can be explained by a PGE-dCyd to PGE-dUrd conversion. Simular results have been observed by Solomon et al.² although there it was proven that the hydrolytic deamination of 3-HP-dCyd to 3-HP-dUrd ocured during the coarse of the reaction. Our results, although preliminary, may indicate that thermal effects applied during sample preparation can also produce such a conversion. In addition ,the PGE-adducts were analysed by DCI/MS(NH_3) on a Ribermag 10-10B (Nermag S.A.)quadrupole mass spectrometer. Along with the $[\text{MH}]^+$ -ion ,fragment ions were observed at $[\text{RBH}_2]^+$, $[\text{RBH}_2-\text{OH}]^+$, $[\text{S}]^+$, $[\text{S-H}]^+$ and $[\text{S-H}]\text{NH}_4^+$. From the combined data the following structures were deduced:



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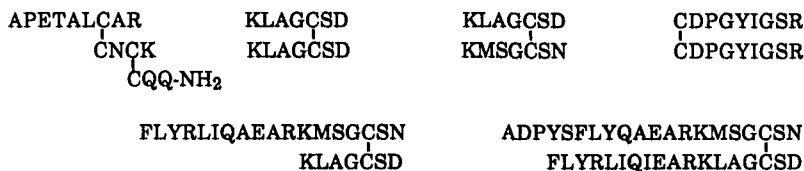
Interchain Disulfide Bond Fragmentations of Dimeric Peptides.

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The assignment of disulfide bonds is one of the most important step in the structural analysis of peptides and proteins. Partial reductions, isomeric combinations of disulfide bonds often alter the biological activity of proteins containing disulfide bonds (1,2). LSIMS could play a key role in the study of peptides linked by one or more disulfide bonds which are typically obtained when a cystine containing protein is enzymatically digested (1-3) but are also naturally occurring (4). Different types of peptides are obtained by such degradations:

- single peptides with one or multiple intramolecular disulfide bonds.
- peptides with independant chains linked together by disulfide bonds.

Only a few reports are available on the second type of peptide. We have therefore synthesised a variety of homo and heterodimeric peptides to study their fragmentations under LSIMS conditions in various matrix. The molecular weight of these synthetic interchain disulfide peptides is ranging from 1 to 6 kDa (see a few examples bellow).



We report here on the main fragments observed on this type of cystine containing, double or triple chain peptides. Also source spectra were compared with the MIKES (with and without CID) and the main fragments identified and compared.

All these peptides yielded a clear molecular ion but also intense fragment peaks corresponding to the different chains. These intense fragments are generated by a cleavage at the level of the disulfide bond of the cystine which appeared therefore to be more fragile in these conditions than the peptide bonds. It is therefore possible to determine the molecular weight of each individual chains. This is particularly useful since it avoids a preliminary chemical reduction of the peptide to determine the mass of the different chains.

Such fragmentations were already reported on rather low molecular weight peptides with interchain disulfide bonds (3). We showed that this fragmentation is also preferential on large peptides up to 6 kDa. For example the peptide presented on fig. 1 yielded a clear $[MH]^+$ and two major fragments at m/z 2097.4 and 2621.6. These values correspond to the expected $[MH]^+$ for the two monomeric chains resulting from a putative reduction by the matrix. But our study showed that these two peaks were in fact generated by a source fragmentation of the molecular ion at m/z 4716.4. This preferential source fragmentation was observed for all peptides we studied up to 6 kDa.

MIKES on the molecular ions also produced major fragments corresponding to cleavages at the disulfide bond level (Fig. 2). Use of CID enhanced the S-S fragment ions by 4. In addition, two peaks at +33 and -33 mass units from the disulfide bond fragment were spontaneously formed and correspond to carbon-sulfur bond cleavage. The detailed

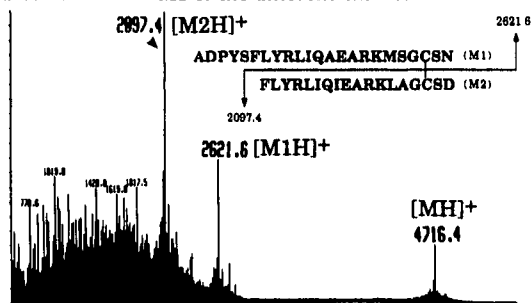


Figure 1. LSIMS of a dimeric peptide in thioglycerol. $[MH]^+ = 4716.4$.

mechanism of these fragmentations are under investigation, but it seems that the source fragmentation and the MS-MS fragmentation are following different patterns.

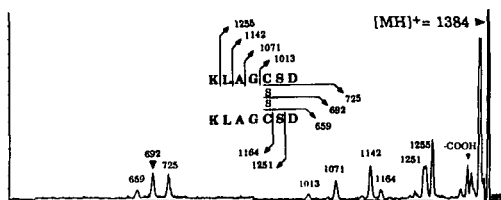


Figure 2: MIKE spectrum of molecular ion $[MH]^+$ 1384 from a homodimeric peptide.

A time dependant study in various matrix was realised for several peptides as shown on Fig. 3. The relative intensity of the fragments and the molecular ion were constant over 3 to 5 minutes (15 to 25 scans) but was varying from one matrix to another. Reduction solely induced by the matrix was therefore insignificant or slow.

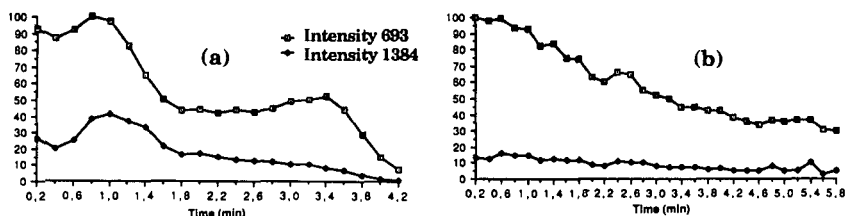


Figure 3: Relative intensities of the dimeric peptide $KLAGCSD \ KLAGCSD$ ($MH^+ = 1383.7$) and of the disulfide bond fragment M1 ($MH^+ = 693.3$) vs time in thioglycerol (a) and glycerol (b).

In thioglycerol, glycerol and DTT/DTE we also observed peaks at $[M+matrix-2+H]^+$ corresponding to matrix covalently bonded to peptide. Formation mechanisms of such ions must involve reactive species formed at the surface of the matrix. An analogy could be done with the formation of reactive species formed from sulfides and disulfides under UV light, X rays or electrons (1,5).

Conclusion: When several peptide chains are linked together by disulfide bonds the mass of the individual peptide chains is usually given by the main LSIMS fragments (see example on figure 4). No chemical reduction is needed.

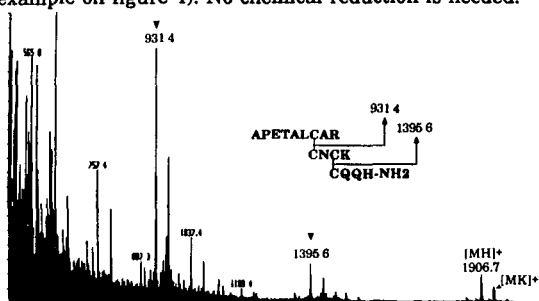


Figure 4: Triple chain peptide, $[MH]^+ = 1906.7$, obtained from the tryptic digest of Apamine. The peptide ($10\mu g$) was previously dissolved in AcOH 5%. Matrix: thioglycerol.

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Quantification of 1,25-dihydroxyvitamin D₃ by stable isotope dilution using combined high performance liquid chromatography-thermospray mass spectrometry.

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Introduction: Combined gas chromatography-mass spectrometry (GC-MS) has been used in the past to study aspects of vitamin D₃ metabolism (e.g., ref. 1). While these methods provide useful data, lengthy sample preparation procedures are required. Furthermore, thermal degradation of the derivatized compounds often occurs at the temperatures required for GC analysis (>200°C), complicating chromatographic profiles and compromising sensitivity.

Recently combined thermospray liquid chromatography-mass spectrometry (LC-MS) has proved to be a suitable alternative to GC-MS for analysis of many polar and thermally labile low molecular weight (<1000 Da) compounds in biological matrices (see refs. 2-4). For this reason we examined the thermospray ionization behaviour of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and developed a stable isotope dilution assay based on selected ion monitoring thermospray LC-MS.

Methods:

Biological sample preparation: 25-hydroxyvitamin D₃ (10μg per tube) was incubated with 5-10mg of either guinea pig or sheep renal mitochondrial protein. The incubation medium contained 0.05M Tris/acetate buffer pH 7.4, 0.01M MgCl₂, 0.012M isocitrate and 1.6μg ml⁻¹ NN'-diphenyl-p-phenylenediamine. Incubations were performed for 15-60min under air at 37°C and were terminated by addition of acetonitrile. The internal standard (40ng of [26,26,26-³H]₃1,25(OH)₂D₃) was added at this stage. Precipitated protein was removed by centrifugation and the supernatant was applied to a Waters C₁₈ Sep-Pak cartridge which had been prewashed with 3ml methanol and 3ml water. The sample was washed with 3ml methanol/water 30/70 (v/v) and eluted with 3ml acetonitrile. Extracts were dried and reconstituted in the mobile phase described below.

Analytical method: The instrumentation used was a Waters model 600MS liquid chromatograph equipped with a 15cm x 0.46cm i.d. C₁₈ Nova-Pak column, interfaced with a Vestec model 201 Thermospray LC-MS system. A thermospray compatible mobile phase suitable for isocratic elution of 1,25(OH)₂D₃ was selected (0.1M ammonium acetate pH 6.5 in methanol/water 80/20 (v/v)). A solution of 1,25(OH)₂D₃ (2μg ml⁻¹) in the mobile phase was pumped directly into the mass spectrometer at a rate of 1ml min⁻¹ to allow selection and optimization of the most useful ionization mode. The [M+H-H₂O]⁺ fragment ions of 1,25(OH)₂D₃ and [26,26,26-³H]₃1,25(OH)₂D₃ (m/z 399 and 402) were chosen as the most suitable for use in quantitative selected ion monitoring (SIM) analyses. Standard mixtures containing 40ng of the stable-labelled internal standard and varying amounts of 1,25(OH)₂D₃ were analyzed by thermospray LC-MS using SIM and a standard curve was derived by linear regression analysis. Incubation extracts were analysed in the same way and the amount of 1,25(OH)₂D₃ formed was estimated from the standard curve.

Results: The tuning solution was used to compare the overall signal intensity generated by 1,25(OH)₂D₃ in six different operating modes (pure thermospray, filament-on and discharge-on with either positive or negative ions detected). The positive ion, filament-on mode yielded the most useful data (mass spectrum shown, Figure 1). By SIM at m/z 399 the limit of detection (S/N 2:1) was 250pg injected on-column. A standard curve across the range 0-40ng 1,25(OH)₂D₃ derived by least-squares linear regression analysis showed good linearity (r² = 0.997). The incubation extracts

generated readily measurable responses in the m/z 399 channel at an elution time corresponding to that of authentic $1,25(\text{OH})_2\text{D}_3$ (Figure 2). The quantities present, estimated from the standard curve, are listed in Table 1. Due to chemical noise the practical limit for reliable quantification in biological extracts was 3ng. In some cases earlier eluting species were also observed in the m/z 399 channel (Figure 2). The species eluting at 3.4 min had a retention time identical with that of $24,25\text{-dihydroxyvitamin D}_3$. The more hydrophilic compound (2.7 min) probably corresponds to $25,26\text{-dihydroxyvitamin D}_3$ but an authentic standard was not available for comparison.

Summary:

Combined thermospray LC-MS is a suitable alternative to GC-MS for the detection of $1,25(\text{OH})_2\text{D}_3$ in biological samples, with the advantage that minimal sample preparation is required. By selected ion monitoring of the $[\text{M}+\text{H}-\text{H}_2\text{O}]^+$ fragment ion it was possible to detect as little as 250pg of $1,25(\text{OH})_2\text{D}_3$ injected on-column. Using a stable isotopically labelled analogue as internal standard a linear and reproducible calibration curve for quantification in the range 0-40ng was established. The method was applied to the study of *in vitro* 1α -hydroxylation of $25\text{-hydroxyvitamin D}_3$ by guinea pig and sheep renal mitochondria.

Table 1. Amounts of $1,25\text{-dihydroxyvitamin D}_3$ formed by guinea pig and sheep renal mitochondria.

Incubation (time)	Experiment A	Experiment B
Guinea pig (30min)	22.0ng	28.2ng
Sheep (15min)	23.6ng	16.7ng
Sheep (60min)	17.9ng	23.0ng

Figure 1.
Positive ion filament on
thermospray mass spectrum
of $1,25\text{-dihydroxyvitamin D}_3$.

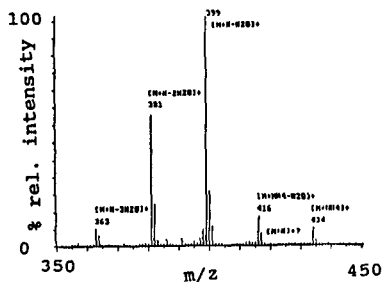
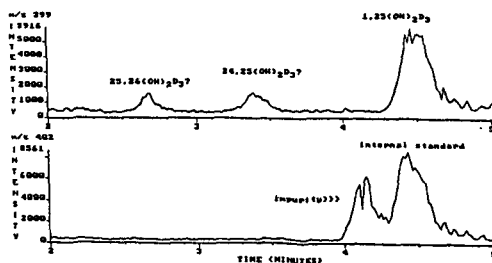


Figure 2.
SIM profile from extracted
supernatant of guinea-pig renal
mitochondrial incubation with
 $25\text{-hydroxyvitamin D}_3$.



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THE ANALYSIS OF NONIONIC, CATIONIC, ANIONIC
AND AMPHOTERIC SURFACTANTS USING THERMOSPRAY MS/MS
AND FAB MS/MS

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The analysis of surfactants has significant industrial and some environmental importance. Various spectroscopic, chromatographic and colorimetric tests which are available for the analysis of surfactants must address complexities resulting from: the diverse range of samples they appear in, the low levels of surfactants present, various classes of surfactants and the distribution of the chain lengths on both hydrophilic and hydrophobic ends.

We report here an analytical scheme for a wide range of surfactants utilizing a colorimetric screening test followed by thermospray (TSP) MS/MS or FAB MS/MS, which is specific, sensitive, and rapid since it requires little, if any, extraction and no chromatographic separation. MS/MS is used for the characterization of surfactants and to distinguish surfactants from other components in complex industrial samples.

A particular type of a surfactant is best analyzed by a specific MS technique and polarity. For quick characterization it is helpful to know the type prior to analysis. We have found the colorimetric test based on a dimidiobromide-disulphine blue mixed indicator [1] useful to screen for the type of the surfactant present. Appropriate mass spectrometric techniques can then be successfully applied for quick characterization of the surfactant.

Non-ionic surfactants produce a series of abundant pseudo-molecular ions with the thermospray interface by discharge ionization with 100% methanol as the mobile phase. Figure 1 shows a TSP mass spectrum of a complex mixture of polyethoxylated alcohols obtained by direct sample introduction (bypassing the HPLC column). The presence of a series of ions separated by 44 and 14 mass units is indicative of the varying chain lengths of the hydrophilic ethyleneoxy group ($n=2$ to 12) and the hydrophobic alkyl chain ($R=C_9-C_{11}$), respectively. Some unsaturated alkyl groups are also present. Tandem mass spectrometry (MS/MS) can be applied to select any ion from the complex mixture and obtain structural information (Figure 2).

To-date, thermospray also appears suitable for the characterization of amphoteric surfactants which provide reproducible spectra with molecular weight information. Investigation with additional amphoteric is being pursued to fully understand their behavior under thermospray conditions and their response to the colorimetric screening test.

Characterization of non-ionic surfactants by FAB was found to be less sensitive than thermospray in our hands. FAB-MS/MS on the other hand, is the preferred method for the analysis of both cationic and anionic surfactants. TSP-MS with discharge ionization was also found to be satisfactory for anionic surfactants. Cationic surfactants are prone to the loss of alkyl groups under thermospray conditions [2].

The above methods are extremely rapid, as HPLC separation of the surfactant components is not required. The presence of all the protonated molecular ions of the components with the hydrophobic and hydrophilic group distribution in one spectrum allows one to identify the components at once by knowing the identity of one of the ions in the mass spectrum.

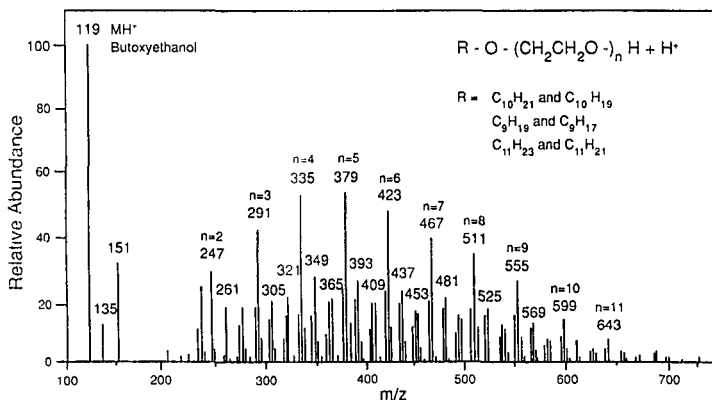


Figure 1 Thermospray mass spectrum of a complex mixture of polyethoxylated alcohols obtained by applying discharge ionization.

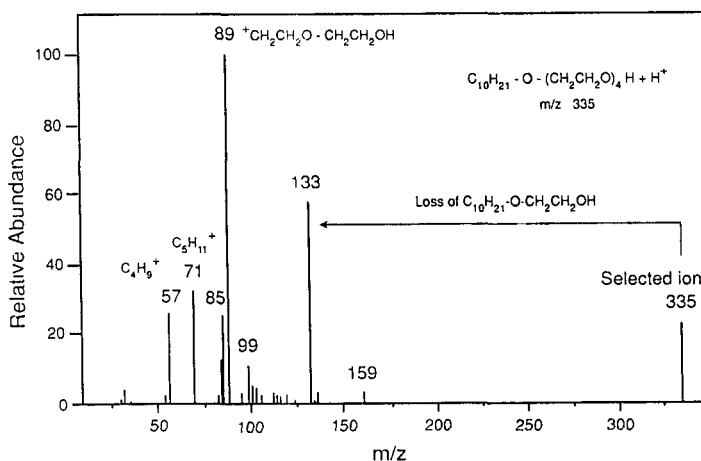


Figure 2 Collision-induced dissociation (CID) daughter spectrum of m/z 335 at 30 eV collision with argon.

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Therospray LC/MS Interface
for
Time-of-Flight Mass Spectrometry
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Ion focusing in time-of-flight mass spectrometry requires the ions to be formed in a carefully defined location at a particular point in time. On the other hand, ions are formed continuously in a therospray source. These two apparently incommensurate demands can be met if the therospray ions can be manipulated into packets of ions that are defined in space and time. Rather than trying to accomplish this by manipulating the ion formation conditions, we have chosen to modify the ion source external to the therospray ion exit region and before the Ion Focus Pulse (IFP) grid.

We require that the therospray ions enter the ion focusing region only at times that are consistent with application of the IFP. This is accomplished by adding two lens elements between the therospray ion exit and the IFP. The position of and potentials on these grids are determined by ion trajectory considerations. The first, the Backing Grid (BP), is close to the therospray ion exit and is maintained at ground potential in order to define a potential plane. The second, the Therospray Retarding (RTH) grid is intermediate between the BP and IFP grids. It is normally biased, but pulsed to allow ion entry into the focus region. These two lens elements and the presence or absence of a pulse on the RTH grid define 3 regions of interest for ion trajectories into the ion focus region:

Region 1 - RTH pulse ON. Ions are accelerated toward RTH grid by a field E_1 . Ions present initially receive the full effect of the RTH pulse, while others, which pass through BP after the start of the pulse, receive proportionately less acceleration. Ions experience acceleration for a time $\alpha \tau_p$ where τ_p is the duration of the RTH pulse and α is a fraction.

Region 2 - RTH pulse OFF. E_1 is such that ions of $m/z = m^0$ and $\alpha = 1$ reached the RTH grid just as the pulse shut off. All other m^0 ions experience deceleration from the biasing of the RTH grid by field E_2 . There is a value of α_c , for which m^0 ions experience so little acceleration in Region 1, that they, and all ions with $\alpha < \alpha_c$ cannot pass the RTH grid.

Region 3 - RTH pulse OFF. m^0 ions with $\alpha > \alpha_c$ have passed beyond the RTH grid and are accelerated by the field, E_3 , resulting from the RTH grid bias. Ions of m^0 and $\alpha = 1$ just exit a 1mm wide area at the center of the ion focus region, the "sweet spot", at τ_{A^0} . Other well focussed ions, with $\alpha = \alpha_b$, will just enter the "sweet spot" as those with $\alpha = 1$ exit.

The time after the end of the RTH pulse, τ_{A^0} , for an m^0 ion with $\alpha = 1$ to transit the RTH grid and the top of the sweet spot,

determines the delay required between the RTH pulse and IFP. The analogous time for an ion with $\alpha = \alpha_b$ is τ_3 . These times are calculated from the equations given below, obtained from solution of the trajectory expressions for each of the three regions of ion motion.

The magnitudes of the fields E_1 and E_2 can be adjusted proportionately so that various values of m/z follow the trajectory calculated for m^0 . In addition, the relative values of these two fields for a particular m^0 , determine that fraction of ions, γ , which have passed the RTH grid that are in the "sweet spot" when the IFP is applied. For ions of $m/z=100$, an RTH pulse

length of 1 usec, and with the distances BP- RTH (d₁) and RTH-IFP (d₂) set at 0.16 and 0.635 cm respectively, a 5.3V RTH pulse height coupled with a 2V bias leads to $c = 0.52$, $b = 0.878$ and $a = 0.25$. That is 25% of the ions passing the RTH grid should be well focused. For this same focus efficiency at m/z 5600, an RTH pulse height of 297V with a 112V bias would be required. Good quality 1 usec pulses of this magnitude can be obtained.

Results to date are confined to thermospray solvent ions, and the effectiveness of this system has been demonstrated in principle. Work continues to obtain spectra a reasonable values of higher m/z .

$$\gamma_A^0 = \gamma_P \left(\frac{E_1}{E_3} \right) \left[\sqrt{1 + \left(\frac{E_3}{E_1} \right) \frac{2 m d_T'}{e E_1 \gamma_P^2}} - 1 \right]$$

$$d_T' = d_1 + \frac{1}{2} d_2 + 0.05$$

$$\gamma_3 = \gamma_P \left(\frac{E_1}{E_3} \right) \left[\alpha - \frac{\gamma_2}{\gamma_P} \left(\frac{E_3}{E_1} \right) \right] \left[\sqrt{1 + \frac{2 e E_3 d_2'}{m v_2^2}} - 1 \right]$$

$$d_2' = d_1 + \frac{1}{2} d_2 - 0.05$$

$$E_3 = -E_2$$

$$\gamma_2 = \left(\frac{E_1}{E_2} \right) \left[1 - \sqrt{1 + \frac{E_2}{E_1} \left(1 - \frac{1}{\alpha^2} \right)} \right] \alpha \gamma_P$$

$$v_2 = \frac{e E_1}{m} \left[1 - \left(\frac{E_2}{E_1} \right) \left(\frac{\gamma_2}{\alpha \gamma_P} \right) \right] \alpha \gamma_P$$

$$\alpha_c = \sqrt{\frac{E_2}{E_1 + E_2}}$$

α_B determined from plot of $\Delta = \gamma_A^0 - (\gamma_2 + \gamma_3)$
vs α when $\Delta = 0$.

**USE OF DEUTERATED SOLVENTS IN THERMOSPRAY LC/MS TO DISTINGUISH
CLUSTER COMPOSITION, ADDUCT FORMATION, DIMERIZATION AND
EXCHANGEABLE PROTONS**

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INTRODUCTION: Thermospray (TSP) spectra can be difficult to interpret, especially the spectra of complete unknowns. The "soft" nature of the the TSP ionization process yields few or no fragment ions. Both molecular ions and pseudomolecular ions are commonly observed in both positive and negative ion modes. Rearrangement ions, adduct ions, cluster ions and clusters of analytes are commonly observed. The ambiguous interpretation of these spectra is compounded by the qualitative and quantitative changes due to filament, discharge electrode and instrument parameters. This poster illustrates how a simple procedure using either a "plug" or a "sandwich" of an appropriate deuterated solvent may be employed in certain cases to determine whether an observed ion is a molecular ion, an adduct ion or a cluster ion. The technique also provides information about the number of exchangeable protons on a given molecule.

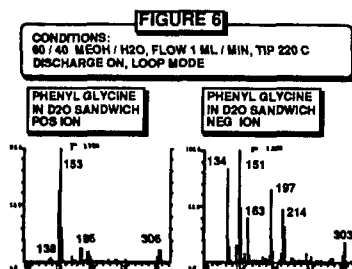
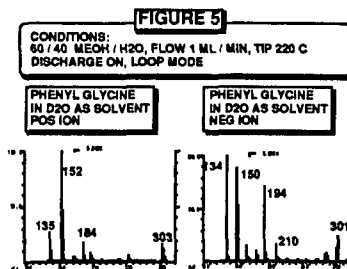
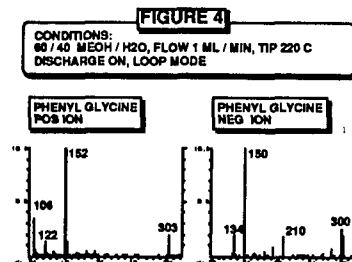
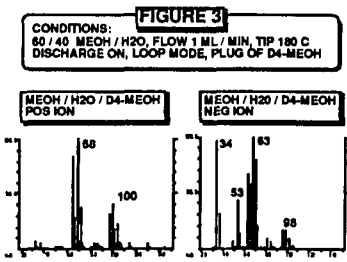
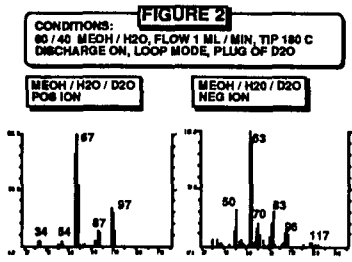
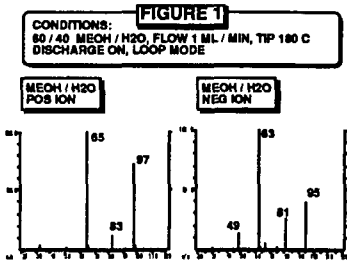
METHODS AND PROCEDURES: All TSP spectra were obtained using a Vestec TS101 TSP interface installed on a Finnigan 4500 MS/DS. Samples were introduced via the "direct loop" procedure -- i.e. no HPLC column in line and manual injection using a 50 μ l fixed loop. The isocratic mobile phase was delivered to the Vestec interface at a flow of 1ml/min. Analytes passed from the injector to a U.V. detector in order to monitor injections. Specific experimental procedures are discussed separately for each set of experiments described below or are included in figure legends.

SOLVENT CLUSTER EXPERIMENTS: The spectra shown were obtained in "loop" mode at a flow of 1.0 ml/min. and a TSP probe tip temperature of 180° C. The mobile phase was either 60/40 methanol/water or 60/40 acetonitrile/water as indicated. A "plug" of 50 μ l of deuterium oxide or D₄-methanol was introduced into the mobile phase via manual injection. The non-deuterated control experiment (figure 1) shows methanol clusters at m/z =65 and 97 in positive ion detection and at m/z= 63 and 95 in negative ion. The ions at 83 in positive and 81 in negative ion represent methanol-water complexes. The addition of a "plug" of D₂O (figure 2) results in proton exchange with the methanol clusters and methanol-water complexes. The methanol clusters in positive ion shift to 68 and 99 amu indicating the addition of 1 deuterium atom and 1 proton exchange per methanol molecule. The methanol-water complex displays the incorporation of D₂O into the complex. The negative ion data also show proton-deuterium exchange. Using D₄-methanol the hydroxy deuterium exchanged with the mobile phase, resulting in methanol clusters at m/z= 71 and 106, indicative of D₃-methanol (figure 3). Negative ion data were consistent with the positive ion proton exchanges.

ANALYTE CLUSTER EXPERIMENTS: Thermospray spectra often have ions at twice the nominal mass of a given analyte. Use of deuterated solvents can help distinguish a TSP formed dimer from a preformed dimer. Under the TSP conditions indicated, phenylglycine (MW=151) exhibits ions at m/z=152,302 and 303 in pos ion monitoring and at m/z 150 and 300 in negative ion monitoring (figure 4). When the phenylglycine is injected in a plug (figure 5) or in a "sandwich" of D₂O-made by injecting 50 μ l of D₂O immediately before and after the sample itself (figure 6), the spectra show isotope patterns indicative of deuterium exchange. In this case the isotope pattern is consistent with a thermospray phenylglycine cluster ion rather than a dimer. The number of exchangeable protons for two phenylglycine molecules is the number of proton-deuterium exchanges observed in the dimer. The sandwich technique described by Siegle (1) yields a more robust exchange than the plug technique.

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OPTIMIZATION OF SENSITIVITY AND INFORMATION IN THERMOSPRAY LC/MS

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Introduction

Many applications of thermospray LC/MS in our laboratory are concerned with qualitative analysis, i.e. confirmation or identification of structures. Two aspects play an important role in solving this type of problems:

- * sensitivity: because small amounts of sample are available and people are interested in the minor components.
- * information content: obtain both molecular weight information and useful fragmentation for structure elucidation.

These aspects are discussed in this contribution using some recent examples and applications.

Experimental

All experiments were performed on a Finnigan MAT TSQ-70 mass spectrometer equipped with a Finnigan MAT thermospray interface. The ion source contains a repeller electrode opposite to the sampling cone. Two LKB 2150 HPLC pumps controlled by an LKB 2152 HPLC controller were used for solvent delivery.

Sensitivity

Several experimental parameters have been investigated with respect to their influence on sensitivity, e.g. ionization mode, solvent composition, flow-rate, vaporizer temperature, repeller potential. The most important parameter determining the sensitivity is the analyte itself. Differences in sensitivity of five orders of magnitude are observed with various compounds.

In thermospray buffer ionization best signals are mostly obtained at repeller potentials between 50 and 100 V, while in discharge-on mode intense protonated molecules are observed at low repeller potentials and fragmentation at high repeller potentials.

Information

Structural information can be obtained from thermal decomposition, repeller-induced fragmentation and by collisionally induced dissociation. Depending on the type of molecule one or more of these methods can be successful. Pitfalls may also be present. Two examples:

1. Mitomycin C gives next to the protonated molecule at $m/z=335$ a thermal hydrolysis product at $m/z=278$ (after loss of HNCO) (see figure 1 for structures)
2. Cyano-compounds analyzed can either lose HCN or give a hydrolysis product ($-\text{CN} \rightarrow -\text{OH}$, apparent loss of 9 amu) (see figure 2 for reactions and figure 3 for some spectra)

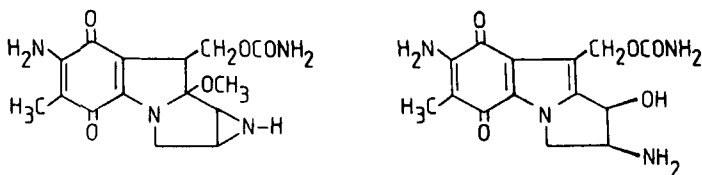


Figure 1: Structure of mitomycin C and its hydrolysis product, formed at higher vaporizer temperatures.

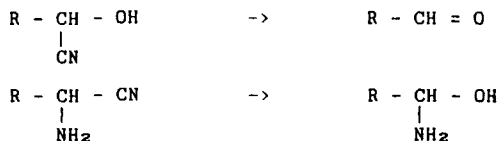


Figure 2: Thermal decomposition of cyano-compounds.

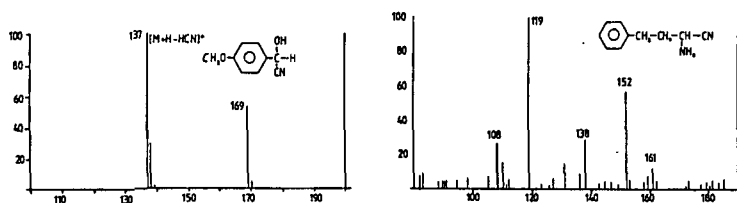


Figure 3: Thermospray mass spectra of cyano-compounds.

Low flow thermospray

Preliminary experiments with low-flow thermospray (0.25 ml/min of methanol) in discharge-on mode with (substituted) polycyclic aromatic hydrocarbons indicate that it is possible to obtain charge exchange type spectra under these conditions. The charge exchange behaviour is more clearly seen at higher vaporizer temperatures. For these thermally stable compounds higher vaporizer temperatures are no problem. Experiments will be continued and results will be reported in due course.

The Effect of Ion Source Configuration on the Gas-Phase Ionization and Ion-Evaporation Components of Thermospray.

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INTRODUCTION

To better evaluate the gas-phase ionization and ion evaporation mechanisms in thermospray, a set of five compounds were subjected to thermospray MS analysis on three different ion sources. The response for each compound was related to the efficiency of the ionization process. This information was then used to determine which ion source configuration was optimum for a particular compound class. The ion sources investigated were the conventional, or transverse, thermospray ion source, an on-axis thermospray ion source, and an ion source of counter-flow design. The five compounds used for these investigations were amonafide, imipramine, secobarbital, T-2 toxin and Direct Violet 32. These compounds were chosen because of their varying degrees of thermospray sensitivity and their different mechanisms of ionization.

EXPERIMENTAL

A Vestec model 201 dedicated LC/MS system (Houston, TX) was used for all experiments. The liquid chromatograph was an SSI (State College, PA) model pump. A Hewlett-Packard model 9100 data system (Palo Alto, CA) was used for all data collection and to control all the mass spectrometric parameters. The mobile phase used in all experiments was 0.1 M aqueous ammonium acetate. Modifications performed on the conventional thermospray ion source to obtain the on-axis and counter-flow ion sources are shown diagrammatically in Figures 1 and 2, respectively.

RESULTS AND DISCUSSION

On-Axis Ion Source: The on-axis ion source exhibited good sensitivity for those compounds which are ionized in the gas-phase. The $(MH)^+$ ions of imipramine and amonafide, and the $(M-H)^-$ ion for secobarbital, gave intermediate response compared to the transverse and counter-flow designs. A typical response curve for these compounds is given in Figure 3. For T-2 toxin, the on-axis ion source gave the highest response. No response was observed for the $(M-2Na)^{2-}$ ion of Direct Violet 32 (Figure 4). These observations are consistent with efficient gas-phase ionization generating $(MH)^+$, $(M-H)^-$ and $(MNH_4)^+$ ions.

Transverse Ion Source: The results indicate that this ion source is very efficient for detecting ions formed by gas-phase ionization. The transverse source gave the highest relative response for imipramine, amonafide and secobarbital which are ionized by proton transfer reactions (Figure 3). The transverse source also gave good results for the $(MNH_4)^+$ adduct ion of T-2 toxin. The transverse source gave a weak signal for the $(M-2Na)^{2-}$ ion of Direct Violet 32 (Figure 4). In water, and 0.005 M aqueous ammonium acetate, a higher signal was obtained.

Counter-flow Ion Source: A schematic of the counter-flow ion source is given in Figure 2. The counter-flow ion source, like the on-axis ion source, was designed to increase sensitivity for ions generated directly from the condensed phase (i.e. those that are produced by ion-evaporation). This is essentially done by increasing the residence time of the droplets inside the ion source. The increased residence time allows ions to evaporate from the charged droplets that would otherwise be swept past the ion sampling region. The longer residence time of the droplets in this region also increases the probability for ion evaporation to occur. The back flow of hot vapor also

aids in neutral evaporation from the droplet thus, increasing the charge-per-unit area on the droplet which is necessary for ion-evaporation.

The increase in ion-evaporation is reflected in the dramatic increase in response for the $(M-2Na)^{2-}$ ion of Direct Violet 32 (Figure 4). This increase is also aided by a decrease in gas-phase neutralization reactions which is directly related to the lower pressure within the counter-flow ion source. The lower ion source pressure is also responsible for a dramatic decrease in the sensitivity for compounds ionized by gas-phase ionization.

CONCLUSIONS

The conventional ion source works best for those compounds that are ionized by proton transfer reactions in the gas-phase. Very good sensitivity was obtained for compounds having high gas-phase acidities or basicities using this ion source. The model compounds used to measure gas-phase ionization efficiency were amonafide, imipramine and secobarbital.

The on-axis ion source gave best results for T-2 toxin indicating enhanced response for those compounds which have low proton affinity but ionize by gas-phase ionization to form ammonium adduct ions. It appears that gas-phase proton transfer reactions occur in the initial jet expansion region near the vaporizer exit. This region is most efficiently sampled by the transverse ion source. Adduct ion formation is a less energetic process and may require less energetic collisions which occur downstream from the initial jet expansion. In this case, the on-axis ion source would sample these ions more effectively.

The counter-flow ion source increases sensitivity for ions generated directly from the condensed phase by increasing the residence time of the thermospray droplets inside the ion source and by decreasing gas-phase neutralization reactions. Full-scan mass spectra (m/z 300-750) of Direct Violet 32 were easily obtained on two micrograms of dye injected. Selected ion monitoring results for the $(M-2Na)^{2-}$ ion of Direct Violet 32 gave signal-to-noise ratios of more than ten-to-one with two nanograms of the dye injected.

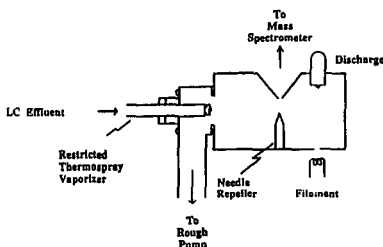


Figure 1. On-axis ion source.

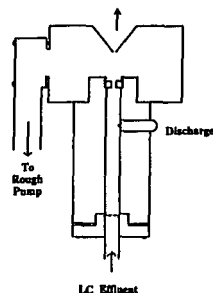


Figure 2. Counter-flow ion source.

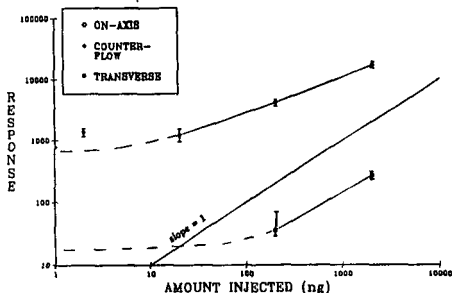


Figure 3. Log-log plot of response vs amount of imipramine injected.

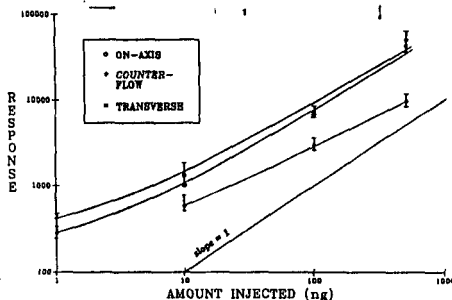


Figure 4. Log-log plot of response vs amount of Direct Violet 32 injected.

DESIGN AND PERFORMANCE OF A NEW COMBINED THERMOSPRAY-EI LC-MS SYSTEM

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Performance data are presented on a new interface between liquid chromatography and EI mass spectrometry which efficiently transports the sample to the mass spectrometer while removing almost all of the solvent without introducing significant loss in chromatographic efficiency. A block diagram of the system is shown in Figure 1. It employs a thermospray probe to partially vaporize and nebulize the LC effluent. Vaporization is completed in the heated spray chamber, and most of the solvent vapor is removed in novel countercurrent flow gas diffusion separator. (Fig. 2) Further solvent vapor reduction and carrier removal to reach an appropriate EI ion source pressure is accomplished using a 2-stage momentum separator. Carrier flow is typically 2 L/min of He, but only 1-2 mL/min (STP) enters the ion source along with less than 1 ppm of the solvent vapor (Fig. 3), and except for the most volatile materials, about 70% of the sample. (Fig. 4) Total dispersion due to the interface is typically 1.6 sec (Fig. 5), which is somewhat less than that introduced by the on-line UV detector. Response is independent of LC flow up to at least 1.5 mL/min (Fig. 6), and dependence of solvent composition is minimal. (Fig. 7)

All heating is controlled from a standard thermospray vaporizer controller and heater power is automatically adjusted for flow and composition changes without operator intervention. Library searchable EI spectra are routinely obtained with less than 100 ng of sample injected on column, for samples within the volatility range of the technique, and SIM detection limits are usually in the subnanogram range. Instrument sensitivity is comparable to GC-MS, but detection limits may be somewhat poorer in some cases due to interference from solvent impurities and because of reduced sample mass flow rate caused by lower chromatographic efficiency of LC vs. capillary GC. The interface is compatible with all LC separations (except the MS does not tolerate nonvolatile buffers) and all EI mass spectrometers, including benchtops.

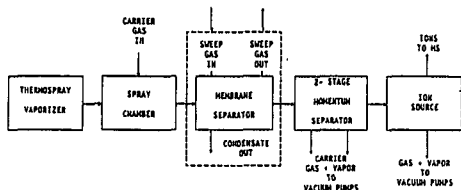


Fig. 1

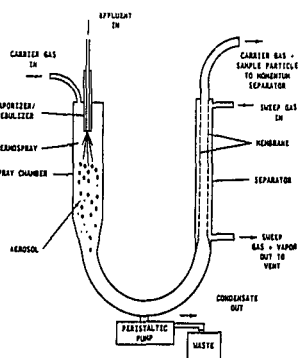


Fig. 2

VESTEC UNIVERSAL INTERFACE

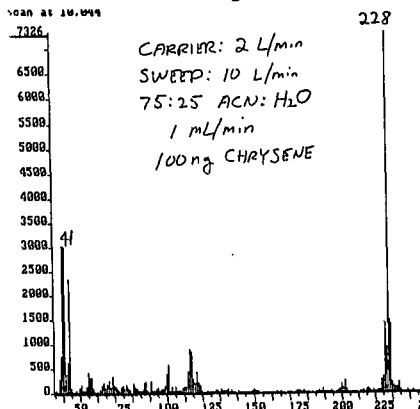


Fig. 3

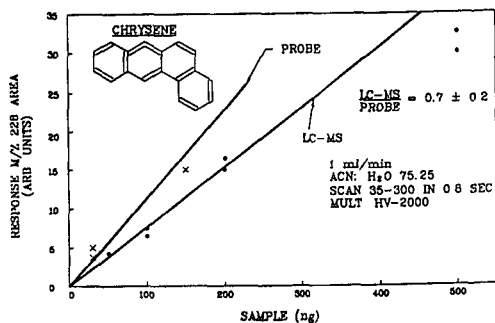


Fig. 4

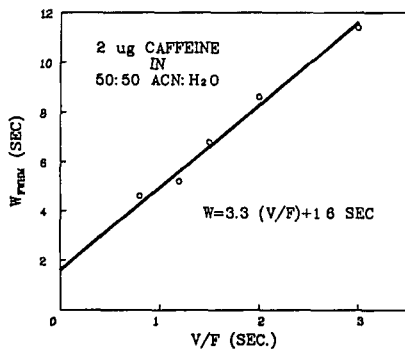


Fig. 5

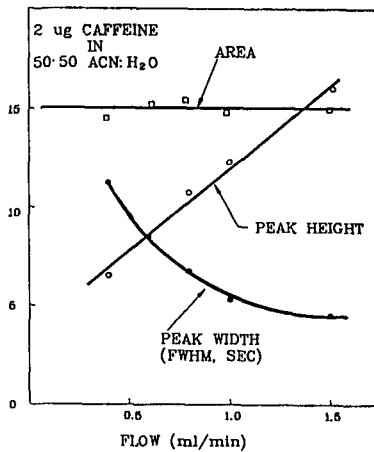


Fig. 6

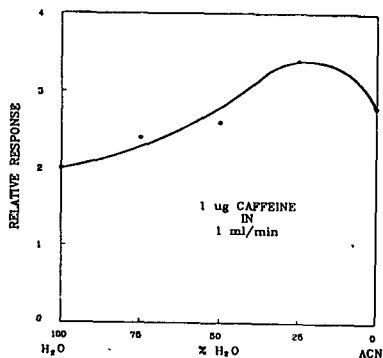


Fig. 7

APPLICATION OF THERMOSPRAY HPLC/MS TO ORGANIC SYNTHESSES

Lily Yang, Keystone Environmental Resources, Inc., Houston, TX 77063

Thermospray HPLC/MS has been widely applied to environmental science, pharmaceuticals, biochemistry and drug analyses since 1981. Dr. M. L. Vestal et al. first introduced the technique.

This paper presents the application of Thermospray HPLC/MS to organic syntheses. Because of the unique characteristics and the practical calibration method developed⁽¹⁾, Thermospray HPLC/MS can be used to identify and confirm the final product in a synthetic mixture. Figure 1 shows an example of the confirmation. Protonated molecular ion at m/z 264 provides significant evidence of the formation of the compound expected by the organic chemist.

Thermospray HPLC/MS has been successfully applied to the organic synthesis of histidine from prebiotic precursors. Intermediates such as Imidazole-4-acetaldehyde, Imidazole-4-ethanol, and Imidazole-4-glycol were first identified by Thermospray HPLC/MS⁽²⁾. The analytical results encouraged the continuation of the synthetic process, although the histine was not found in these synthetic mixtures. When the final product was identified in a very small yield (Figure 2), the Thermospray HPLC/MS once again directed the synthetic route. Based on two years efforts, the final product, histidine, was successfully obtained and confirmed (Figure 3). The formation of histine from prebiotic precursors was further confirmed with HPLC/MS/MS⁽³⁾.

These results point out the importance of HPLC/MS in organic syntheses.

This work began at the chemistry department of the University of Houston, was finished at Vestec Corporation, and was summarized at Keystone Environmental Resources, Inc.

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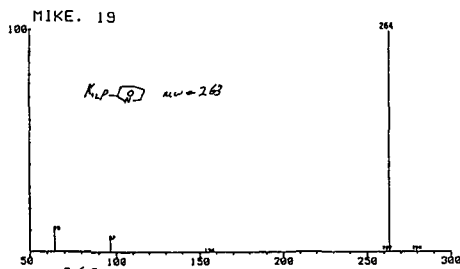


Figure 1: Thermospray mass spectrum of the synthesized organic compound with molecular weight 263.

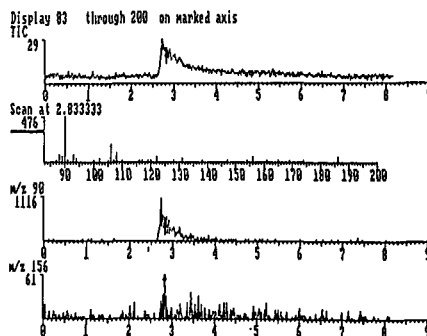


Figure 2: Thermospray mass spectrum and Ionchromatograms of the synthetic Histidine mixture. Ions at m/z 156 indicates the formation of Histidine.

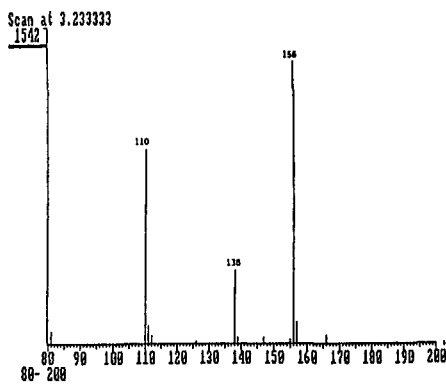
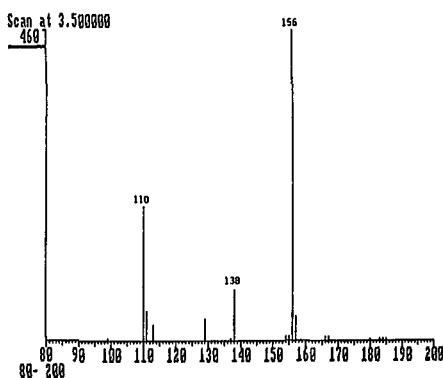


Figure 3: (Left) Mass Spectrum of synthesized Histidine by thermospray HPLC/MS.
(Right) Mass Spectrum of Authentic Histidine by thermospray HPLC/MS.

OPTIMIZATION OF ON-LINE ENZYMATIC HYDROLYSIS HPLC/MS
FOR PEPTIDE MAPPING AND DISULFIDE BOND LOCATION

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The use of immobilized forms of enzymes offers a number of advantages including: a) potentially higher enzyme stability than soluble forms, b) controlled reaction kinetics, and c) fewer reaction by-products. To realize the many advantages attending the use of immobilized enzyme combined with HPLC/thermospray MS, the immobilized enzyme must exhibit high activity and stability. Optimal conditions as well as accepted operating range of conditions for a variety of peptidases including trypsin, chymotrypsin, V-8 protease, thermolysin, and carboxypeptidase, A, B, and Y were determined. Immobilized enzyme activity is dependent on pH, temperature, solvents and buffers used for the hydrolysis. Extremes in any one condition can irreversibly destroy the bioreactor activity. Hydrolysis at conditions far from optimal can lead to no hydrolysis or partial hydrolysis of a peptide, providing limited useful information on the peptide.

The effect of pH on the activities of the immobilized enzymes indicate that the endopeptidases have an optimum pH between 7.5 and 8.0, carboxypeptidase A from pH 7.0-7.5 and carboxypeptidase Y from pH 5.5-6.5. Furthermore, when the pH was adjusted 1-2 units from the optimal range for a period of 1-4 hours, 90% of the activity could be regenerated when conditions were returned to optimum, indicating enzyme stability. However, exposure to pHs above 10 and below 4 often resulted in an irreversible loss in enzyme activity.

The temperature of the immobilized enzyme bioreactors affects the enzyme activity according to the activation energy of the reaction, the effects on mass transfer, and the effects on enzyme structure. Column temperatures of 40-50°C could increase activity by a factor of 2 to 3 compared to the activity measured at 20°C.

Enzymatic activity is strongly dependent on the choice and percentage of organic solvent in an aqueous solution. To achieve suitable resolution and elution, organic modifiers must be added to an aqueous solution for reversed-phase chromatography. Isopropanol, acetonitrile, and tetrahydrofuran (25% v/v in H₂O) solutions do not appreciably affect the activities. Use of ethanol or methanol shows nearly a factor of 2 loss in activity for trypsin and carboxypeptidase B. Acetone exhibited the greatest denaturing power resulting in nearly complete loss of activity. The 0-30% organic range appears to be suitable for bioreactor operation. Often low percentages of organic component (2-15%) can result in higher enzyme activity, perhaps due to increased structural flexibility of the enzyme when low percentages of organic are present. Solutions containing 15-30% organic phase resulted in losses of enzyme activity (typically less than 50% loss of activity). At organic percentages greater than 30% the enzyme activity further decreased, presumably due to structural changes in the enzyme.

Usually the best enzymatic activity was found using aqueous solvents with tris or phosphate buffers. However, these buffers must be substituted with volatile buffers for suitable thermospray MS operation. Buffers including ammonium acetate, ammonium formate and ammonium hydroxide all exhibit similar enzyme activity compared to tris. Additional experiments indicate ammonium acetate concentrations between 0.1M to 1M resulted in the highest immobilized enzyme activity. Buffer concentrations at 0.01 M or lower resulted in a factor of 2 or more loss in enzyme activity as well as reduced thermospray MS sensitivity.

The combination of immobilized peptidase bioreactors HPLC/thermospray MS can provide an unequivocal, rapid method for identification of disulfide bond position in proteins.

Such analytical methodology would greatly facilitate studies of disulfide bond formation pathways in the folding mechanisms of proteins. Selection of the proper endopeptidase in the case of most fully reduced peptides or proteins will yield peptide fragments containing only one cysteine. This is usually true for thermolysin or combinations of chymotrypsin, trypsin or V-8 protease generated peptides from vasopressin, BPTI and somatostatin. Comparison of the molecular masses of peptides obtained from the native peptide to those from the reduced peptide will thus identify the disulfide bond position in peptides of known primary structure. For example, the tryptic hydrolysis of native somatostatin resulted in a thermospray spectrum showing ions at m/z 741 and 935 (Figure 1A). The latter ion can be attributed to the disulfide bond linkage of the tryptic fragments T_1 and T_3 . The reduction of the disulfide bond and subsequent thermospray MS analysis showed ions at m/z 378, 741, and 558 for tryptic fragments T_1 - T_3 (Figure 1B). The absence of the ions at m/z 935 and the presence of ions at m/z 378 and 558 indicated the location of the disulfide bond. While somatostatin is a trivial example, this procedure was used to determine the disulfide bond location in BPTI.

This work was supported by the National Institute of Drug Abuse, Grant No. 5 R01 DA 04202-02.

Figure 1. Trypsin Hydrolysis Thermospray HPLC/MS
Analysis of Somatostatin

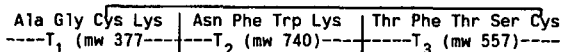


Figure 1A.

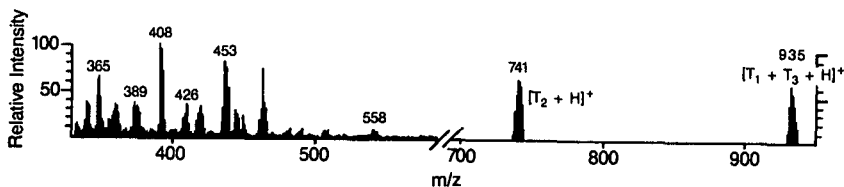
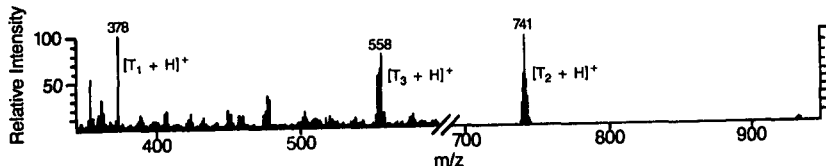


Figure 1B.



- 1A) Native Somatostatin
1B) Reduced Somatostatin

ANALYSIS OF ANTIBIOTICS USING SUPERCRITICAL FLUIDS AND HPLC/MS TECHNIQUES

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Introduction

During the past few years there has been a growing concern about residues of antibiotics and other pharmaceutical substances in agricultural products such as milk and meat. This concern has led to the search for more rapid and simple methods for the determination of such drug residues. The current work examines the utility of supercritical fluids for the extraction (SFE) and chromatography (SFC) of tetracycline antibiotics. Cephalirin, another antibiotic of interest which was not successfully chromatographed using supercritical fluids, was analyzed by HPLC and HPLC/MS to determine its metabolites and its presence in bovine milk.

Experimental

SFE of tetracyclines were performed using 12% methanol in CO₂. A 0.2 mL stainless steel extraction cell held at 40°C was loaded with the tetracycline samples and dynamically extracted with 8 mL of supercritical fluid at 5500 psi. A crimped 0.001 mm stainless steel tube or a 25 mm i.d. fused silica tube was used to maintain supercritical conditions. The outlet was placed in methanol to dissolve the extracted components as the supercritical fluid was expanded. The methanol was evaporated and the sample was redissolved in 0.5 mL of methanol prior to analysis by SFC or MS. SFC separations were performed on a 30% biphenyl column using pure CO₂ with flame ionization detection (FID). Mass spectra of the tetracyclines were obtained on a particlebeam interface on an HP-5988A quadrupole MS. The interface was operated at a desolvation temperature of 40°C, source temperature of 250°C under EI conditions (70 eV).

The experimental conditions for the analysis of cephalirin were as follows: Extraction of cephalirin from milk was accomplished by using ultrafiltration with a 10,000 dalton cutoff filter. A 0.5 mL milk sample was diluted with an equal volume of acetonitrile:methanol:water (2:1:2), mixed and centrifuged at 3000 G for 30 minutes. The ultrafiltrate sample was used directly for HPLC or HPLC/MS analysis. The HPLC analysis was performed using a microbore phenyl spheri-5 cartridge (22 cm x 2.1 mm) with a 20% acetonitrile, 5% methanol solution containing 0.005 M dodecanesulfate and 0.5% H₃PO₄. The mobile phase flow rate was 0.4 mL/min. An ultraviolet photodiode array detector (200 nm - 360 nm) was used to monitor the separation. HPLC/MS was performed on a Finnigan MAT 4800 with a thermospray interface. The HPLC conditions were changed to 6.5% isopropanol in 0.03 M ammonium acetate solution with 0.5% acetic acid. The separation was performed on a phenyl spheri-5 (22 cm x 4.6 mm) cartridge at 1.3 mL/min. The thermospray interface was operated under ion evaporation conditions with a vaporizer temperature of 135°C and a source temperature of 270°C.

Results and Discussion

Analysis of tetracyclines:

Tetracycline could be extracted using 12% methanol in CO₂. Extraction recoveries greater than 75% were achieved for solution standards. Tetracycline showed poor recoveries (less than 10%) when using pure CO₂, probably indicating poor solubility in supercritical CO₂. However, SFC separation of tetracycline could be accomplished using pure CO₂ on a 30% biphenyl column. Tetracycline, oxytetracycline and chlorotetracycline

have retention times of 20.9, 20.6, and 21.2 minutes respectively, making their simultaneous detection difficult. Separation of tetracycline using pure CO₂ enabled the use of FID for their detection, permitting determinations down to 0.1 ng. The identity of tetracyclines were confirmed by particlebeam HPLC/MS. Particlebeam interface permitted acquisition of EI spectra which showed [M]⁺ ions as well as numerous fragment ions, permitting compound confirmation.

Analysis of cephalixin:

Ultrafiltration provided a quick and easy method to extract cephalixin from milk. The mean recovery of cephalixin from spiked milk within 50-1000 ppb range was 85%. The ultrafiltrate could be directly analyzed by HPLC/UV down to 10 ppb (0.5 ng) with minimal interference from the milk matrix. The retention time for cephalixin was 6 minutes. The best UV sensitivity for cephalixin was obtained at 291 nm, the UV max for the antibiotic. The UV chromatogram also showed a peak at a retention time of 4 minutes which exhibited a similar UV spectrum as cephalixin. This possible metabolite of cephalixin was identified by thermospray HPLC/MS.

Thermospray HPLC/MS proved specific for cephalixin and permitted the tentative identification of the metabolite as desacetyl-cephalixin. The thermospray spectrum of cephalixin (mw 423) exhibited an [M+H]⁺ ion at m/z 424 and fragment ion at m/z 382, 364, 338, 317, 304, 268, 252, 209, and 168. The first three fragments were losses of CO₂ and H₂O while the ions at m/z 209 and 168 are protonated fragment ions from the opening of the ring cleavage of the cephalixin ring. Desacetyl-cephalixin (mw 381) exhibited an [M+H]⁺ ion and the same fragment ion (below m/z 380) as cephalixin.

Desacetyl-cephalixin was detected in-vivo and in-vitro in bovine milk and in serum. The in-vivo ratio between cephalixin to desacetyl-cephalixin correlated closely with in-vitro incubation of bovine milk and serum. However, the metabolite was not formed in pasteurized milk, probably due to the destruction of an esterase responsible for the metabolism of cephalixin.

The quantity of cephalixin and desacetylcephalixin could be determined by HPLC/UV and confirmed by thermospray HPLC/MS in bovine milk. The quantity of each component found in bovine milk various times after intramammary administration is presented below:

Time after administration (hrs)	Cephalixin (ppm)	desacetyl-cephalixin (ppm)
16	17.0	16.5
24	1.3	1.8
40	0.02	ND
48	0.01	ND
64	<0.01	ND
72	<0.01	ND
96	<0.01	ND

ND=not determined due to matrix interference.

Only the 16 hr and 24 hr samples were confirmed by thermospray HPLC/MS. Clearly, cephalixin can be found in milk 48 hours after its administration.

This research was supported by the Food and Drug Administration, Cooperative Agreement No. FD-U-000244-0.

ANALYSIS OF CATIONIC HEAVY METAL ALKYLATES BY THERMOSPRAY LC/MS

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The analysis of metals in environmental and biological samples becomes increasingly important. Due to the growing knowledge about the metabolic pathways, it is not longer sufficient to determine the amount of a metal in a biological fluid or tissue. More important are the informations about their transport mechanisms in organisms; this means in such a context the analysis of element-organic species.

The main problem appears to be the separation from the matrix, since a wide variety of different complexes may be formed (cationic, anionic, coordinative a.s.o.). Therefore, a mass spectrometer is the ideal detector for such a separation and LCMS on-line instrumentation is most promising.

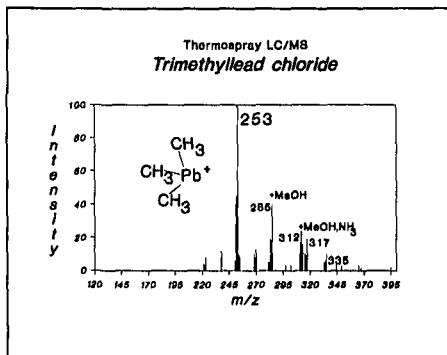


Fig. 1

Our objective is evaluation of the scope and the limitations of Thermospray-LCMS for the analysis of element-organic species.

The compounds of this study have been chosen with respect to toxicology, importance in technical products and their abundance in the environment. The trimethyllead cation is one of the most toxic lead derivatives formed by demethylation from the gasoline additive tetramethyllead; its analysis in biological fluids is difficult and we have used thermospray successfully in a cooperative study already [1]. The organo-tin compounds are used as fungicides (triphenyltin acetate = "Fentin-acetate") and stabilizers for plastics, polyurethan foams and paints in large amounts and some of them or their environmental breakdown products are known as highly toxic.

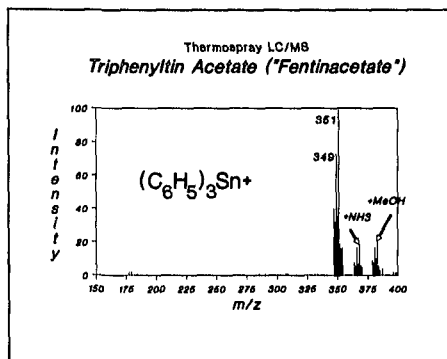


Fig. 2

We have used our self constructed Thermospray-LCMS [2][3] system

comprising a single focusing MAT CH5, a probe with replaceable 50 micron nozzle and a home made LC/MS ion source. For the chromatography, a syringe ISCO LC-2600 precision pump was employed and a RP-column (LiChroCart 125-4, Merck, Darmstadt). The solvent was NH_4OAc buffer (0.1M) in 20% Methanol/80% H_2O or 80% Methanol/20% H_2O for the organotin compounds adjusted with acetic acid to pH 4.5. The typical flow was 1.1 ml/min.

For the full spectra, a MAT 90 equipped with the Thermospray ion source was used. The samples were introduced without a column and the solvent was 100% Methanol with 0.1M NH_4OAc ; the flow was set to 1.5 ml.

Discussion

It becomes evident that the detection of mono-cationic organolead and organotin compounds is possible with high sensitivity and specificity, although adduct-ions with ammonia and methanol are formed. There is little fragmentation; results from MS/MS experiments indicate a rather complex fragmentation pattern (loss of H_2 , CH_2 , CH_4 a.s.o). For the dicationic species the most abundant ion is the adduct with the acetate anion; no further clusters of remarkable intensity are recorded. The trichloro monobutyltin compound was not detected. In the future, the isotopic pattern may serve as a chemometric detector for the species in more complex matrices.

In conclusion we have shown that thermospray LC/MS is a very powerful technique for the analysis of metal organic species. The first experiments indicate that the detection and quantitation of organolead and organotin compounds is possible with linear responses down to the low ng level absolute. The identification of such compounds with the help of their isotopic pattern allows the analysis of complex mixtures of biological samples. With modified LCMS systems in the near future the detection limit will approach the picogram range.

Acknowledgements

We are grateful to P. Dobberstein, Finnigan MAT, Bremen, for measurement of the full spectra on a Finnigan MAT 90 mass spectrometer. Financial support from the BMFT, Bonn, and the MWF, Düsseldorf, is gratefully acknowledged.

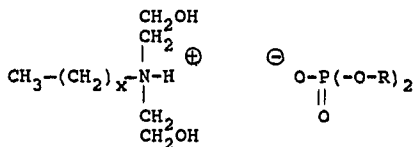
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HPLC-MS ANALYSIS OF COCONUT AMINE SALTS OF ORGANIC PHOSPHORIC ACIDS

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Arthur D. Little, Inc., Cambridge, MA 02140

Coconut amine salts are fatty amine salts which have wide applications as surfactants and biocides. They typically contain an alkyl homologous series (x) on the amine portion of the molecule as shown below.



In some cases it is desirable to determine the identity of the species present in products comprised of these particular salts. We have utilized plasmaspray LC-MS to identify the components in a product containing coconut amine salts of organic phosphoric acids.

Experimental Conditions

Equipment

HPLC: Waters Nova-Pak C₁₈ Column (3.9 mm x 15 cm)
Waters 600-MS Multisolvant Delivery System
Mass Spectrometer: VG-70 SEQ with VG Thermospray/Plasmaspray
LC-MS Interface

Chromatographic Conditions

85% Methanol/15% 0.1M Ammonium Acetate in Water
Isocratic 1 mL/minute

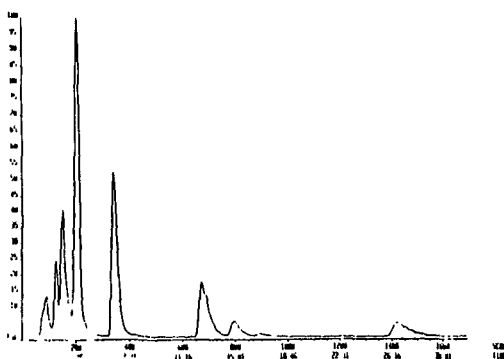
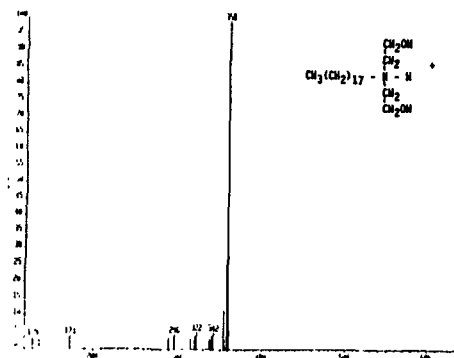
Mass Spectrometry Conditions

Mass Range Scanned: 800-120 amu
Scan Speed: 1.0 sec/decade; 0.3 sec interscan delay
Ion Source Temperature: 200°C
LC-MS Interface Probe Tip Temperature: 300°C

Solutions of the following were prepared at 10mg/ml concentrations and analyzed (10uL) by plasmaspray LC-MS under the conditions described.

1. di-ethyl hexyl phosphonic acid (DEPA)
2. mono-ethyl hexyl phosphonic acid (MEPA)
3. coconut amine neutralized salt of DEPA
4. coconut amine neutralized salt of MEPA
5. Product containing mixtures of #1-4

The coconut amine neutralized salts contain a homologous series with the carbon chain length ranging from C_8 through C_{18} . Analysis by LC-MS of the product mixture resulted in separation and identification of approximately 10 species. The mass spectra could distinguish between these components by virtue of their carbon chain length. Shown below is an example of a typical plasmaspray mass spectrum for these compounds. Also shown is the LC-MS chromatographic profile for one of these products.



By full scanning, the method was adequately sensitive to detect approximately 50 ng per component. Since these products occur as mixtures (no standards are available) but contain no chromophores, LC-MS was needed to identify the species present. Selected ion monitoring would obviously have improved on the sensitivity, of the method but was not necessary for this work.

Analysis of Environmentally Significant Compounds by Thermospray EI LC-MS Winn, D.H., Vestal, M.L., Vestec Corporation, 9299 Kirby, Houston, TX 77096

There have been numerous methods developed for both GC and GC-MS for a number of environmentally significant compounds. However, a greater number of these compounds are amenable to analysis by HPLC than GC. The lack of an HPLC detector that can definitively identify compounds in a mixture has been lacking up till the advent of a reliable interface for EI LC-MS. With Thermospray EI LC-MS it is possible to combine the versatility of HPLC and the confirmational capabilities of a mass spectrometer.

Data from the adaptation of EPA method 610 for the analysis of polynuclear aromatic Hydrocarbons, (PAH) by Thermospray EI are presented. The data presented shows that it is possible for routine quantitation of target compounds as well as confirmation of its presence.

Figure 1 shows a chromatogram of a mixture containing 25 ng of chrysene. It is possible to extract an individual ion chromatogram and spectrum to show the compound's presence. Further comparison can be to a user generated library spectrum, Figure 2, or from a standard reference library, Figure 3.

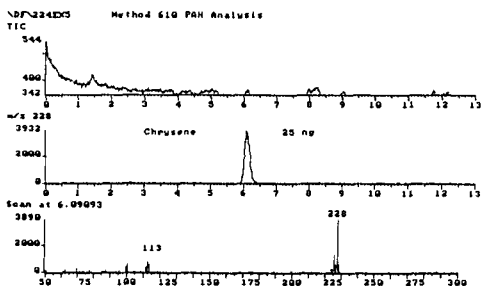


Figure 1.

Figure 2.

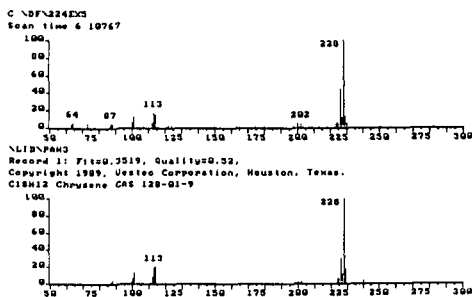
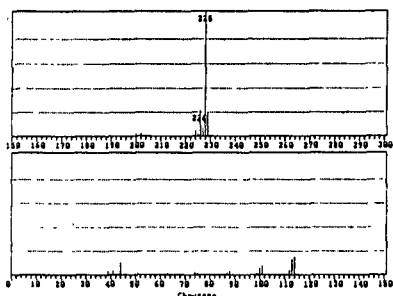


Figure 3.



Just as target compounds can be identified and confirmed, with Thermospray EI it is possible to identify total unknowns in a mixture, Figure 4.

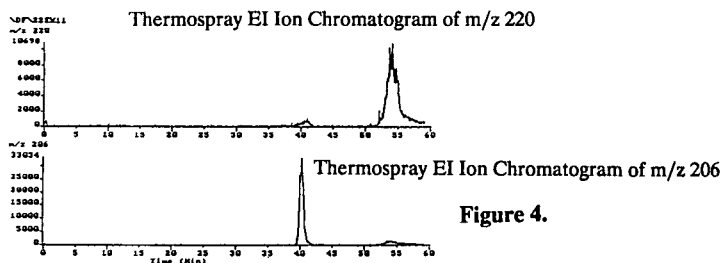
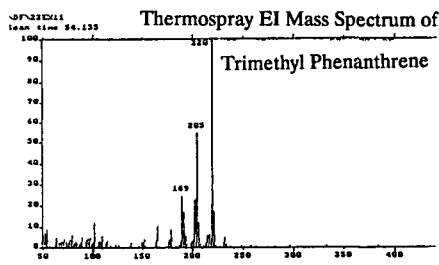
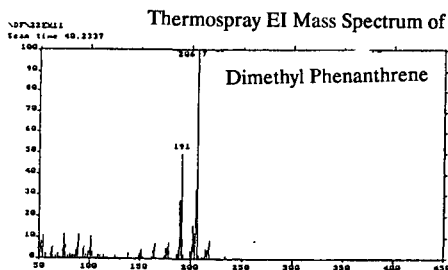
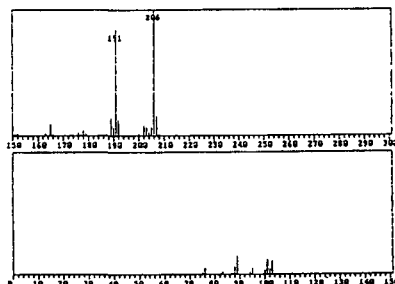


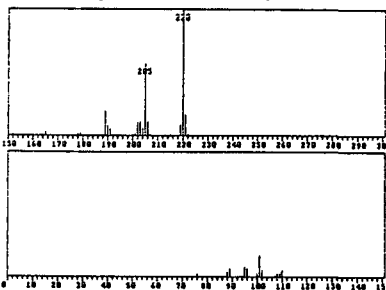
Figure 4.



NIST Mass Spectrum of Dimethyl Phenanthrene



NIST Mass Spectrum of Trimethyl Phenanthrene



From this work, it will be shown that Thermospray EI is a dependable means and reliable means for the analysis of not only PAH mixtures as well as many other environmentally significant compounds.

Peptide Mapping Using Thermospray LC-MS/MS

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Peptide mapping is often used in the biotechnology industry to confirm that the structures of proteins produced by recombinant DNA technology are identical to reference proteins and to ensure that there are no inter-lot variations of protein structure. The method involves cleavage of a protein into smaller peptide fragments using an enzyme or chemical reagent. Confirmation of samples is based on correspondence of the number of peaks and their retention times; hence, a highly accurate and reproducible HPLC system is essential. Because peptide maps generally contain 30 to 100 peaks, problems arise with instrument-specific differences in gradient elution, especially when shallow gradients are required. Nonretained and overlapping peaks can also make the analysis difficult.

Thermospray (TSP) LC/MS is ideally suited to alleviate these chromatographic problems and to generally increase the information available in the HPLC analysis of peptide mixtures. Previous studies, however, have been limited to full scan spectra to m/z 2000 (1,2). With the extended mass range available on current triple stage quadrupole instruments, full scan spectra can be recorded to m/z 4000. Furthermore, MS/MS spectra can be recorded on lower mass fragments. In this paper, an evaluation of this technique in the analysis of a tryptic digest of β -lactoglobulin A is described.

Experimental

These experiments were done on a Finnigan MAT triple stage quadrupole mass spectrometer and a Finnigan MAT TSP LC/MS ion source. The peptide mixture (10 nmol, purchased from Applied Biosystems) was separated on a 250 x 4.6-mm Aquapore RP-300 column (Applied Biosystems). The peptides were eluted at a rate of 1.2 ml min⁻¹ over 30 min with a linear elution gradient of 0% to 100% of buffer B. Buffer A was 0.1% TFA; buffer B was 70% acetonitrile in 0.1% TFA. The TSP conditions were: ion source block, 190°C; vaporizer, 85°C; and aerosol, 160°C. MS/MS spectra were recorded using 0.8 mTorr of Argon with a collision energy of -35 eV.

Results and Discussion

These experiments indicate that TSP LC/MS has a number of advantages over other MS methods for peptide mixture analysis. First, TSP LC/MS is compatible with the chromatographic systems most often used by the peptide chemists: gradient elution with TFA buffers and flow rates of 1 to 2 ml min⁻¹. Figure 1 shows the UV trace at 214 nm and the TSP total ion current. Spectra were obtained for all but two of the expected tryptic fragments, including two peptide fragments with molecular weights in the 2000 to 3000 u range. The spectra consist of abundant singly protonated and doubly protonated molecular ions with little fragmentation. Figure 2 is a TSP LC/MS spectrum of tryptic fragment T4, a 20-amino acid residue peptide. The peak at m/z 2313.8 corresponds more closely to the largest peak in the isotopic cluster and not the average molecular weight of 2314.7 u. A reduction of quadrupole resolution would result in a centroid of the entire molecular ion cluster (average molecular weight determination), as well as increased sensitivity. A TSP spectrum of the two tryptic fragments T5 and T18 connected by a disulfide bond was also obtained. The spectrum contains both a doubly protonated molecular ion of m/z 1390 and a singly protonated molecular ion of m/z 2780.

TSP LC-MS/MS spectra are recorded for smaller molecular weight tryptic fragments. Figure 3 is a collisionally activated dissociation (CAD) spectrum of tryptic fragment T2, with a MH^+ equal to m/z 673. The sequence ions shown in Figure 3 correspond mostly to Y and B type ions. Above mass 900 u, it becomes increasingly difficult to record MS/MS spectra of tryptic fragments during the time frame of a chromatographic peak. For example, for tryptic fragment T10 ($MH^+ = 1065$), no significant fragmentation of the molecular ion is achieved under these CAD conditions. An increase of the center-of-mass energy by using Xenon instead of Argon as a collision gas facilitates fragmentation of these higher mass ions.

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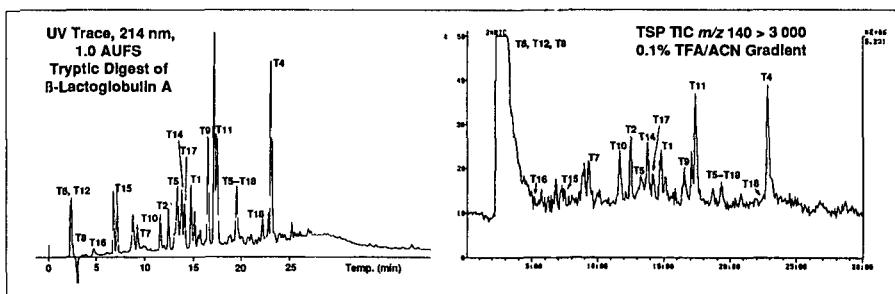
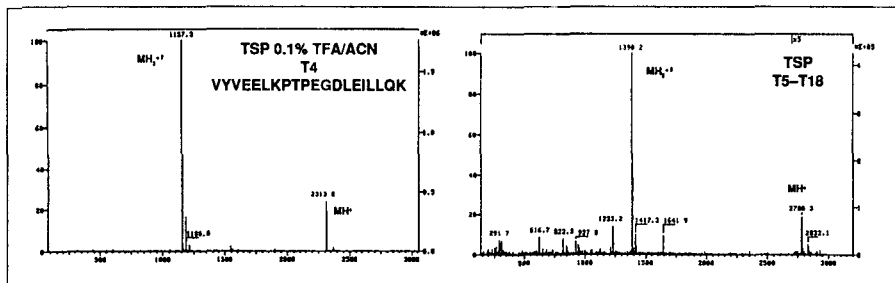


Figure 1.



A FACILE METHOD FOR THE PREPARATION AND LC/MS CHARACTERIZATION OF BENZYLOXYCARBONYL METHYL ESTERS DERIVATIVES OF REDUCED GLUTATHIONE, CYSTEINE AND CORRESPONDING XENOBIOTIC-THIOETHER CONJUGATES

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Introduction

Formation of glutathione-S-conjugates is an important mechanism for the detoxification of many xenobiotics. The xenobiotic-thioether conjugates may be excreted as such in bile or further metabolized to cysteine (CYSH) and mercapturic acid conjugates and excreted in urine. Recently, we utilized an approach of derivatizing xenobiotic-glutathione conjugates to their benzyloxycarbonyl methyl ester derivatives (CBZM). This approach facilitates the isolation of these compounds from biological matrices and assist in their subsequent purification prior to mass spectrometric analysis. The derivatives are then analyzed by FAB/MS/MS. The collision induced dissociation (CID) daughter ion spectra recorded showed several diagnostic fragment ions that are specific for these types of derivatized glutathione conjugates.¹ In the present study, we report a simple method for the preparation of synthetic standards of CBZM derivatives for the xenobiotic-thioether conjugates to aid in mass spectral confirmation of biologically formed metabolites. We have also investigated the use of thermospray liquid chromatography/mass spectrometry (TSP LC/MS) for analysis of the CBZM derivatives of xenobiotic-glutathione conjugates, cysteine conjugates and the methyl ester derivatives of mercapturic acid conjugates.

Experimental

Synthesis:

1. The bis-benzyloxycarbonyl methyl ester derivatives of GSSG or cystine were prepared by adding a 6 molar excess of benzylchloroformate to a vigorously stirred solution of either compound in sodium carbonate buffer (pH 9.0). Excess chloroformate was extracted out with methylene chloride. The solution was acidified, the derivative extracted out with ethyl acetate and treated with excess anhydrous methanolic HCl at room temperature for 10 h to yield the methyl esters.
2. Reduction of the bis-derivatives to the CBZM thiol was carried out in refluxing acetonitrile using one equivalent of dithiothreitol (DTT) as reducing agent. DTT and DTT-oxidized were removed by evaporation of acetonitrile by addition of water. The derivatives recovered by filtration.
3. The methyl ester derivative of N-acetylcysteine and N-[2H₃-acetyl]cysteine were prepared from the methyl ester derivative of cystine followed by acetylation with a mixture of pyridine/acetic anhydride or [2H₆]acetic anhydride (4:1) for 10 min at room temperature. Excess reagents were removed by rotary evaporation.
4. The CBZM derivatives of xenobiotic-thioether adduct were prepared by gradual addition of the synthetic xenobiotic electrophile in acetonitrile to a solution of the CBZM thiol in acetonitrile. Triethylamine was used as a catalyst in case of reaction with styrene oxide.

LC/MS:

Thermospray (TSP) LC/MS spectra were generated using a Vestec 201 instrument (Vestec Corp., Houston, TX) with a directly heated probe vaporizer. TSP probe control and tip temperatures were 115 and 174°C, respectively, and were adjusted to maintain a jet temperature of 204°C. The source block was normally held at 265-285°C. The HPLC system consisted of an LKB 2150 pump and LKB 2152 controller unit (LKB, Bromma, Sweden). Separations were achieved using an Ultrasphere C18 column (15 cm x 4.6 mm, i.d.; 5µm particle size) with a mobile phase consisting of 40% acetonitrile in aqueous 0.1 M ammonium acetate (flow rate 1.2 ml/min). Analyses were carried out in the "filament on" mode (filament current 350-400 µA).

Results and Discussion

In addition to the advantages described above for the derivatization strategy, the synthetic procedure described for obtaining the CBZM thiols provided several advantages: 1. The disulfides are commercially available in pure form and therefore provided an ideal method for protecting the thiol group. 2. The derivatization proceeded smoothly and rapidly in quantitative yield. 3. Using DTT as the reducing agent provided a reactant that is water soluble and therefore together with its oxidized form can be easily removed after the reaction. 4. The presence of oxidized DTT at the end of the reaction keeps the thiol in the reduced form and can be removed at any time before reacting the thiols with the xenobiotic electrophile. 5. The reaction with xenobiotic electrophiles is a rapid one step reaction although in some cases has to be catalyzed.

Our evaluation for TSP LC/MS for analysis of these types of compounds indicated that the spectra obtained provided somewhat similar information to that obtained by collision induced dissociation (CID) in FAB/MS/MS. However, for the CBZM-glutathione type compounds the spectra obtained showed either a weak [M+H]⁺ ion (Fig. 1) or no [M+H]⁺ ion at all, as in the case of methylisocyanate glutathione conjugate. Still, the spectra show diagnostic ions that would be taken as indicator for the presence of a glutathione conjugate.

The CBZM-cysteine conjugates (Fig. 2) and the methyl ester derivative of the mercapturic conjugates behaved favorably in TSP and gave several diagnostic ions that indicated the presence of either species. The spectra also showed both the protonated as well as the ammoniated molecular ions. The derivatization procedure described allows for screening for the presence of both type of compounds in the same biological sample without

need for any HPLC purification following the derivatization. The preparation of deuterated analogs of such compounds allows for their use as internal standards for quantifying these conjugates by selected ion monitoring. This work was supported by grants from NATO and the National Institutes of Health (DK30699).

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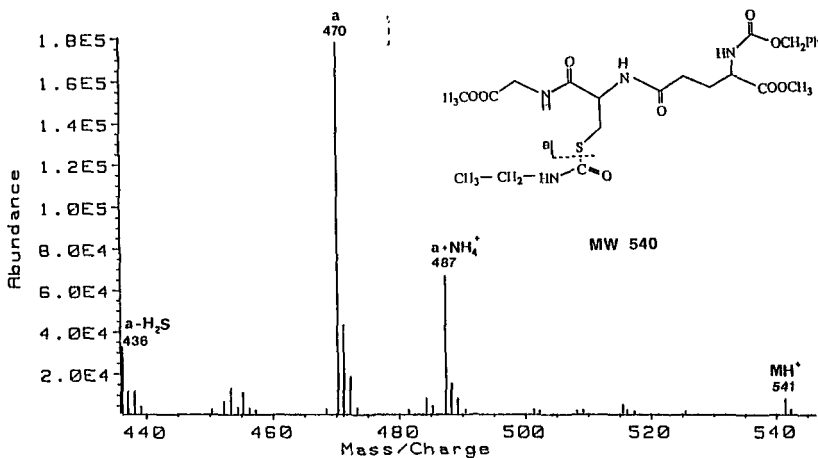


FIGURE 1. TSP LC/MS SPECTRUM OF THE SYNTHETIC CBZM DERIVATIVE OF S-(N-ETHYLCARBAMOYL)-GLUTATHIONE CONJUGATE.

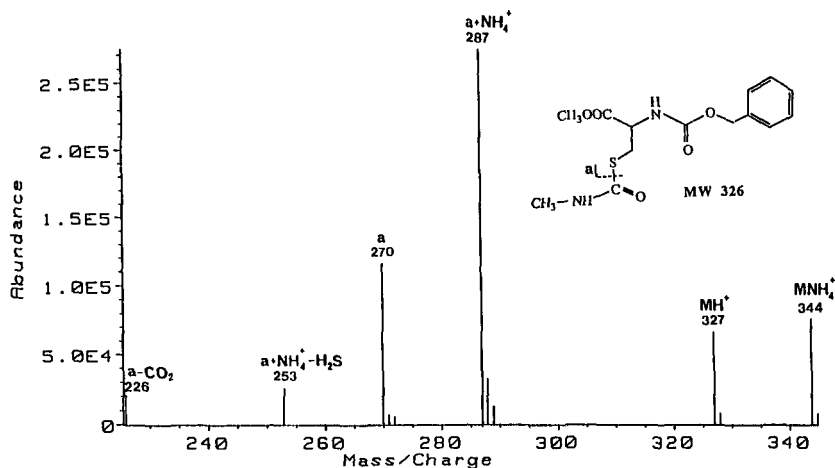


FIGURE 2. TSP LC/MS SPECTRUM OF THE SYNTHETIC CBZM DERIVATIVE OF S-(N-METHYLCARBAMOYL)-CYSTEINE CONJUGATE.

STRUCTURE DETERMINATION OF IMPURITIES IN A SYNTHESIS
OF A PROTECTED DIAZABICYCLOHEPTANE BY LC/MS

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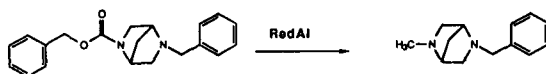
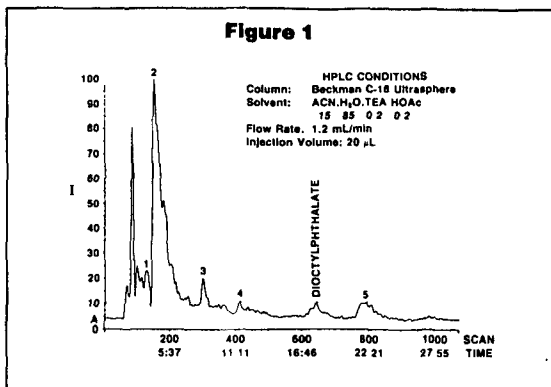
LC/MS is a useful method for rapidly identifying unknown compounds in reaction mixtures. Diazabicycloheptanes are useful in many organic syntheses. In the present case, it was desired to prepare the diazabicycloheptane as shown in scheme 1. It was of particular importance to make the N-methyl product as shown in scheme 1 without making any of the des-methyl analog as the des-methyl analog would react in later synthetic steps to produce unwanted side-products. Plasmaspay LC/MS was employed to determine the presence of impurities in the reaction product. Elemental compositions of the compounds present in this mixture were confirmed by high resolution EI and high resolution FAB.

The total ion chromatogram of the diazabicycloheptane mixture is shown in Figure 1. Five reaction products and a small amount of dioctylphthalate were identified. Compound 1, which was the impurity sought for, was identified as the des-methyl impurity. Compound 2 was the desired product. Compound 3 was generated from reaction of an impurity in a previous synthetic step with RedAl. The origin of compound 4 is not known. However, it is possible that 4 may have arisen from reaction of compound 1 with an impurity in a previous synthetic step. Compound 5 was derived from reaction of benzyl amine with the CBZ derivative shown in Scheme 1. This compound is unreactive with RedAl.

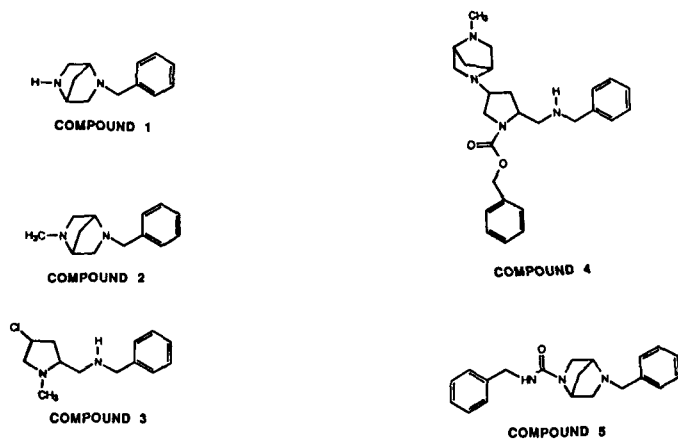
The elemental composition of compounds 1, 2, and 5 were obtained by scanning high resolution EI of the reaction mixture. The elemental composition of compound 4 was determined by high resolution FAB of the reaction mixture. Compound 3 did not yield a molecular ion by EI. Although an ion at $m/z = 239$ was observed in the low resolution FAB mass spectrum, it was too weak to obtain high resolution data on. The isotopic distribution of the molecular ion observed in the plasmaspay mass spectrum of this compound suggested that the compound contained a chlorine atom. Searching the scanning high resolution data for this isotopic distribution yielded an ion at $m/z = 118$. Based on the molecular ion data and the high resolution data of the ion at 118 amu, we are proposing the structure of compound 3.

Conclusion

LC/MS was used as a rapid method for identifying five compounds in a synthetic reaction mixture. None of the compounds identified were preparatively separated prior to analysis, which greatly reduced the analysis time. Once molecular ions were established by LC/MS, their elemental compositions were determined by high resolution (again on the mixture). Using this procedure allowed us to determine the structure of four compounds in the mixture and propose the structure of a fifth. An undesired compound (compound 1) was determined to be present which indicated that this reaction should not be used to prepare fully protected diazabicycloheptanes.



SCHEME 1



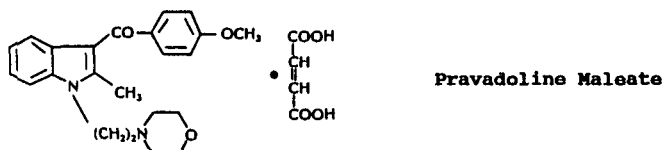
INVESTIGATION OF PRAVADOLINE METABOLITES BY THERMOSPRAY MASS SPECTROMETRY

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Pravadoline maleate is an orally active non-opioid analgesic.

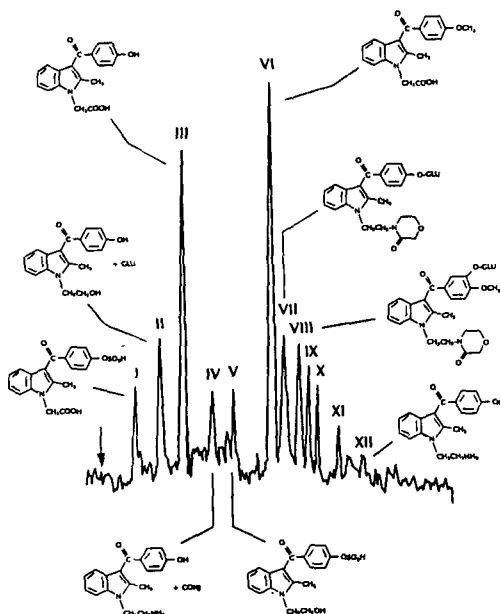


Radiolabeled studies in mice, rats, dogs, monkeys and humans have shown that the compound is well absorbed from the gastrointestinal tract, is cleared almost entirely by metabolism and is subject to moderate levels of pre-systemic metabolism in most species. The objective of these studies was to determine the metabolic profile of pravadoline in a number of species and to structurally characterize the metabolites.

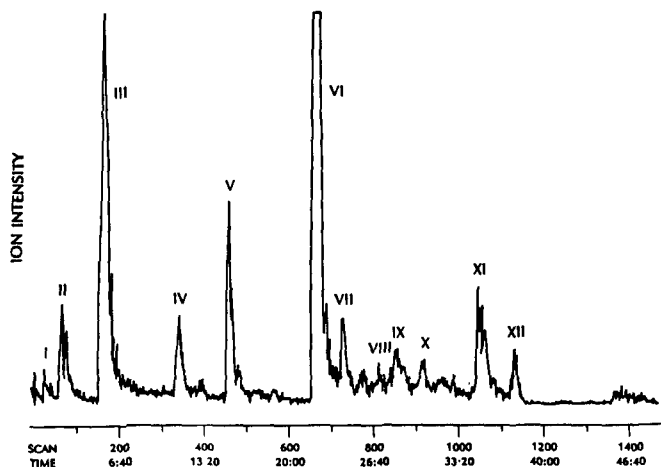
A Varian 5500 HPLC was interfaced with a Ramona radioactivity monitor and a Finnigan 4500 mass spectrometer equipped with a Finnigan TSP interface. Optimum operating parameters included 0.05 M ammonium acetate/acetonitrile gradients, a 1.5 ml/min flow rate and jet and vaporizer temperatures of 230°C and 120°C, respectively. Qualitative metabolic profiles of urine, bile and/or deproteinized plasma samples from a variety of species were obtained by direct injection onto the TSP-LC/MS system. Representative data, a radiogram of a 0-24 hr human urine specimen and the corresponding reconstructed TSP ion chromatogram are shown below. In most cases, metabolites were also isolated and purified by gradient reverse phase HPLC. Structural assignments were confirmed through comparison of their electron impact mass spectra and relative retention times with those of synthetic metabolites.

The results demonstrate the utility of TSP-LC/MS for rapid, direct analysis of drugs and their metabolites in biological fluids. With gradient conditions, metabolites of varying polarity can be analyzed in a single run. Interfacing a high pressure radioactivity monitor with the system eliminates the need for HPLC effluent splitting. The sensitivity of TSP ionization (Filament off) is highly dependent on the analyte. Although TSP spectra are useful for molecular weight determinations, limited fragmentation may preclude unambiguous identification; this can be supplemented by EI or MS/MS analysis.

RADIOGRAM OF 0-24 hr HUMAN URINE



RECONSTRUCTED ION MASS CHROMATOGRAM



METABOLISM AND CARCINOGENICITY OF HAIR DYES:
A TSP LC/MS STUDY

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and
F.W. Kari, S.M. Driscoll, and K.M. Rudo, TRTP, NIEHS, RTP, NC 27709

H.C. Blue 1 and H.C. Blue 2 are nitrophenylenediamine dyes that have been used in the hair color industry. These two closely-related permanent hair coloring agents are representative of a large class of dyes which fall into two groups based on their biological activity. H.C. Blue 1 induces significantly greater hepatocarcinogenicity than does H.C. Blue 2, producing cancer in 96% of the female mice to which it was fed, while Blue 2 is not carcinogenic even at 3 times the dose used for the Blue 1 studies (1).

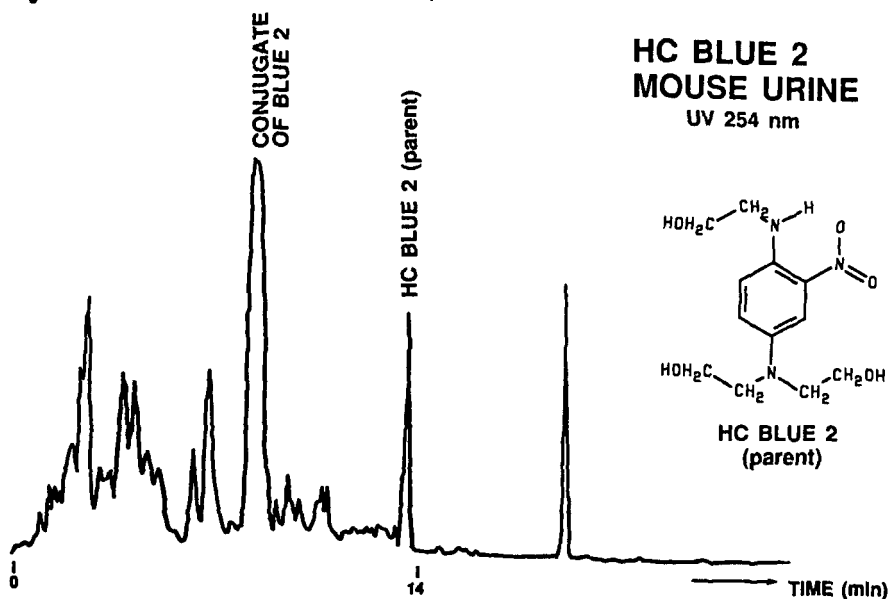
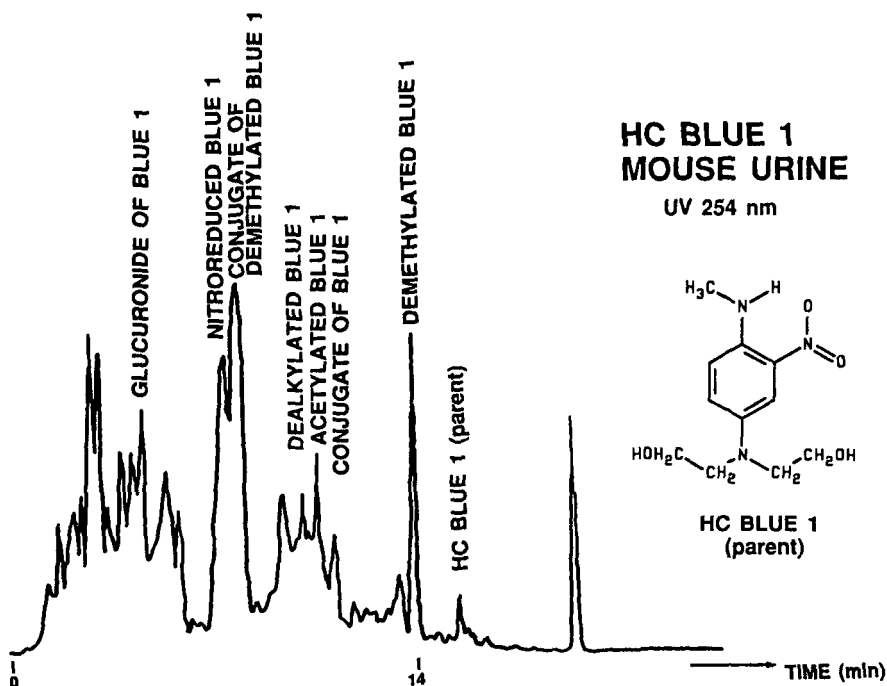
In order to explain these carcinogenicity differences, which appear to relate to differences in metabolism (2), an LC/MS study was initiated to identify the urinary metabolites. Blue 1 forms many more metabolic products than does Blue 2. Thus far, TSP LC/MS of Blue 1 metabolites has provided evidence for an acetylated analog and a demethylated product. Demethylated Blue 1 was the only one of the metabolites which was present in sufficient quantity to be detected by TSP in gradient analyses of whole urine. For the other metabolites, fractions had to be collected and concentrated.

A metabolite in which the NO₂ group has been reduced to the amine has also been identified. In similar systems, amines have been implicated in pathways leading to carcinogenicity, often via N-acetylation (3). In addition, three conjugates of Blue 1 have also been detected: one is a glucuronide conjugate, one appears to be a conjugate of the parent Blue 1, and one is a conjugate of demethylated Blue 1.

The identification of the demethylated Blue 1, the acetylated Blue 1, and the nitroreduced Blue 1 metabolites has been confirmed by synthesis and comparison of TSP spectra. The identity of the glucuronide conjugate has been confirmed enzymatically.

In contrast, the non-carcinogenic Blue 2 is not extensively metabolized to demethylated, nitroreduced, or acetylated forms. Instead, Blue 2 is metabolized to one major product which may be a conjugate, whose structure is still under investigation.

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HIGH SENSITIVITY AND STRUCTURAL ANALYSIS OF DYES
BY THERMOSPRAY, PARTICLE BEAM IONIZATION AND
CAD TECHNIQUES*

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INTRODUCTION

Dyestuffs are of major environmental interest because of their widespread use as colorants. Synthetic intermediates, byproducts, and degradation products of these dyes could be potential health hazards due to their toxicity or carcinogenicity or both. In addition, dyes form about 10 percent of those new or imported compounds that are reviewed under the U.S. Environmental Protection Agency's (U.S.EPA's) Premanufacturing Notice (PMN) program.

Thermospray high performance liquid chromatography/mass spectrometry (TSP-LC/MS) has been found to be a suitable technique for the analysis of nonvolatile dyes.^{1,2} One of the drawbacks of TSP-LC/MS is that one obtains mainly protonated molecules and adduct ions, and this information may not be sufficient for structural elucidation of dyes of unknown structure. While tandem mass spectrometry (MS/MS) has been used to deconvolute fully the structural information contained in the TSP mass spectra of dyes³, it would be of great utility to analyze dyes with a single quadrupole LC/MS system and obtain structural information.

The purpose of this present work is to refine further the LC/MS technique for the detection and analyses of dyes in environmental samples. The new methodology should have the following characteristics and capabilities: high sensitivity, molecular weight determination, and structural information.

EXPERIMENTAL

A Finnigan TSQTM45 was used with three different configurations of LC/MS instrumentation. For the first two types of experiments a Vestec thermospray LC/MS interface and ion source were used. A modification to the commercial ion source block was made by adding a wire-repeller connected to an external power supply. The wire-repeller was flush with the base of the ion source block and operated at 200 - 250 V for the sensitivity-enhancement experiments. For repeller-induced collision activated dissociation (CAD) experiments, the repeller was inserted 4 mm into the ion chamber and was operated at 400 V.

An Extrel Corporation ThermaBeamTM particle beam (PB) LC/MS interface was attached to the triple quadrupole mass spectrometer in the other mode of operation. A machined adaptor made mainly of Vespel and partly of brass was used to fit the ThermaBeamTM interface to the mass spectrometer. This adaptor was heated from 200 - 250°C.

RESULTS AND DISCUSSION

The limits of detection of some commercial dyes are reported in Table 1. The detection limits for the dyes with the wire-repeller under thermospray ionization are compared to the limits under particle beam introduction on our

*NOTICE: Although the research described in this article has been supported by the United States Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

system. Also, included in this table are six of the dyes that were previously obtained under thermospray ionization with an ion source without a repeller. The configuration with the wire-repeller shows the greatest sensitivity. Enhancement factors of between 10 and 400 are observed over thermospray ionization without a repeller. The limits of detection for the wire-repeller mode are better than the particle beam results by factors of 20 to 16,000.

Three different types of spectral information were compared for use in structural interpretation. Tandem mass spectrometry was used to generate daughter ion CAD spectra from the thermospray ionization of dyes; particle beam electron impact spectra were generated from the same dyes; and repeller-induced CAD spectra were obtained in the thermospray ion source. The repeller-induced CAD spectra show fragmentation characteristic of both CAD MS/MS spectra and the particle beam EI spectra.

Tandem mass spectrometry in conjunction with the particle beam introduction of Basic Green 4 was able to confirm the existence of doubly-charged ions.

Table I - DETECTION LIMITS FOR COMMERCIAL DYES

<u>Dye</u>	<u>Structure</u>	<u>MW</u>	<u>Dye Content (%)</u>	<u>TSP (w/o repeller) Detection Limit (ng)</u>	<u>TSP (with repeller) Detection Limit (ng)</u>	<u>PB LC/MS Detection Limit (ng)</u>
Disperse Yellow 5	1	324	C.30	-	5	500
Disperse Orange 13	2	352	C.15	150-200	20	5500
Solvent Red 3	3	292	100	-	10	200
Disperse Orange 3	4	242	C.20	-	2.5	50
Disperse Red 13	5	348	C.25	-	10	4400
Solvent Red 23	6	352	C.85	50	5	500
Disperse Brown 1	7	432	C.25	-	10	2700
Disperse Red 1	8	314	C.30	-	0.1	250
Disperse Orange 25	9	323	C.20	-	10	500
Disperse Blue 79	10	624	100	250-500	10	300
Basic Green 4	11	329	C.98	20	0.67	700
Disperse Blue 3	12	296	C.20	20	0.05	800
Fluorescent Brightener 236	13	389	100	25-30	4	600
Solvent Red 49	14	442	C.97	-	1	500

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THERMOSPRAY-LC/MS POSITIVE AND NEGATIVE
IONIZATION MODES OF CHLORINATED HERBICIDES

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INTRODUCTION

The goal of this study was to find a way to detect the chlorinated herbicides (free acid form) and their esters by thermospray-liquid chromatography/mass spectrometry (TSP-LC/MS). The potential of TSP-LC/MS to analyze for chlorinated phenoxyacetic acids at the ppb level had previously been shown by Voyksner using the "filament on" mode with negative-ion detection. During the course of this investigation various ionization modes were examined to find the mode which would produce not only the greatest sensitivity but also structural information. The positive (wire-repeller enhanced) and negative (discharge on) ionization modes, daughter ion collision activated dissociation (CAD), and wire-repeller induced CAD in the positive ion single quadrupole mode were examined. The application of a wire-repeller in the thermospray source has greatly improved the limits of detection for the thermospray positive-ion mode.

EXPERIMENTAL

A Finnigan MAT TSQTM45 was interfaced to a Spectra-Physics SP8700XR gradient pump and an ISCO LC-5000 syringe pump for (postcolumn flow) via a Vestec Thermospray system. A wire-repeller was inserted into the ion source opposite the ion exit orifice. Normally, the repeller was operated at 225 V in the positive ion mode and 0 V in the negative ion mode. Negative ion spectra were acquired with the discharge on and filament off. Single quadrupole collision activated dissociation spectra were obtained when this repeller was operated at 400 V and inserted 4 mm into the ion chamber. Separation of analytes was achieved using a flow of 0.4 mL/min through a 15 cm x 2.1 mm Supelcosil 5- μ m LC-18 analytical column (Supelco, Bellefonte, PA) with a linear gradient elution program of 100% water to 100% methanol in 10 minutes with a 15 minute final hold.

The CAD experiments were conducted at a collision energy of 20 eV with argon at a pressure of approximately 1 mTorr.

RESULTS AND DISCUSSION

The herbicides investigated in this study are listed in Table 1 along with their molecular weights, positive and negative mode quantitation ion, and their limits of detection (LOD). They are all chlorinated phenoxy acids except dalapon, which is a chlorinated aliphatic acid, and dinoseb, which is a nitrated phenol. The positive ion mode gave the ammonium adduct ion ($M+NH_4$)⁺ as the base peak in all cases; for two compounds, 2,4-DB and dinoseb, the ($M+H$)⁺ ion is present. No ions were detected for dalapon.

Little structural information is obtained from the positive ion spectra; therefore, three options to the single quadrupole positive ion mode were tested: (1) the negative ion "discharge on" mode; (2) daughter ion CAD (MS/MS)

* On sabbatical leave from the Weizmann Institute of Science, Rehovot, Israel.

**NOTICE: Although the research described in this article has been supported by the United States Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Table I - CHLORINATED HERBICIDES MOLECULAR WEIGHTS AND THEIR LIMITS OF DETECTION IN THE POSITIVE AND NEGATIVE ION MODES

COMPOUNDS	M.W.	POSITIVE MODE	LOD (NG)	NEGATIVE MODE	LOD (NG)
		QUANTITATION ION		QUANTITATION ION	
DALAPON	142	ND	-	141(M-1) ⁻	11 ± 4
DICAMBA	220	238(M+NH ₄) ⁺	13 ± 5	184(M-HCl) ⁻	3.0 ± 1
2,4-D	220	238(M+NH ₄) ⁺	2.9 ± 1	219(M-1) ⁻	48 ± 25
MCPA	200	218(M+NH ₄) ⁺	116 ± 8	199(M-1) ⁻	28 ± 5
DICHLORPROP	234	252(M+NH ₄) ⁺	2.7 ± 1	235(M-1) ⁻	8.4 ± 3
MCPP	214	232(M+NH ₄) ⁺	5.0 ± 2	213(M-1) ⁻	12 ± 4
2,4,5-T	254	272(M+NH ₄) ⁺	169 ± 8	218(M-HCl) ⁻	6.5 ± 5
SILVEX	268	286(M+NH ₄) ⁺	162 ± 1	269(M+1) ⁻	43 ± 30
DINOSEB	240	228(M+NH ₄ -NO) ⁺	24 ± 12	240(M) ⁻	19 ± 6
2,4-DB	248	266(M+NH ₄) ⁺	3.4 ± 2	247(M-1) ⁻	114 ± 38

spectra of the (M+NH₄)⁺ ions; and (3) wire-repeller induced CAD spectra in the positive ion single quadrupole mode. The results from our work in the negative ion (discharge on) mode gave a fairly intense (M-H)⁻ ion, along with significant fragmentation in most cases. Losses of Cl and (CH₂)_xCOOH from the (M-H)⁻ ion form the majority of the fragment ions. An interesting ion observed in our work and not in Voyksner's experiments is the (M+H)⁻ ion. The ion is clearly observed at m/z 269 for 2,4,5-TP (Silvex, Figure 1) after subtracting the ³⁷Cl isotopic contribution from the (M-H)⁻ ion. Yinon had previously reported this ion, (M+H)⁻, in the isobutane negative chemical ionization spectra of two nitrated explosives. This ion is apparently formed from the ion-molecule reactions with M⁺ or (M-H)⁻.

Characteristic spectral patterns were generated in the MS/MS mode of operation of the triple quadrupole mass spectrometer for these chlorinated herbicides. Peaks resulting from the loss of OH (or NH₂), Cl, and (CH₂)_xCOOH·NH₄ from the ammoniated species comprise the majority of the ions in the spectrum.

Attempts to increase further the sensitivity in the thermospray positive ion mode, with the wire-repeller, were made by increasing the voltage. The wire-repeller was also moved further into the thermospray stream and closer to the sample cone orifice. Instead of increasing the sensitivity this resulted in additional fragmentation ions. Figure 2 shows the single quadrupole CAD spectra of 2,4-DB generated with the wire-repeller at 400 V. The resultant spectra was similar to those obtained by electron impact. The EI spectra of 2,4-DB, from the NBS library, and the wire-repeller CAD spectra both show a major ion at m/z 162, the resultant loss of (CH₂)₃COOH from the main structure. Also both spectra display a m/z 98, which is possibly from the opening of the ring with a chlorine left attached, C₅H₃Cl.

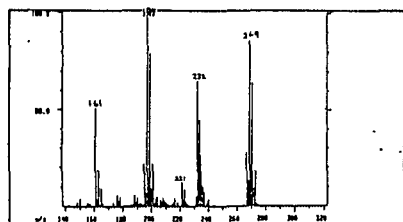


Figure 1-NEGATIVE ION SPECTRUM OF SILVEX

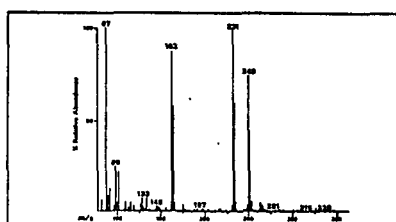


Figure 2-REPELLER CAD OF 2,4-DB

**THERMOSPRAY LC-MS ANALYSIS OF AN INSECTICIDE CONTAINING A SERIES OF
NEREISTOXIN ANALOGS**

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INTRODUCTION

Nereistoxin (4-N,N-dimethylamino-1,2-dithiolane) a naturally occurring neurotoxin isolated from the marine segmented worm *Lumbriconereis heteropoda* (1) has been found to inhibit neuromuscular transmission in some insects (2). Nereistoxin and compounds analogous to nereistoxin, such as Evisect (5-N,N-dimethylamino-1,2,3-trithiane), and Duo-Sewan (7-N,N-dimethylamino-1,2,3,4,5-pentathiocane) (3,4) are a series of polythiacycloalkanes that are useful as insecticides (5). Because of the low UV absorptivity of these compounds, analytical methodologies using high performance liquid chromatography (HPLC) for their analysis and characterization have been limited. Thermospray (TS) is a mass spectrometric (MS) ionization technique that is amenable for coupled HPLC-MS analysis (6,7). In this report we describe the direct HPLC-TSMS analysis of an insecticide sample from China containing these polythiacycloalkanes.

EXPERIMENTAL

Chemicals

A crude sample of formulated pesticide containing a series of Nereistoxin analogs was synthesized by Guo *et al.* (4) of Guizhou Chemical Industry Institute, Guiyang, China.

HPLC-TSMS system

Chromatography was achieved using a conventional Cyano-propyl bonded-phase analytical column (Beckman Instruments, Inc./Altex Scientific Operations). The mobile phase was aqueous buffer/acetonitrile (80/20, v/v). The aqueous buffer was 0.1 M ammonium acetate and was adjusted to pH 5 with acetic acid. The mobile phase was pumped at 1.0 mL/min by a model LC-5000 syringe pump (Isco). The dissolved pesticide sample was injected in the system with a model 7125 septumless injector (Rheodyne) equipped with a 20 μ L loop.

Mass spectral analysis was performed with a model R1010C quadrupole mass spectrometer equipped with a thermospray source (Delsi-Nermag) and thermospray interface (Vestec). Typically, the control temperature was 132°C, and the tip temperature was 192°C. The TS source was operated in the discharge-off and filament-off modes. Mass spectra obtained over the mass range m/z 100 to m/z 550 at a scan rate of 2 s per scan were recorded and processed with a Finnigan/Inco 2300 data system.

RESULTS AND DISCUSSION

HPLC-TSMS, using the conditions described above, was used to analyze a crude pesticide sample which contained a series of polythiacycloalkanes. The sample was dissolved in aqueous solution and its

components resolved using reversed-phase HPLC and detected by on-line TSMS operated in the positive ion mode. The results of this analysis were a set of four resolved HPLC peaks (Figure 1). The TS mass spectrum of the major HPLC peak seen at 4.8 min is shown in Figure 2. This mass spectrum shows essentially only the base peak at m/z 150 which corresponds to the $[M+H]^+$ ion for Nereistoxin. The second major peak (RT=5.2 min.) seen in Figure 1 corresponded to Evisect which has an elemental composition of $C_5H_{11}NS_3$ and a molecular weight of 181. The TS mass spectrum for this compound (Figure 3) included the $[M+H]^+$ ion as the base peak. Two minor HPLC peaks are seen at 5.5 and 6.1 minutes. TS mass spectra of these minor components showed m/z ions at 214 and 246, respectively, indicating an increase of 32 and 64 in molecular weights compared to Evisect. The latter corresponds to the $[M+H]^+$ ion for Duo-Sewan which contains two additional sulfur atoms, relative to Evisect. The compound seen eluting at RT=5.5 min was not identified.

CONCLUSIONS

These data demonstrate HPLC-TSMS to be a useful tool for the analysis and characterization of pesticides that contain nereistoxin or its structural analogs which are typically difficult to analyze using conventional HPLC detectors. The methodology may therefore be applicable for the determination of polythiacycloalkane-type insecticides and their metabolites or degradation products in biological samples

ACKNOWLEDGMENTS

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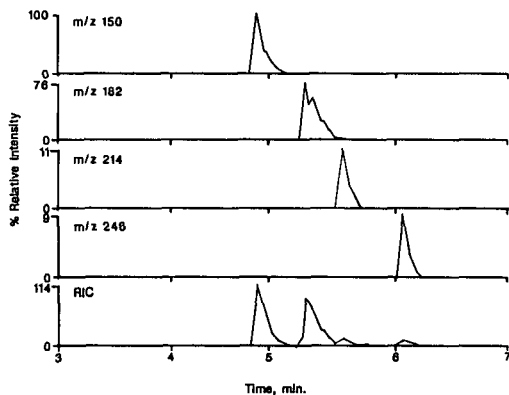


Figure 1. HPLC-MS mass chromatograms of a crude insecticide sample obtained from China.

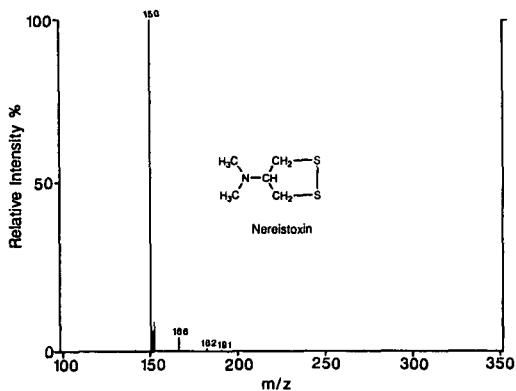


Figure 2. Thermospray mass spectrum of component eluting at 4.8 min. (Tentative assignment is nereistoxin)

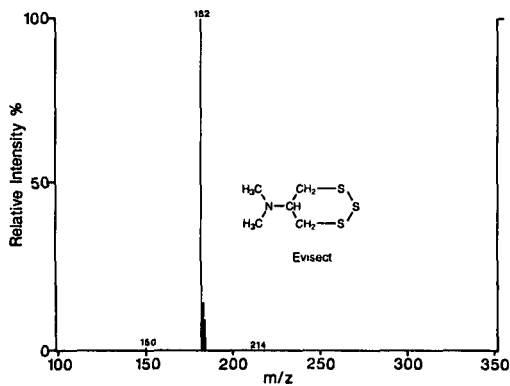


Figure 3. Thermospray mass spectrum of component eluting at 5.0 min. (Tentative assignment is Evisect)

GC/MS and LC/MS in the Analysis of NPPD ("Spy Dust")
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NPPD (5-(4-Nitrophenyl)-2,4-pentadien-1-ol, Figure 1) has reportedly been used by the Soviet Union as a "spy dust" for tracking of U.S. embassy personnel in the USSR. In field use, "spy dust" would be sprayed or deposited on surfaces that embassy personnel frequently touch, applied in amounts so small that the material was essentially invisible. The "spy dust" would then be transferred by touch, allowing for the embassy employee to be tracked and thus monitor his/her activities.

The analytical method developed for NPPD¹ is based on a standard spot test technique which relies on a color reaction to detect aromatic and alpha, beta-unsaturated aldehydes. In a methanol solvent, naphthoresorcinol is used as the color reagent with HCl to catalyze the color reaction. A pink-to-red-violet color is produced proportional to increasing concentrations of NPPD. In the field, detection procedures consist of wiping the surface to be tested with a cotton swab moistened with methanol and developing the color reaction in a test tube. As little as 150 ng NPPD/100 cm² will yield a color reaction visible to the eye. At 506 nm with a spectrophotometer, the sensitivity can be increased to less than 20 ng/cm².

For a particularly critical field sample, confirmation of the identification provided by the color test was sought. The initial technique used for the confirmation of identification was capillary GC/MS, using a 30m DB-1 fused silica capillary column (0.32 mm, 1 μ film thickness). At levels of 100 ng injected on the column, a reasonably well-shaped chromatographic peak was observed (Figure 2) and a mass spectrum characteristic of NPPD was obtained (Figure 3). However, at levels near the concentration level of interest, the molecular ion at mass 203 was not observed and the base peak of the mass spectrum (mass 128) was very difficult to distinguish from other residues present in the sample. Calibration of the instrument over this concentration range for quantitative analysis could not be performed with acceptable reproducibility. On-column injection was attempted, with and without the use of chemical ionization techniques, but a characteristic (M+1)⁺ ion was not obtained and the reproducibility did not improve significantly. The identification could not be confirmed and accurate quantitative analysis could not be performed.

Because of the critical nature of the wipe sample in question, the use of LC/MS as a technique for the confirmation of the compound identification was explored. The mass spectrometer used was a VG 12-250 quadrupole mass spectrometer fitted with a Vestec model 201 thermospray source for LC/MS analysis. The LC/MS analysis was performed in methanol:water. A clear extracted ion current profile (Figure 3) was observed at low concentrations and a molecular anion at mass 203 was the major peak of the mass spectrum (Figure 4). Using this technique, the confirmation of NPPD in wipe samples can be confirmed at 20 ng/cm².

¹Jameson, C.W., Moseman, R.F., Collins, B.J., and Hooper, N.D., Anal. Chem., 58 915A (1986).

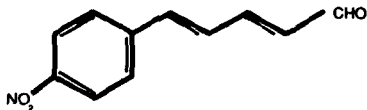


Figure 1 NPPD (5 (4 Nitrophenyl) 2,4 Pentadien-1Al)

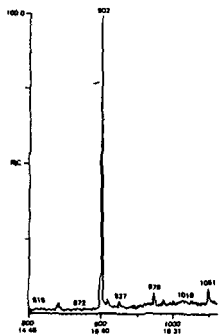


Figure 2 Reconstructed Ion Chromatogram of NPPD at 100 ng

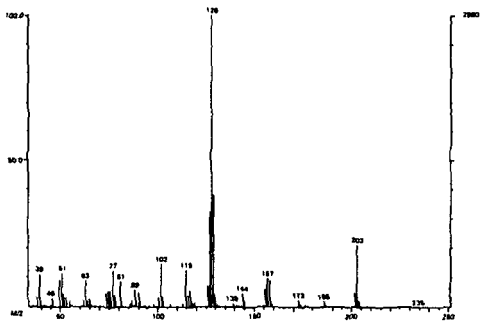


Figure 3. Electron Ionization Mass Spectrum of NPPD at 100 ng

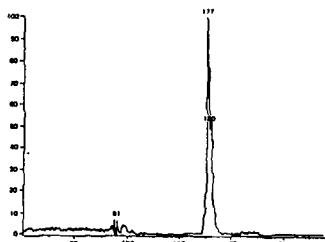


Figure 4 Extracted Ion Current Profile (mass 203) of LC/MS Analysis of NPPD

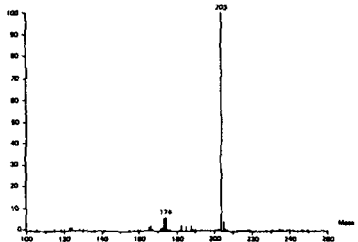


Figure 5 Negative Ion Mass Spectrum of NPPD Obtained by LC/MS

SYNTHESIS AND CONJUGATION OF A NEW CARBOHYDRATE DERIVATIVE
WITH FEMTOMOLE NCI-MS SENSITIVITY

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The detectability of oligosaccharides by HPLC has benefited from their conjugation with photoaffinity groups by reducing-end glycosylation (pyridinylamine) (5) or reductive amination (aminobenzyl ethylester) (1). These reagents have also found some use in structural studies employing FAB-MS, but MS sensitivity for carbohydrate samples remains a problem.

SFC-MS brings new opportunities for the enhancement of oligosaccharide detectability through selective derivatization and negative-ion chemical ionization (NCI). Improved sensitivity using NCI has been demonstrated with the pentafluorobenzyl (PFB) derivative of prostaglandin analogs (2). The same derivatives have been used to study cyclooxygenase products (3) and greater than 100-fold increases in sensitivity (compared to EI) were reported for lipoxigenase products (4).

We have prepared a new reagent that combines the sensitivity observed with the PFB derivative to the capability for reducing-end conjugation utilized in the preparation of the PA and ABEE derivatives. This is the pentafluorobenzyl ester of p-aminobenzoic acid (PFBAB). This product was purified by HPLC and characterized by mass spectrometry. We have conjugated the reagent to numerous N-linked glycans, mono-, di-, and oligosaccharides by both glycosylation and reductive amination. Although the glycosylation of the PFBAB derivative to carbohydrates results in anomeric products, it has interesting advantages over the reduced analog (5). Most important of these is the retention of the PFBAB label under conditions of periodate oxidation (6).

Results and Discussion

PFBAB was synthesized by condensation of p-aminobenzoic acid (PABA) with pentafluorobenzyl bromide (PFB-Br), Scheme 1. The subsequent conjugation of this product was investigated using glycosylation and reductive amination approaches. Carbohydrate conjugation was evaluated under aqueous and nonaqueous conditions using numerous samples. A more quantitative study was carried out with maltoheptose, fucose, and N-Acetylglucosamine. Nonaqueous conditions gave yields approximating 90% for maltoheptaose and 80% for GlcNAc with reaction times of 1/2 hour. These results are preliminary and further work to maximize glycosylation conditions are underway. SFC-MS sensitivity studies were conducted with known amounts of PFBAB-derivatized fucose under NCI conditions by sample dilution. Full scan spectra were obtained (800 amu) for each injection at a rate of 10 s/d. The spectrum in Figure 1 represents a dilution to 55 fmol and the selected ion plot for the (M-PFB)⁻ ion is shown in Figure 2.

SFC-MS in combination with NCI and PFBAB-derivatized carbohydrate samples appears most promising. Further signal enhancing techniques, such as isotope carrier, array detection, and single-ion monitoring suggests further improvements in sensitivity may be possible.

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Scheme 1

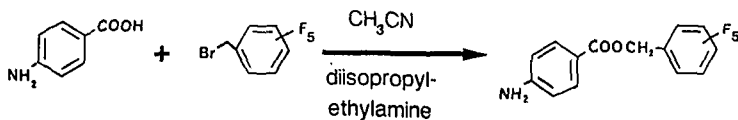


Fig 1

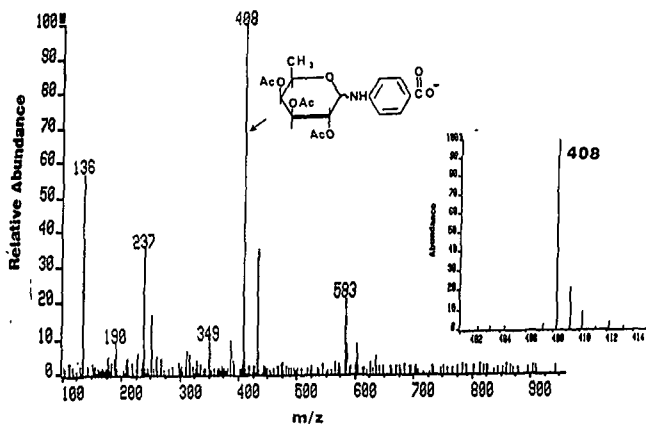
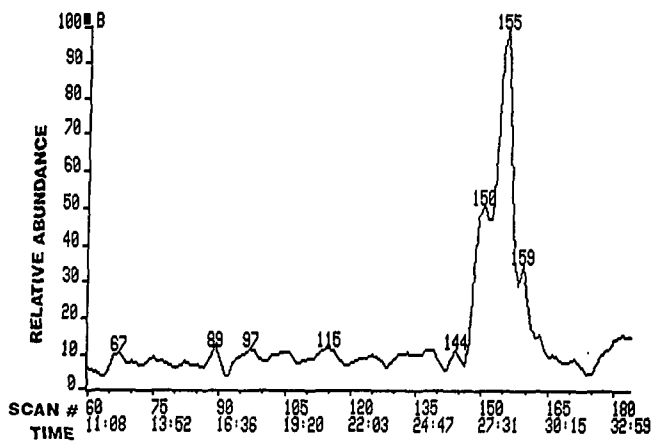


Fig 2



Interfacing Chromatographic Techniques to
Multiphoton Ionization Mass Spectrometry

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The advantages of MultiPhoton-Ionization (MUPI) in comparison with conventional ionization-techniques in mass spectrometry has been shown in recent years [1]. In addition to Laser-Desorption (LD) chromatographic methods have been coupled to MUPI-MS volatilizing thermally labile and involatile molecules [2]. This methods allows to create mass spectra over long time periods by using extraction-techniques. We think that by using the high sensitivity of MUPI the detection of molecules after chromatography could also be drastically increased. The Golay equation for open tubular columns furthermore shows that the same separation efficiencies, in the same analysis time, can be achieved with GC, SFC and LC, provided the diameter of the column is reduced along with the corresponding mobil phase diffusion coefficients. Smaller column dimensions obviously have a detrimental effect on sample capacity. In analytical instruments this problem can be overcome by making use of more sensitive detectors. In more practical terms, the above also means that new mass spectrometers with greatly enhanced sensitivity are required to perform SFC-MS and especially LC-MS [3] with the same separation efficiency we routinely have in capillary GC-MS. The increase in sensitivity should preferably not be achieved with ionization techniques that lead to mass spectra with greatly diminished information content when compared to EI-spectra. It is our opinion that time-of-flight mass spectrometry with laser induced multiphoton-ionization could fulfill these requirements. The high potential sensitivity of this type of mass spectrometer has not yet been realized due to the small area of overlap (in space and time) with the pulsed ionizing laser beam [4]. Our approach to this problem is to continuously accumulate sample molecules from a chromatographic column onto a substrate and to pulse-desorb the sample by a CO₂ laser at a frequency of 2.5 Hertz. The desorbed "packet" of sample molecules is transferred to the ionization volume by the gas jet to coincide adsorption substrate that is transparent to the desorbing laser radiation, we aim to achieve both spatial and "time" focusing of the sample for maximum overlap with the pulsed ionizing beam.

The prototype of the Bruker TOF 1 mass spectrometer was coupled to a Carlo Erba VEGA Series 2 gas chromatograph equipped with a Rheodyn injection valve model no. 7410 (1µl loop). SFC grade CO₂ was delivered by a Brown-Lee SFC pump. A schematic diagram of the designed continuous SFC-MS-Inlet-System is shown in Figure 1.

The Mach disk of the supersonic beam of CO₂, which emerges from the ca. 1µm diameter orifice at the end of the fused silica transfer line, seems to lie very close to the jet tip. This prevents the sample from moving through the skimmer and into the ionizing region in a dense, well defined beam. When the AgCl cylinder is placed around this beam the amount of sample reaching the ionizing beam doubles. Samples completely lost from the beam is evidently accumulated on the inner wall of the cylinder with subsequent pulsed desorption by the CO₂ laser. This results in an additional 5-fold increase in the pyrene concentration during the desorption peak. With pyrene as our test compound, first results indicate that a 10-20 -fold signal enhancement can be obtained with the simple concentrator/pulse desorption-interface without any prior optimization. Without that interface we detected a amount of 3 ng fluorene by a signal to noise ratio of 6:1.

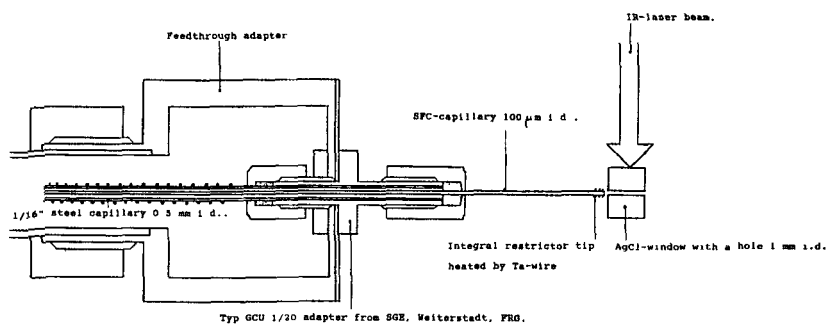


Figure 1: Continuous SFC-MS-Inlet-System using an integral restrictor heated by resistance wire at the very end of the tip.

Acknowledgement.

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**SUPERCritical FLUID CHROMATOGRAPHY/CHEMICAL IONIZATION
MASS SPECTROMETRY IN A THERMOSPRAY SOURCE**

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Supercritical fluid chromatography (SFC) represents a new method for introducing nonvolatile, thermally labile and high mass molecules into a mass spectrometer. Interfacing SFC with mass spectrometry poses many of the same problems encountered with liquid chromatography/mass spectrometry, the most critical of which are the high vapor loads and cooling effects of the evaporating liquids. Since the thermospray source was designed to overcome these problems it is an ideal starting point for developing an SFC/MS interface. The purpose of this study was to design an interface that would be simple to build and operate, then evaluate the performance of the interface with several anticancer drugs that represented a wide range of polarities. Anticancer drugs were chosen for this study because they represent a group of drugs which are thermally unstable and generally nonvolatile, making them model compounds for describing the conditions that are necessary for drug analysis by SFC/MS.

A supercritical fluid chromatograph (Suprex Corp.), consisting of a syringe pump, column oven and controller was used to maintain SFC conditions. Carbon dioxide was used for the supercritical fluid was modified with an organic solvent via a high pressure injection valve (Valco). Sample introduction was through a Valco high pressure electronically actuated valve with a 1 μ l injection volume. SFC conditions were maintained until reaching the ion source by a 50 cm long piece of 0.25 mm id fused silica capillary with an integral restrictor. The flow of exiting gas from the restrictor was 200 ml/min when SFC conditions were 400 atm with an oven temperature of 80 °C. Under these conditions the source pressure was 0.5-0.6 torr.

A highly modified Finnigan 1015 mass spectrometer was used for all experiments. Briefly it consisted of a differentially pumped vacuum housing with oil diffusion pumping, quadrupole electronics upgraded by Extrel and a Technivent data system. A Vestec thermospray source and controller were used for all experiments. The source temperature was kept at 200 °C and the probe temperatures were 120 °C and 80 °C for the tip and control, respectively. An original thermospray probe was modified (fig. 1) such that it would fit into the thermospray source and operate with all thermospray electrical connections. The principal modification was to replace the original metal capillary with two larger inner diameter metal capillaries that would permit fused silica capillaries to be inserted.

The SFC/MS interface described in this experiment uses a thermospray ion source and modified thermospray probe to provide a simple and convenient method for interfacing SFC with mass spectrometry. We have demonstrated that chemical ionization conditions are observed and that the optimum conditions are dependent on the source pressure, with a maximum occurring at approximately 1.0 torr. Chemical ionization mass spectra were recorded for thiotepa, cyclophosphamide, diaziquone (fig.2, A) and mitomycin C (fig.2, B). While CI mass spectra were obtained for all the anticancer drugs in our study, more efficient chromatography could be achieved by adding a gradient of organic modifier to the mobile phase. The lower limit of detection was determined to be 50 ng of the test compound diaziquone, which produced a signal to noise ratio of 5:1 during full scan (135-500 amu) operation. Finally, use of the discharge electrode for ionization in place of filament ionization increases the overall sensitivity by a factor of 10, without causing increased fragmentation.

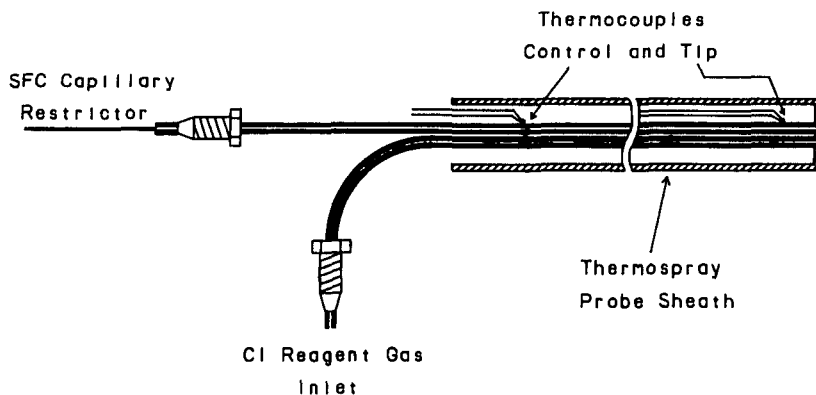


Figure 1. Schematic illustration of the SFC/MS probe.

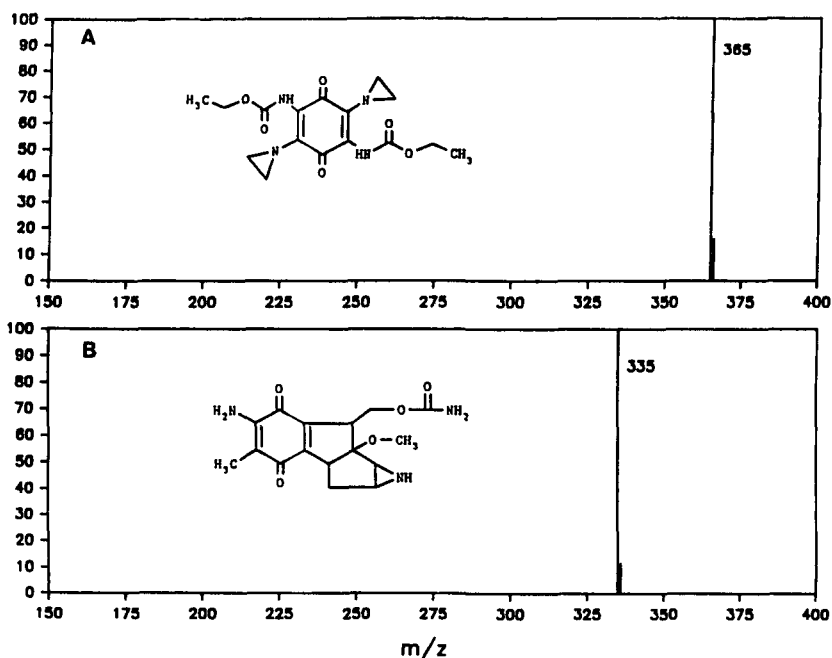


Figure 2. SFC/CI mass spectra of diaziquone (top),
and mitomycin C (bottom).

ELECTRON IMPACT MASS SPECTRA WITH SFC/MS

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Supercritical fluid chromatography/Mass spectrometry (SFC/MS) has become an important analytical technique for separation and identification of mixtures. Sample introduction with the fluid phase presents high pressure in the mass spectrometer. Therefore the chemical ionization (CI) mode is usually chosen. However, CI mass spectra give mainly molecular weight information which makes it difficult to interpret unknown mass spectra. EI mass spectra are of particular interest to those who need appropriate structural information for the identification of unknown compounds.

An SFC/MS system has been constructed through additions and appropriate modifications to a Hewlett-Packard (HP) 5985B GC/MS instrument. Supercritical fluid conditions are effected by transport of liquid carbon dioxide mobile phase through a Lee Scientific pump, Model 501, to the injector and then into a 10-m 50-um ID SB-biphenyl fused silica capillary column. The exit of the column was connected to a 50-um ID frit restrictor (Lee Scientific). Two approaches to a successful coupling of the resultant effluent to the MS system in order to yield EI mass spectra have been demonstrated. In one configuration, a Scientific Glass Engineering (SGE) open-split GC/MS interface is used to eliminate excess mobile phase. Inside the heated interface is a heated frit restrictor connected to a deactivated, uncoated, 110-um ID fused silica capillary column (also heated) which functions as a transfer line into the ion source of the mass spectrometer. An alternate configuration designed to accommodate the introduction of higher molecular weight components omits the open-split interface and locates the frit restrictor inside the ion source. A specially fabricated, stainless steel rod, heated by the ion source heater, has a conical opening into which the restrictor tip is positioned and also heated. In both configurations, a Balzer turbomolecular pump, Model 510, is used for the ion source.

The sample preparation consisted of dissolving the sample in an appropriate solvent for injection. Carbon dioxide was used to sweep the sample onto the GC capillary column in the oven. The mass spectrometer was operated in the EI mode at 70 eV energy. The source temperature was maintained at 200-300°C. An HP 1000 computer system with RTE-A data system was interfaced to the mass spectrometer. Selected compounds and samples were examined with the SFC/MS system. EI mass spectra were satisfactorily produced without ion-molecule contributions or carbon dioxide clusters. The resulting spectra can thus be searched against a conventional mass spectral library for the identification of supercritical fluid-separated components.

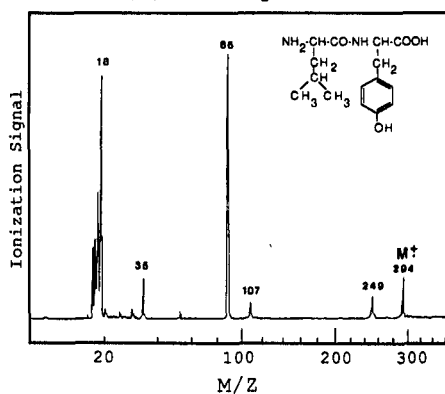
HIGH PRESSURE LIQUID NH_3 INJECTION OF POLAR MOLECULES INTO A MASS
SPECTROMETER WITH RESONANT TWO-PHOTON IONIZATION DETECTION

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The University of Michigan, Ann Arbor, MI 48109.

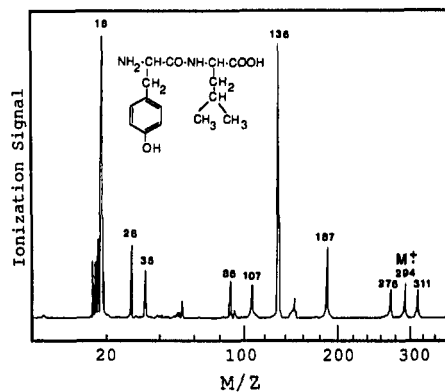
High pressure (> 200 atm) liquid NH_3 has been investigated as a means of volatilizing polar nonvolatile molecules for injection into a TOF mass spectrometer. The NH_3 is rapidly heated to supercritical conditions ($\sim 140^\circ\text{C}$) before expansion so that a gaseous supersonic jet results and detection is performed by resonant two-photon ionization (R2PI) at 266 nm in a TOFMS. There are several key features of this technique. Liquid NH_3 can be used to dissolve polar nonvolatile molecules at room temperature and provided the residence time in the high temperature region is short (< 1 sec), then decomposition can be minimized. Using liquid NH_3 injection with R2PI detection, relatively soft ionization mass spectra have been obtained for highly polar nucleosides such as adenosine and guanosine where strong M^+ and BH^+ ion peaks were observed with little additional fragmentation. Soft ionization mass spectra have also been obtained for steroids such as estrone and estradiol and also for di- and tripeptides. By increasing the laser power, characteristic fragmentation is observed where the dipeptides leu-tyr and tyr-leu can be distinguished for example. A second key advantage of this method is the enhanced selectivity of laser ionization in supersonic jets. Although a high density jet and chamber pressure ($\sim 5 \times 10^{-5}$ torr) result from high pressure NH_3 injection, the NH_3 solvent remains completely transparent and is not ionized by the laser radiation. This allows the ability to produce either soft ionization or extensive fragmentation without physical separation of the analyte from the NH_3 solvent. In addition, based upon the supersonic expansion method cold wavelength spectroscopy of tryptamine and several dipeptides as a function of laser wavelength has been obtained for unique spectral identification in a mass spectrometer.

Figures 1a and b show R2PI mass spectra of the dipeptide isomers Leu-Tyr and Tyr-Leu injected into the TOFMS using high pressure liquid NH_3 (150 atm). The pulsed nozzle was held at 150°C in order to produce a supercritical fluid expansion with subsequent R2PI at 266 nm. In these experiments, the power was raised to 5×10^6 W/cm² to increase the observed signal. In Figure 1, the molecular ion is observed as well as several fragments distinctly characteristic of each isomer. These fragments are characteristic of MPI and are induced by the increased laser power required to enhance the signal because the solubility of these compounds in liquid NH_3 may be the limiting factor here. The fragments are not those characteristic of thermal decomposition, which might be otherwise produced due to the high temperature in the valve. The short residence time in the valve allows injection under supercritical fluid conditions without significant decomposition. The result is that R2PI/MPI of small peptides can be studied for identification and structural analysis by liquid NH_3 injection without significant interference of the solvent. The NH_4^+ peak observed in the mass spectrum is due to charge-exchange in the TOFMS acceleration region.

(A) . Leu-Tyr



(B) . Tyr-Leu



SFC-MS OF HIGH MOLECULAR WEIGHT GLYCOCONJUGATES

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Structural characterization of compounds from biological tissues often presents problems in availability and difficulty in purification. Thus, there is always great interest in methods with improved specificity and sensitivity. SFC is a technique which presents great potential for the separation of complex biological mixtures, as well as important advantages for structural characterization by mass spectrometry interfacing. In view of this potential, we have applied SFC-MS to the compelling problem of structural analysis of oligosaccharides (1). Recent work in the characterization of these molecules has made use of a wide array of analytical techniques, including HPLC with varying column supports and phases, sample derivatization, multi-column separations, and a variety of sensitive detectors, including mass spectrometry. This report describes an SFC-MS study of a series of high mannose oligosaccharides, (obtained from glycoproteins by Endo H treatment), and from animal urine samples, (mannosidosis). These mixtures were used as a paradigm for development of an SFC separation scheme applicable to more complex glycans.

Experimental

SFC was performed on a model 501B supercritical fluid chromatograph (Lee Scientific, Inc.) equipped with a flame ionization detector, and a pneumatically actuated submicroliter (0.2 μ l) injection valve (Valco Instrument Co. Inc.). Pump cylinder and injection valve temperatures were held at 10 $^{\circ}$ C. Capillary columns had inner and outer diameters of 50 and 375 microns respectively, and were coated with a film thickness of 0.25 μ m (Lee Scientific, Inc.). Mobile phase pressure and flow were maintained by fabrication of the integral restrictor described by Guthrie and Schwartz. Chemical ionization MS was performed on a VG ZAB-SE high resolution mass spectrometer, (VG Instruments), operating at 8 kV. The ion source was modified for dedicated CI operation by blocking of all inlet ports, and use of a narrower exit slit. The SFC-MS interface has been described previously (1).

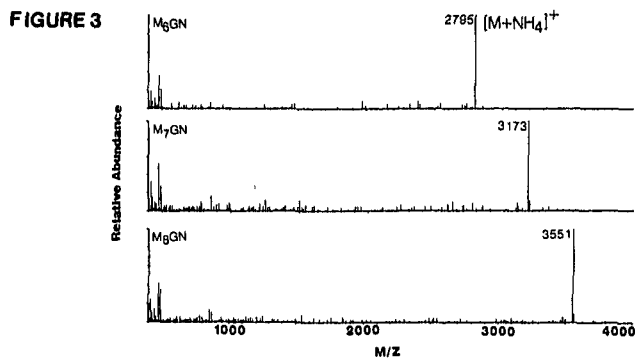
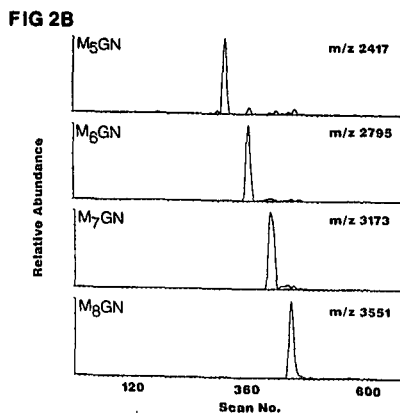
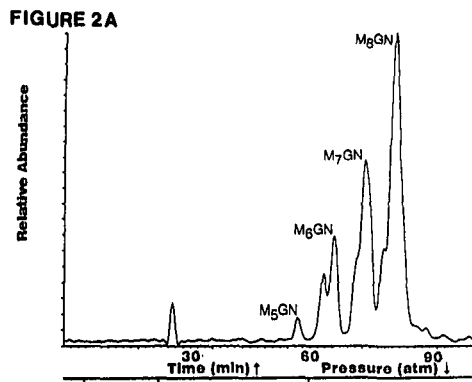
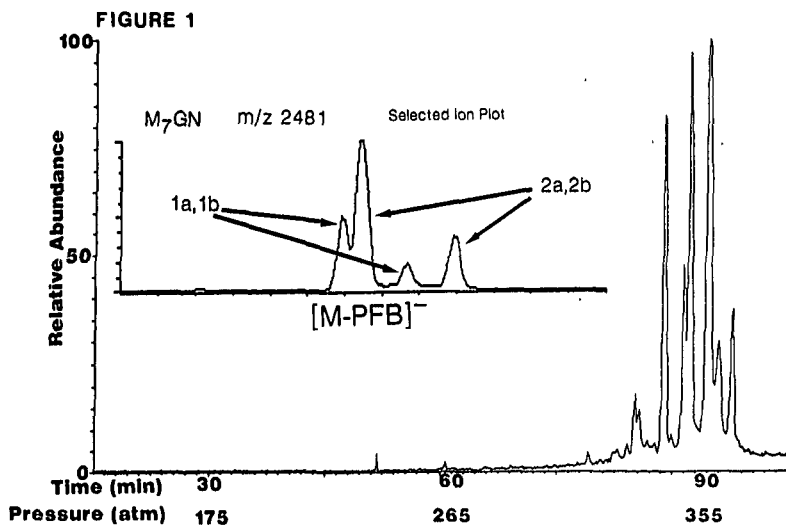
Results

The chromatographic efficiencies of a series of derivatives, encompassing silyl and methyl ethers, and acetyl and polyfluorinated esters, have been evaluated, utilizing a variety of column stationary phases, including 5% phenyl, 30% biphenyl, and 50% cyanopropyl/methyl polysiloxanes. The silylated derivative provides good chromatographic resolution according to chain size, but does not enable separation of structural isomers. Overall resolution is slightly poorer with the permethylated derivative, but structural isomers show improved resolution. Chromatograms of the permethylated and silylated derivatives chromatographed on the biphenyl and 5% phenyl columns show similar resolution. Neither the silylated or permethylated derivative resolves well on the cyanopropyl column. However, this stationary phase resolves peracetylated oligomers very well, and is improved considerably by the addition of the pentafluorobenzyl ester of p-amino benzoic acid (PFBAB) to the chain's reducing end. This label is used to generate enhanced sensitivity by SFC-NCIMS (2) and a total ionization plot for a glycan mixture, (Man_nGN, n=5-8), is presented in Figure 1. An insert in Figure 1 shows the selected ion profile obtained when plotting the ion representing (Man)₇GNAC. Figure 2a shows the total ion plot obtained from an identical glycan mixture, (without a reducing-end tag), following sample trimethylsilylation. Selected ion plots for each of the four components is indicated in Figure 2b. Figure 3 presents representative mass spectra for the three major components.

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SUPERCRITICAL FLUID CHROMATOGRAPHY/MASS SPECTROMETRY FOR
CHARACTERIZATION OF COMMERCIAL SURFACTANT MIXTURES

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The development of a probe-mounted capillary direct interface for supercritical fluid chromatography/mass spectrometry (SFC/MS) (1) has extended the utility of SFC for the analysis of commercial nonionic surfactant mixtures. Nearly 800 million pounds of these materials are consumed annually in the United States, seventy-five percent of this in household detergent products. Use of positive and negative ion chemical ionization provides identification and characterization information following efficient capillary SFC separation. Characterization of nonionic surfactant mixtures is enhanced by limiting fragmentation of higher molecular weight species. Contrary to other results (2,3), positive and negative ion mass spectra obtained using this SFC/MS system are influenced by the presence of the chromatographic mobile phase in the ion source. These changes in the mass spectra resulting from the mobile phase complicate the study of the SFC/MS interface and limit application of SFC/MS for evaluation of surfactant mixtures. Chemical ionization conditions in the HP5985 ion source have been studied with selected alkoxylated surfactant mixtures and varied sample introduction methods. Positive and negative ion chemical ionization were performed with and without carbon dioxide or nitrous oxide present. Conditions can be selected to permit production of only molecular species ions, allowing evaluation of interface conditions, sample transfer and system transmission.

Preliminary studies of the probe-mounted capillary direct SFC/MS interface to an HP5985 GC/MS demonstrated capillary SFC/MS could be conducted using conditions developed for SFC/flame ionization detection, with no compromise to MS operating parameters and only a minor loss in chromatographic efficiency. The design permitted rapid changeover from GC/MS or probe/MS to SFC/MS, allowing the SFC/MS interface to be compared to other methods of sample introduction (1). The design also permitted supercritical direct fluid injection, for samples not requiring preseparation. Samples of alkoxylated pyrazoles provided a well-studied mixture (4) for evaluation of chromatographic and interface performance. Although readily separated using supercritical carbon dioxide, detection of mixture components by chemical ionization mass spectrometry following SFC discriminated against higher molecular weight species. One contribution to the apparent discrimination was the single quadrupole analyzer with a stated mass range of 1000 daltons. Increased fragmentation of higher molecular weight materials further complicated system evaluation. Increased fragmentation was also found for higher molecular weight alcohol ethoxylate nonionic surfactants.

Homogeneous samples of normal alcohol ethoxylates were available and used to probe the interface and ionization complications. Positive ion chemical ionization mass spectra produced using methane, isobutane and ammonia were characterized by increased fragmentation when appreciable levels of the common supercritical fluids carbon dioxide and nitrous oxide were present in the ion source. Protonated molecules or ammonium adduct ions remained the base peaks

in the spectra and there was no appreciable rise in the molecular ion (M^+) signal, so it was not clear if the increased fragmentation resulted from charge exchange with the mobile phase gas. Negative ion CI spectra also displayed increased fragmentation and adduct ion formation when SFC mobile phases were present. The exception was chloride-attachment NICI using difluorodichloromethane (FREON-12) as the reagent gas (5). The ion produced for the alcohol ethoxylates was the $(M+Cl)^-$ ion with no fragment ions produced, even with a twofold excess of carbon dioxide present in the ion source.

Capillary supercritical fluid chromatography interfaced with chemical ionization mass spectrometry provides high efficiency separations for characterization of nonionic alcohol ethoxylate surfactants. Contrary to reports for other samples and SFC/MS systems, the presence of carbon dioxide or nitrous oxide in the CI source of the HP5985 system configured for SFC/MS significantly influences the appearance of positive and negative ion mass spectra. When supercritical fluid chromatography or direct fluid injection is employed for characterization of alkoxyated surfactants, the role of the mobile phase during ionization must be considered. Freon-12 as the reagent gas for chloride attachment negative ion chemical ionization appears least influenced by the presence of carbon dioxide and may be the preferred system for obtaining molecular weight and distribution information on commercial alkoxyated nonionic surfactants.

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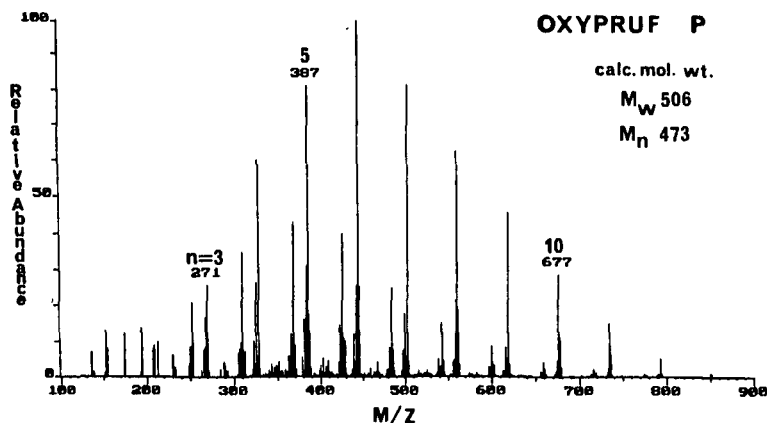
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SUPERCritical DIRECT FLUID INJECTION AMMONIA CIMS



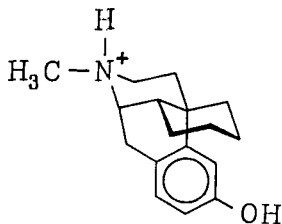
ION SPRAY LC/API-MS OF MORPHINANS

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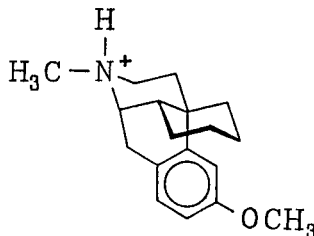
Morphinan derivatives are potent drugs, chemically related to opium alkaloids. They usually exist as pairs of enantiomers, that show a remarkable stereospecific action in the body. The pure d-enantiomers dextrophan and dextromethorphan are converted into their N-methyl derivatives, and are used in pharmacokinetic and metabolism studies in Groningen.

Separations are done by HPLC on C-6 and C-1 stationary phases, the eluents being mixtures of methanol and an aqueous ammonium acetate/acetic acid buffer solution (pH 3.5). Confirmation of the identity of the parent drugs and their N-methyl derivatives takes place by on line LC-UV-MS. In the present study it is essential that thermal demethylation, as reported in thermospray LC/MS of quaternary ammonium compounds (1), is avoided to exclude the ambiguity about the presence and the position of methyl groups. Both the ion spray LC/MS interface (2) and the atmospheric pressure ion source of the NERMAG R 3010 triple quadrupole mass spectrometer (3) operate at room temperature, and thus avoid thermal degradation altogether. The effluent from the UV detector is fed through a splitter, which allows 1-10% of the effluent into the ionspray LC/MS interface. Figure 1 shows the UV and TIC trace of a mixture of 1 and 2 and their N-methyl derivatives. The amounts injected into the LC were app. 300 ng per component. The mass spectra show a complete absence of thermal degradation and fragmentation (Figures 2 and 3, nozzle 70 V). Fragmentation in the free jet expansion region between the API source and the quadrupole mass filter enables us to differentiate between isobaric ions (Figures 2 and 3, nozzle 150 V) In the course of experiments with ^{14}C labelled N-methyl derivatives a degradation product was formed during storage. An unlabelled sample did not suffer from the apparent radiation damage, unless exposed to UV light for a prolonged period. LC/MS has shown that the degradation reaction is a radiation induced oxidation process.

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dextrophan (1)



dextromethorphan (2)

UV absorbance, 280 nm

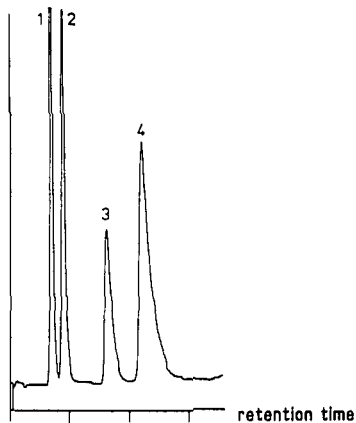
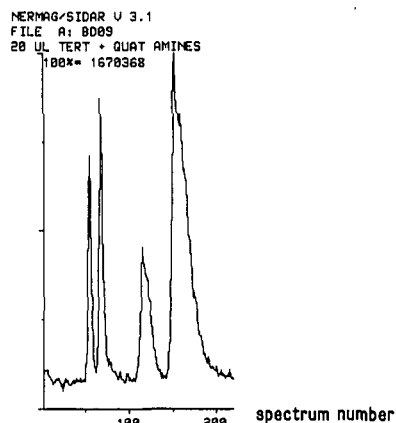


Figure 1

nozzle 70 V

total ion current, m/z 200-300



nozzle 150 V

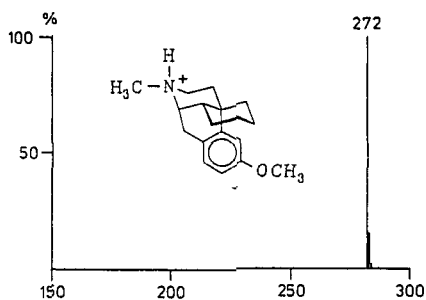
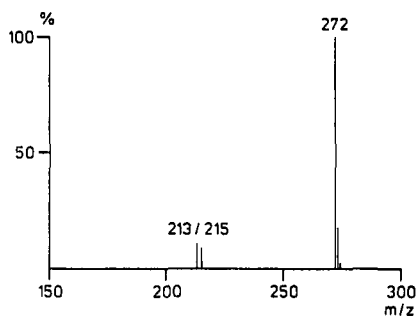


Figure 2

nozzle 70 V



nozzle 150 V

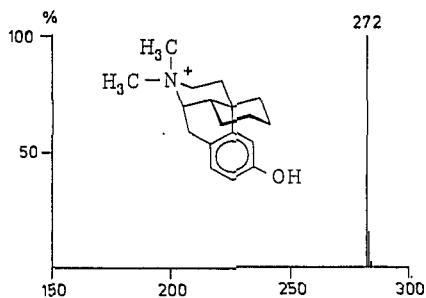
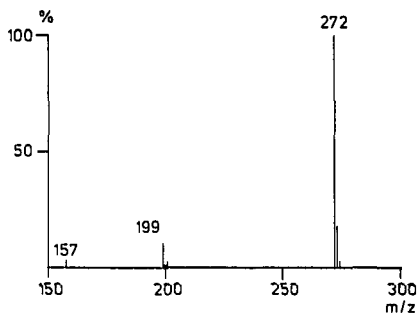
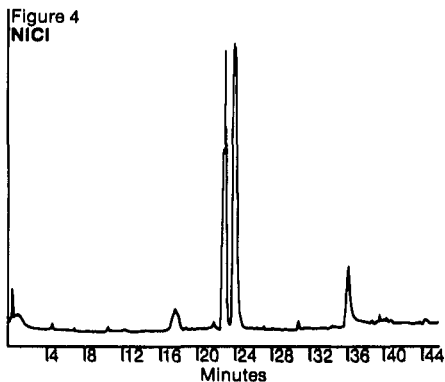
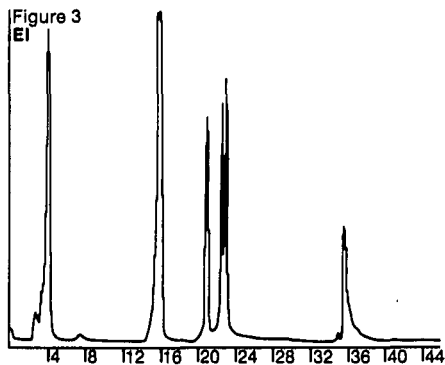
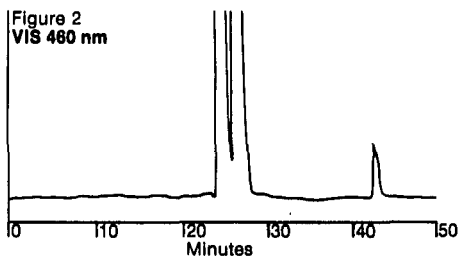
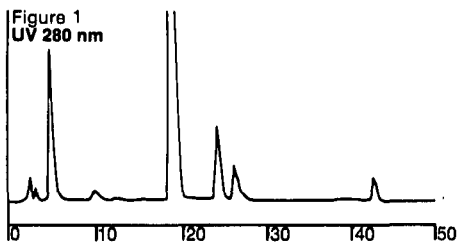


Figure 3





COMPARISON OF THERMOSPRAY AND CONTINUOUS FLOW
FAB LC/MS METHODS FOR THE ANALYSIS OF
ACYL-CoA COMPOUNDS

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Acyl-CoA compounds are important intermediates in the catabolism of various biomolecules, including fats and branched-chain amino acids. Defects in the mitochondrial oxidation of either of these two molecular types can be manifested as Reye's Syndrome or Sudden Infant Death Syndrome (SIDS). It should be possible, given appropriate analytical techniques and methods, to assay specific enzyme activities in the mitochondrial system by determining the appearance or disappearance of acyl-CoA substrates and products. This paper describes a comparative study of thermospray and continuous flow FAB LC/MS methods for the analysis of short and medium-chain (C1 - C8) acyl-CoA compounds.

Methods For continuous flow (CF) FAB, two different instrumental configurations were utilized with a standard pulse-damped HPLC solvent delivery system (Waters 600-MS). The first incorporated an analytical column (Waters Nova Pak C18; 3.9 x 150 mm) with post column flow splitting, and the second a microbore column (Keystone Scientific Hypersil BDS; 1.0 x 250 mm) with pre- and post column flow splitting. With both configurations a dual solvent gradient was employed (A: 0.2 M ammonium acetate/1.75% acetonitrile/2% glycerol; B: acetonitrile) with a two step linear ramp from 100% A to 90% A in 10 minutes, then to 50% A in 20 minutes. The MS system consisted of a VG Trio-3 triple quadrupole instrument which incorporated a VG Dynamic-FAB probe (VG Masslab). Thermospray analyses were accomplished with the same chromatographic system (analytical column; no glycerol) and a standard VG probe.

Results Figure 1 presents the results of the microbore column (Fig. 1A) and analytical column (Fig. 1B) CF-FAB LC/MS analyses of eight short and medium-chain acyl-CoA compounds. These chromatograms represent extracted ion current profiles of the protonated molecular ion (MH^+) of each acyl-CoA. Note that separation efficiency is good with both systems and that chromatographic integrity was maintained throughout the rather severe solvent gradient. Addition of glycerol to mobile phase A appeared to have no effect on the chromatography of either system. CF-FAB spectra of all acyl-CoA compounds analyzed contained molecular weight information in the form of MH^+ , along with structurally significant fragment ions. Sensitivities appropriate to the analysis of biological extracts were obtained (see Figure 2, for example). Thermospray spectra (Figure 3, for example) contained no molecular weight information and numerous fragment ions which were difficult to ascribe to unimolecular processes.

The CF-FAB LC/MS techniques are deemed most appropriate for the analysis of acyl-CoA compounds.

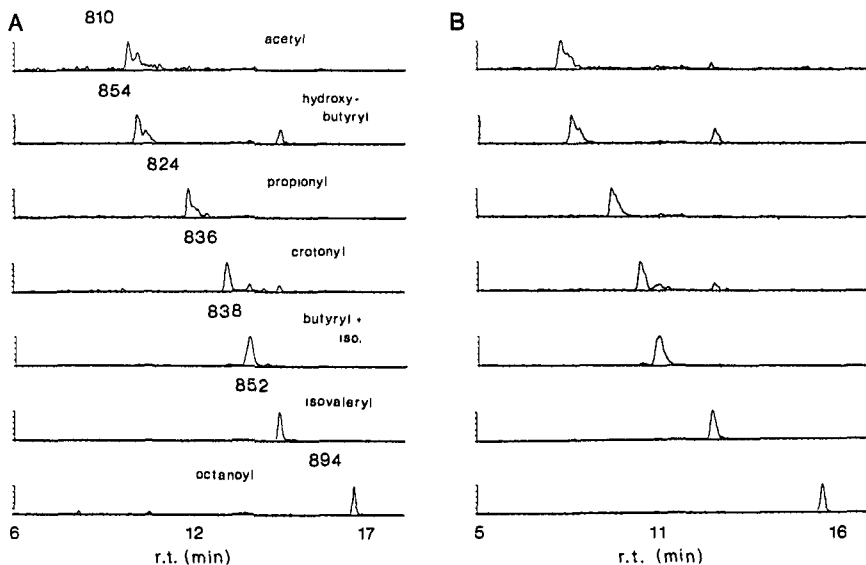


Figure 1. Extracted ion current profiles (MH⁺) from the CF-FAB LC/MS (A-microbore; B-analytical column) analysis of eight acyl-CoA compounds.

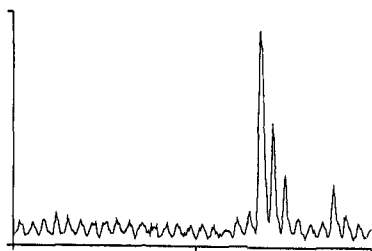


Figure 2. Molecular ion region of a CF-FAB spectrum resulting from the injection of 0.74 nmoles of acetyl-CoA onto the microbore column.

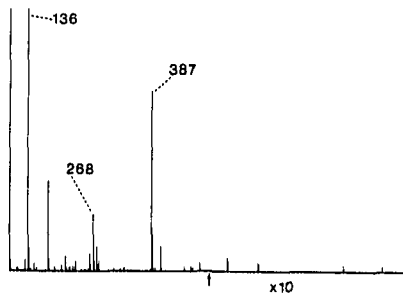


Figure 3. Thermospray LC/MS spectrum of octanoyl-CoA (note lack of molecular weight information).

EFFICIENT INTRODUCTION OF HPLC FRACTIONS INTO A HIGH PERFORMANCE MASS SPECTROMETER A. Cappiello, P. Palma, I.A. Papayannopoulos, K. Biemann, Dept. of Chemistry, Mass. Institute of Technology, Cambridge MA 02139
G. Crescentini, F. Bruner, Ist. di Scienze Chimiche, Universita' di Urbino, 61029 Urbino, Italy

In the determination of the primary structure of proteins by tandem MS, the manipulation of the digest, the transfer of HPLC fractions, etc. is a crucial step that can lead to sample losses and contamination.

In order to minimize sample consumption and for high separation efficiency, dry-packed capillary column (250 μ m ID) and micro gradient system are used. The column is connected to a JEOL HX 110/110 tandem mass spectrometer via a FRIT-FAB interface[®] (JEOL, Tokyo, Japan) which is directly compatible with the low mobile phase flow rate (2-4 μ l/min) of the capillary column, thus eliminating the need for wasteful effluent splitting.

(1) FAB spectra for molecular ion information are obtained by continuously scanning MS-1 over the mass range encompassing the peptides expected.

(2) Alternatively, an off-line multiple loop system is used to trap the peaks eluted from the column and to later introduce them via the FRIT-FAB interface. Some sample band broadening results which is an advantage in MS/MS operations. This approach provides control over the time scale of the experiment because the sample is introduced into the ion source more slowly and at the discretion of the operator, thus facilitating MS/MS operations.

EXPERIMENTAL- Chromatographic equipment: An Applied Biosystems 130A liquid chromatograph was modified in order to reduce the dead volume and to achieve a constant flow rate with linear and reproducible gradients.

A home made capillary column (250 μ m ID x 50 cm), packed with Spherisorb C₈ 5 μ m 300 A pore size was used for the separation of peptide mixtures. The column was directly connected through a fused silica capillary tubing 50 μ m ID to a home made 62 nL micro cell of the UV detector. A fused silica capillary tubing (50 μ m ID) connects the flow cell to the loop system.

Loop system: The loop system consists of a low dispersion injection valve where the loop ports are connected with an electrically actuated multiposition trapping flowpath valve (loop valve) made to hold 8 separate loops (Fig. 1). A 16 loop valve can be added, extending the trapping capacity to 23 loops (one is used as connection line). The loops are made from 0.01" ID teflon tubing, 20 μ L internal volume.

This system can be used both as fraction collector and as sample dispenser. Fractions can either be directly introduced into the ion source via the Frit FAB interface or trapped in consecutive loops by switching the injection valve. The content of each loop is then introduced into the ion source at the convenience of the operator. Samples can also be stored in the loops by injecting them through the syringe port placed in the injection valve and subsequently "pushed" into the ion source by pure mobile phase and sent to the MS.

Two sector operation: Conventional FAB spectra are obtained by scanning MS-1 while the effluent of the column flows directly in the MS ion source.

Four sector operation: In order to obtain MS/MS spectra two different approaches were followed:

a) **ON-LINE.** 50 pmol of the tryptic digest of human hemoglobin α chain were separated in the column (Fig. 2) and directly introduced into the MS via the FRIT-FAB interface. As soon as the precursor ions were detected on the MS-1 detector, the CID spectra were recorded with a microchannel array detector.

b) **LOOP TRAPPING.** In order to avoid a rapid change in sample during CID spectra acquisition, the off-line loop system was employed. The separated peaks of a tryptic digest of 300 pmol of human hemoglobin α chain were collected in six consecutive loops and subsequently injected into the tandem mass spectrometer via the FRIT-FAB interface. A chromatographic peak 40 sec wide (baseline) after being trapped in the loop broadens and lasts in the ion source for about 3 min (half height of the peak envelope). A CID spectrum recorded in this manner is shown in Fig. 3.

CONCLUSION- During a chromatographic analysis two different situations can be encountered:

a) Well separated peaks. In this case the ON-LINE approach is preferable because the time elapsing between peaks suffices for setting MS-1 and MS-2 for the next precursor ion. The sensitivity is high due to the high concentration of the sample during the short eluting time. On the other hand, narrow peaks require rapid scans in order to obtain flatter sample signal.

b) Overlapping peaks. Incomplete separation does not permit ON-LINE recording of CID spectra of more than one component. Loop trapping broadens the chromatographic band and extends the lifetime of the sample in the ion source to allow the recording of consecutive CID spectra. However, spreading out the signal decreases its sensitivity but also allows longer integration times. A very important feature of this way of operation is the decoupling of the separation process and the recording of the mass spectral data which can be carried out at leisure and in any order of priority.

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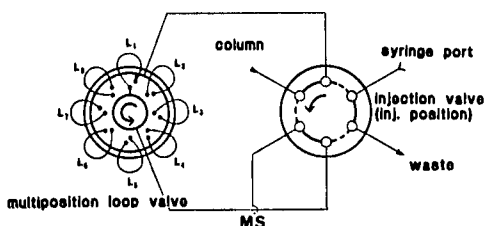


Fig. 1. Multi-loop system. In the load position the injection valve allows filling a selected loop by using a syringe, while the solvent flow by-passes the system. In the injection position an HPLC fraction can be trapped in a loop or the loop content can be pushed by the mobile phase.

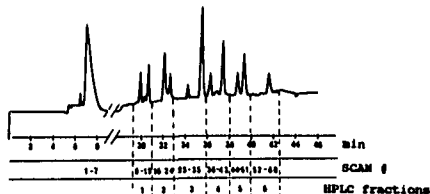


Fig. 2. Chromatogram of a tryptic digest of the α chain of human hemoglobin. 50 pmol (1 μ L) injected onto a C8 dry packed capillary column 250 μ m x 50 cm, 5 μ m particle size, 300 Å pore size. Solvent A: H₂O + 2% glycerol + 0.05% TFA; Solvent B: CH₃CN + 2% glycerol + 0.035% TFA. Gradient used: 0% B to 60% B in 60 min. Flow rate: 3 μ L/min.

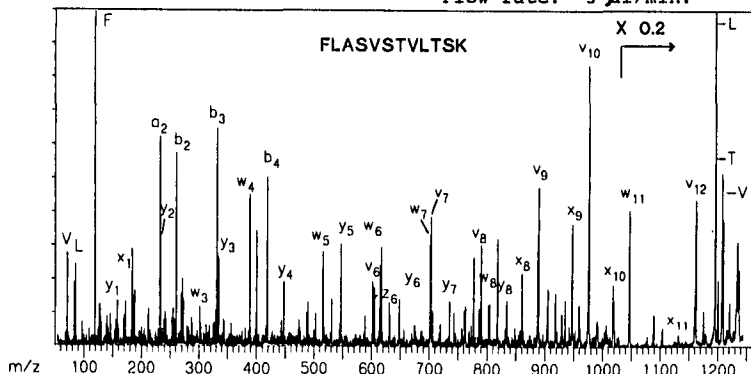


Fig. 3. CID mass spectrum of the tryptic peptide of $(M+H)^+=1252.7$ from hemoglobin α -chain, recorded with a microchannel array detector.

CONDITIONS FOR MICROBORE LIQUID CHROMATOGRAPHY OF PEPTIDES FOR CONTINUOUS-FLOW FAB

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Complex mixtures of peptides are routinely encountered in the course of sequence analysis of proteins and during peptide synthesis. Such peptide mixtures are usually resolved by reverse-phase HPLC and the structures of the components elucidated by amino acid sequence and compositional analysis. We have been exploring the use of on-line liquid chromatography/mass spectrometry to supplement the information available from these traditional approaches.

In initial experiments, a reverse-phase separation method, developed on a conventional HPLC system, was adapted for use with thermospray mass spectrometry. A tryptic digest of β -lactoglobulin A was used as a standard peptide mixture. A 3.9mm X 30cm Delta Pak C18 column (Waters, Milford, MA) was employed and elution performed in 0.02% trifluoroacetic acid at 0.45ml/min with a gradient of 0-70% acetonitrile over 1hr. 0.2M ammonium acetate was added post-column at 0.55ml/min to promote ionization. Protonated molecular ions were observed for 9 of the 16 components expected. All 9 were identifiable at the 0.5 nmol level with signal-to-noise ratios of 10:1 or better, and the mass spectra could be interpreted in the light of the peptide structures at the 2.5 nmol level. However, it was recognized that substantial improvements in sensitivity of detection would probably be difficult with thermospray.

The method was therefore adapted for use with continuous-flow (dynamic) FAB. This required reducing the flow rate to the low μ l/min range. A capillary (microbore) column (320 μ m I.D. X 30cm) was custom packed with the same stationary phase (LC Packings, Zurich), and the same linear solvent velocity was maintained by adjusting the flow rate to 3 μ l/min in order to minimize changes in retention time. Flow was metered by a pair of syringe pumps or by splitting the stream from an M600-MS reciprocating pump (Waters, Milford, MA). An on-line uv detector with a capillary flow cell was employed, and post-column addition of a glycerol-bearing FAB matrix was performed using a concentric transport-tube arrangement with mixing occurring at the FAB probe tip.

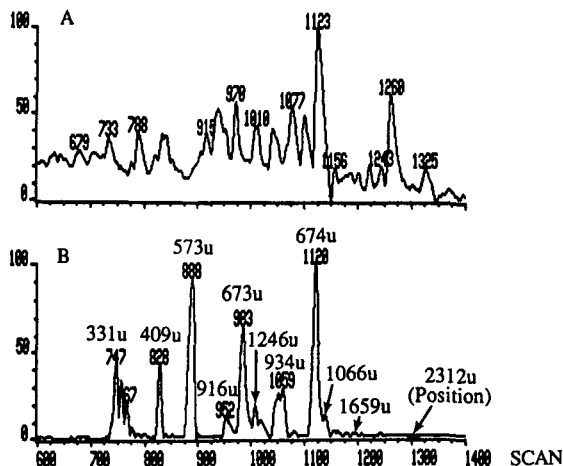


Fig. 1 (A) uv absorption and (B) mass summary chromatograms from LC/FAB/MS analysis of a tryptic digest of 200 pmol β -lactoglobulin A. The mass values indicated are those of the protonated molecular ions for the following peptides: - 331, 139 $\rightarrow$ 141; 409, 136 $\rightarrow$ 138; 573, 71 $\rightarrow$ 75; 916, 84 $\rightarrow$ 91; 673, 9 $\rightarrow$ 14; 1246, 125 $\rightarrow$ 135; 934, 1 $\rightarrow$ 8; 1066, 92 $\rightarrow$ 100; 1659, 149 $\rightarrow$ end; 2312, 41 $\rightarrow$ 60.

All analyses were carried out with digests of 200 pmol of protein, and the protonated molecular ions of 11 of the 16 expected proteins were identified (Fig. 1).

FAB produced less fragmentation than thermospray but the FAB spectra were complicated by the background contributed by the glycerol matrix (Fig. 2).

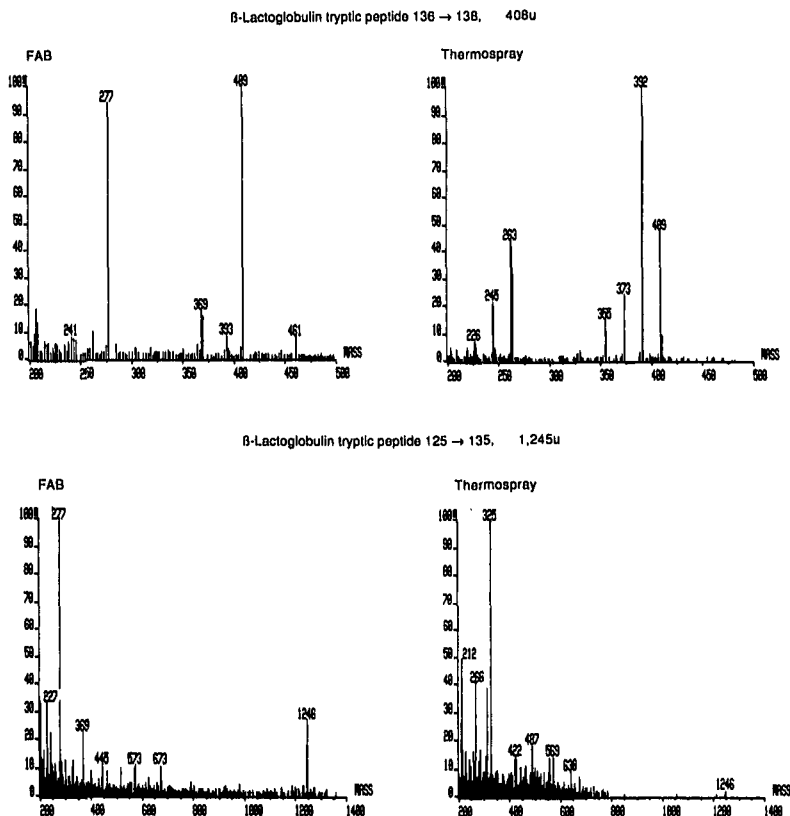


Fig. 2. Comparison of on-column mass spectra acquired by FAB and thermospray, at the 200 pmol and 2.5 nmol level respectively.

Because of the variability of the background contributed by glycerol to the total ion current, the eluting components could not be located reliably by inspection of the total ion current chromatograms, but were identified instead by their uv absorption.

The LC/FAB/MS method was applied to the analysis of digests of insulin B chain by the complex, multisubunit protease, macropain. Of the 15 peptides identified previously by amino acid sequencing, the expected protonated molecular ions of all but those of the three highest mass were identified in a digest of 1.6 nmol of substrate. The results assisted in the identification of 14 sites at which the substrate is cleaved by the enzyme.

ABSORBENT EVAPORATOR FOR CONTINUOUS FLOW LIQUID SIMS

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Continuous flow (CF) - liquid SIMS (LSIMS) or fast atom bombardment (FAB) has become a rapidly developing field in analytical mass spectrometry (1-6). In comparison to static liquid SIMS, this technique provides lower chemical noise. A wide range of solvents, such as methanol, acetonitrile, hexane, and water, may be used. Samples can be introduced into a spectrometer at a shorter duty cycle by eliminating the procedure of withdrawing a probe from the ion source for sample loading and the subsequent re-optimizing operation needed for the probe re-insertion. Since each sample is eluted in a discrete manner, background subtraction and peak ratio calculations can be applied as in chromatographic techniques for quantification (7,8).

In the operation of a CF-LSIMS system where the liquid is delivered through capillary tubing to the LSIMS target surface to form a thin film, one of the handicaps for continuous stable operation is the accumulation of liquid around the probe tip region. This causes such adverse effects as peak broadening from stagnancy of liquid flow, film instability from the freezing of mobile phase in the capillary, and instability of ion source pressure due to dripping and bubbling of mobile phase. We have minimized these effects by installing an absorbent evaporator with extended evaporation surface at a distance from the probe tip. The evaporator is provided with a heat conduction pathway, bypassing the probe tip region, from a large heat sink. Without complicated engineering to control both mass and heat transfer, we are able to operate the continuous flow system near a state of mass transfer dynamic equilibrium without problematic accumulation of liquid for more than eight hours of continuous operation.

A prototype Finnigan (Finnigan Mat, San Jose, Ca) Bioprobe system on a TSQ-70 mass spectrometer and static LSIMS probe tip were used. An unfocused cesium ion gun was from Phrasor (Duarte, Ca). The probe stop in the source block assembly was removed to accommodate the evaporator. The homemade probe was machined from a solid rod of stainless steel. The evaporator was made out of Whatman cellulose extraction thimbles (Whatman International Ltd, Clifton, New Jersey) and force-fitted onto the probe shaft as shown in Figure 1. The thimble shaped evaporator was of 10 mm i.d. x 28 mm length. A pulse-dampened Gilson Model 302 HPLC pump was used to deliver a 20% glycerol/H₂O solution to a 0.5 μ L loop injector and through a 75 μ m i.d. x 90 cm fused silica tubing to reach the probe tip in the ion source. The mobile phase flow rate was about 8 μ L/min. The ion source temperature was 50-55 $^{\circ}$ C. The probe was heated at the probe handle to maintain the temperature of the probe shaft at the entry port of the mass spectrometer at about 40 $^{\circ}$ C. Repetitive injections of 10 ng potassium salt of 3-methoxy-4-sulfatoxyphenylethyleneglycol in 0.5 μ L mobile phase show that the continuous flow system can be operated continuously for up to about nine hours with little deterioration of performance (Figure 2).

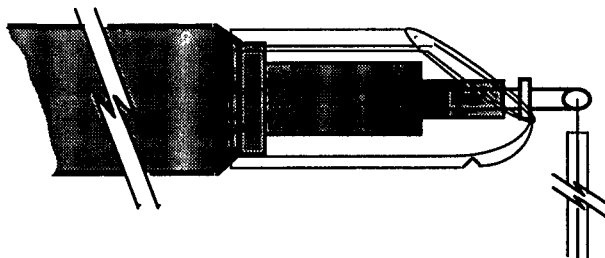


Figure 1. Evaporator and probe assembly

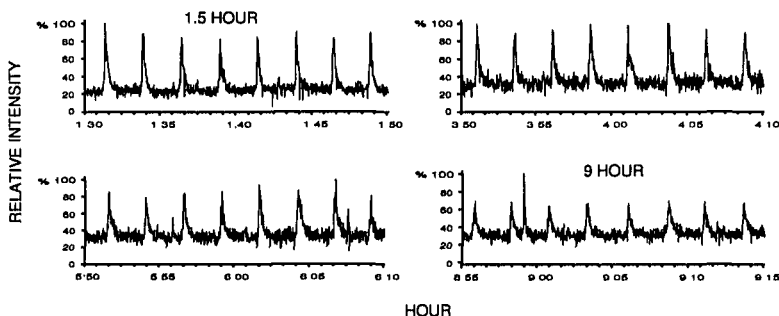


Figure 2. Repetitive injections of 10 ng potassium salt of 3-methoxy-4-sulfatoxyphenylethyleneglycol, Timing started at the beginning of mobile phase flow

There are two engineering principles which this system incorporates. First, the transferring of the mobile phase away from the probe tip region reduces the thermal load on this region. Second, the use of an extended absorbent evaporation surface in contact with a large heat sink increases the rate of mobile phase evaporation at reduced temperature, and improves the overall liquid fluidity. These principles should be adaptable to other CF-LSIMS or FAB mass transfer systems. We anticipate that with adequate amounts of absorbent and evaporation surface, this device will enhance the tolerance of the flow system to various mass transfer and heat transfer control parameter changes. Thus, it should be useful for a wide range of mobile phase compositions and mobile phase flow rates in CF-LSIMS systems.

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Analysis of Nucleoside-PAH DNA Adducts by CID FAB-MS and
Continuous Flow FAB-MS

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The covalent adducts formed when polycyclic aromatic hydrocarbon (PAH) metabolites react with DNA are well suited for analysis by desorption MS techniques. The FAB spectra of these compounds contain $[M+H]^+$ and $[M-Sugar+2H]^+$ ions. Unfortunately, the sensitivity of these compounds to FAB is generally poor. The deoxyribose sugar present in the nucleoside and nucleotide form imparts a large degree of hydrophilicity to the molecule, resulting in a low desorption efficiency, and reduced sensitivity. This fact in combination with the small amounts of adducts recoverable from DNA or cell cultures imposes a serious limitation on the detection and characterization of DNA adducts. The goal of this research was to advance the sensitivity of FAB for the analysis of nucleoside adducts, and to increase the structural information available from the FAB experiment. Two general types of PAHs which have demonstrated carcinogenic activity, aromatic amines and Bay region PAHs, were studied.

The use of TMS derivatives was found to greatly increase the sensitivity of these compounds to FAB. Very high quality spectra could be obtained on as little as 50ng of the aromatic amines. The molecular species is predominantly the TMS_2 derivative were both TMS groups are on the deoxyribose sugar. Thus cleavage of the carbohydrate linkage results in the same mass ion as the underivatized compounds. The enhancement in sensitivity was especially great for the dihydro diol epoxide PAH adducts. Unfortunately the $[M-Sugar+2H]^+$ (AH_2) ions were still too weak to be observed.

Examination of the metastable decay of the PAH adducts showed that the $[M+H]^+$ ions decomposed to yield AH_2 primarily. Ions due to the loss of NH_2 from guanine were also common. Because the AH_2 ions were of high intensity relative to the parent the sensitivity of this transition was evaluated. Detection of 22ng of N-acetyl-N-(deoxyguanosin-8yl)-2-amino fluorene (AAF-GUAN) was possible at S/N=5. Preparation of the TMS derivative of AAF-GUAN resulted in greater than a 10 fold increase in sensitivity. The decomposition of the $[TMS_2+H]^+$ to yield the same AH_2 ion as the underivatized compound (m/z 373) could easily be detected at 4ng (S/N=15). The metastable decay of the AH_2 ions results in a few ions of very low intensity.

High energy collisional activation, with a He target gas, was found to be very useful for inducing fragmentation of the AH_2 ions. Several ions representative of the PAH were present in high abundance, as were ions resulting from cleavage of the guanine base. Figures 1 and 2 show the CID spectra of the AH_2 ions of positional 2-aminophenanthrene-guanine adducts. Visually the spectra are very different. The primary ions stem from expulsion of a cyanamide fragment which contains the phenanthrene structure. In Figure 2 (N^2 adduct) this ion is at m/z 233 rather than m/z 218 ($C8$ adduct), because the amino group of the phenanthrene moiety is not involved in the cyanamide bond. The spectra in Figures 1 and 2 were taken from the TMS derivatives using 200 ng for each.

The use of the continuous flow FAB probe resulted in high quality full scan spectra, of underivatized adducts being generated on as little as 50ng. Figure 3 shows that 10ng of AAF-GUAN can easily be detected by observing the metastable decay of the m/z 489 ($M+H$) to 373 (AH_2). Figure 3 is a B/E scan covering 600 u. Much greater sensitivity could be achieved by scanning a smaller mass range or by using reaction monitoring.

This work was supported by a grant from the Health Effects Institute.

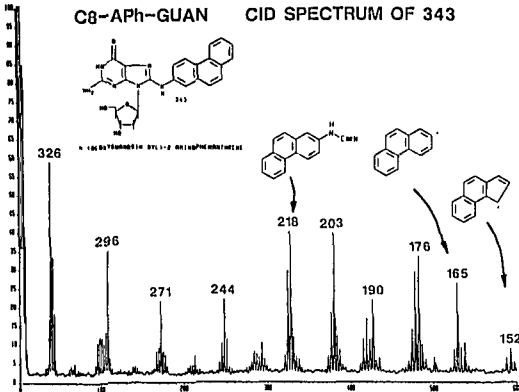


FIG 1

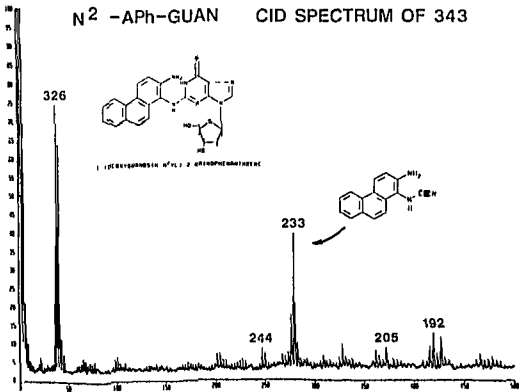


FIG 2

**10ng AAF-GUAN CF-FAB
DAUGHTERS OF 489**

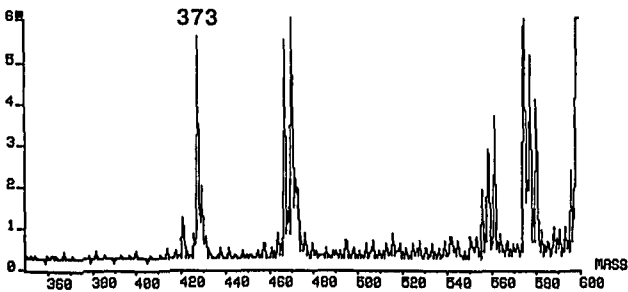


FIG 3

CONTINUOUS-FLOW FAST ATOM BOMBARDMENT LC/MS IN BIOANALYSIS

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Within the rapidly growing research field of interfacing high performance liquid chromatography and mass spectrometry (LC/MS) continuous-flow fast atom bombardment (CF-FAB) receives a growing interest. There are essentially two reasons for this, i.e. the improved performance of CF-FAB as compared to conventional FAB and the possibility to use a "soft" ionization method in LC/MS.

However, the major part of the reports is based on evaluation of the method in flow injection mode and only a minor part is concerned with LC/MS applications. The most important reason for this is the fact that the flow-rate in CF-FAB is limited to a maximum of about 10 $\mu\text{l}/\text{min}$. As a consequence it is necessary to split after the chromatographic separation typically 1:200, which increases the concentration detection limits by a factor of about 200. Miniaturization of the HPLC system to microbore instrumentation reduces the split ratio but does not improve the concentration limits, except for cases in which the sample size is limiting, because the injection volume in miniaturized systems must be decreased correspondingly.

For quantitative analysis on-line miniaturization applying "Phase System Switching" (PSS) is an attractive alternative as we have demonstrated for the moving belt interface and the thermospray interface. The basis for PSS is a coupled column system consisting of an analytical column followed by a trapping column. After trapping the analyte on the second column and a washing step which can be used to remove non-volatile buffers from the mobile phase used in the separation, desorption is achieved with the optimum mobile phase and the optimum flow-rate for the interface used. The latter aspect enables chromatography using conventional columns and the detection at low flow-rates without the need for splitting. The experimental setup and the results for dextromethorphan are given in Figure 1 and 2.

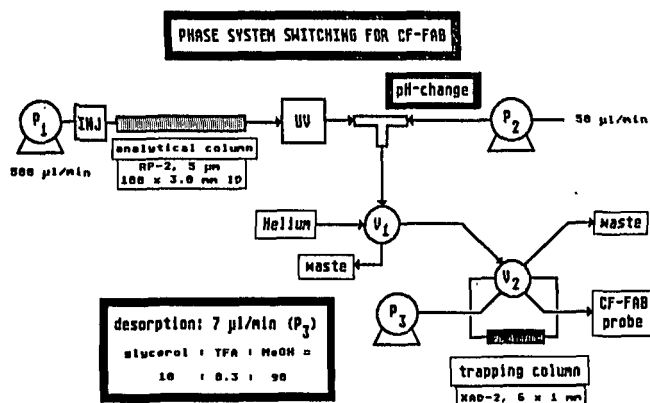


Figure 1. Experimental setup for CF-FAB analysis of dextromethorphan by Phase System Switching.

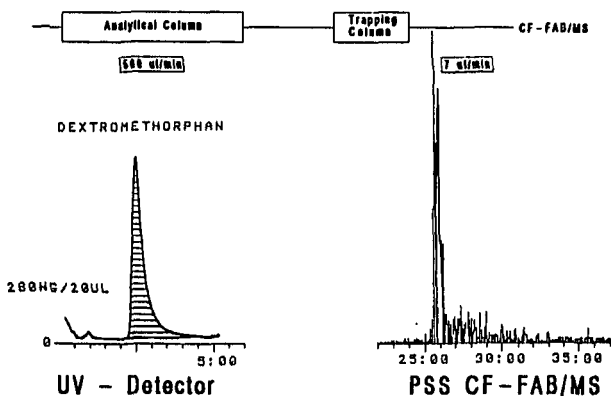


Figure 2. Phase System Switching in CF-FAB for dextromethorphan.

Continuous Flow FAB-MS for Rapid Structural
Comparison of Analogous Therapeutic Proteins.

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Continuous Flow Fast Atom Bombardment (CF-FAB) [1] has been successfully used as an interface technique for Liquid Chromatography Mass Spectrometry (LC-MS). One of the most important growth areas in LC-MS is peptide mapping. In this work enzymatic digests of recombinant IL-2 were mapped using a MAT90 double focusing mass spectrometer and CF-FAB interface, (Finnigan MAT GmbH., Bremen, FRG).

Recombinant human IL-2 expressed in E. Coli was reduced and pyridylethylated (to give S-8-4-pyridylethylcysteine). The sample was desalted by reverse phase HPLC using a Brownlee RP-C18 cartridge, 5µm, 3cm x 2.1 mm internal diameter, equilibrated in 0.1% trifluoroacetic acid/water. The eluent was lyophilised, and the protein (1.6 nmol) dissolved in 0.1 M ammonium bicarbonate, pH 7.8. 800 pmol was digested with trypsin at a ratio of 1/50 w/w at 37°C for 6 hours. The remainder was digested with protease V8 at a ratio of 1/20 w/w.

Chromatography was performed with a Model 130A separations system (Applied Biosystems, Warrington, Cheshire, UK), and a Rheodyne 7410 injector equipped with a 5µl loop. Gradient elutions were performed at 8µl min⁻¹ on a packed fused silica micro-LC column, FUC-330, 5µm, C18, 320µm I.D. x 30 cm (LC Packings, Amsterdam, The Netherlands). Solvent A was 0:10:90:0.1 and solvent B 60:5:35:0.1 acetonitrile / glycerol / water / trifluoroacetic acid respectively.

Figure 1 shows examples of selected mass chromatograms for tryptic fragments obtained from a single injection of 10 pmol. Many other intense peaks were also observed, but rejected on the grounds that they did not elute as sharp chromatographic bands. In total, fragments corresponding to 97% of the sequence were observed, (Figure 2), although the signals for several important fragments were very weak and may not have been recognised as significant had we been studying a true unknown. The spectrum in Figure 3 illustrates the high signal to chemical background ratio characteristic of CF-FAB. The peaks at 562u, 690u, and 910u correspond to tryptic fragments.

These results are in good agreement with those obtained earlier using thermospray ionisation [2]. The principal advantage of using CF-FAB over thermospray is the reduction in sample consumption (10pmol versus 7nmol).

Only in favourable circumstances can complete sequence verification be obtained from FAB mapping of a single enzymatic digest. For this reason, a V8 digest of IL-2 was also analysed which provided complementary data, particularly in the vicinity of residues 55-76 where the tryptic fragment was very weak (Figure 2).

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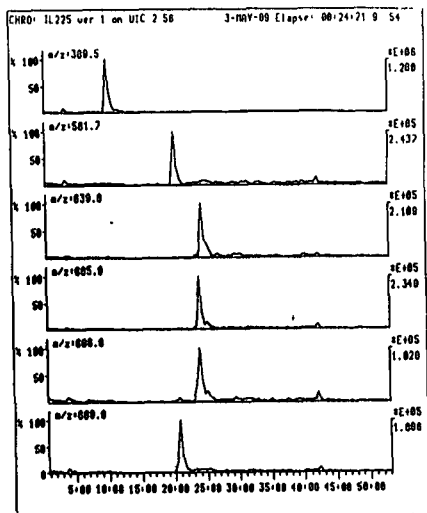


Figure 1

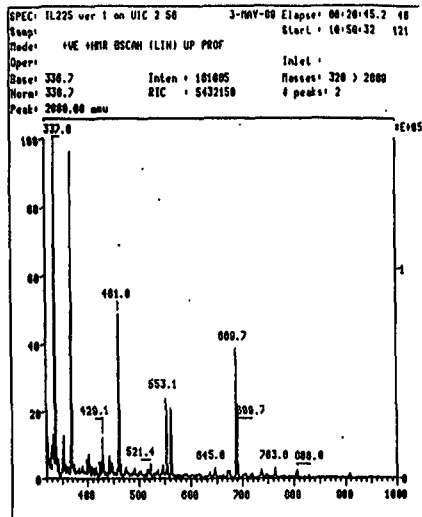


Figure 3

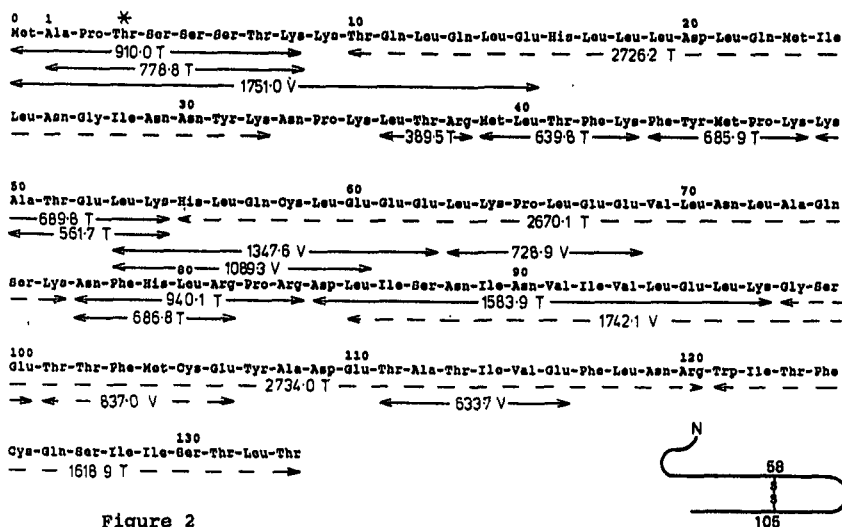


Figure 2

CF-FAB AND MICRODIALYSIS FOR ANALYSIS BLOOD LEVELS OF DRUGS
IN LIVE ANIMALS: MULTIPLE DOSAGE PHARMACOKINETICS

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In this study, we have assessed the ability of the microdialysis and on-line continuous-flow fast atom bombardment (CF-FAB) mass spectrometry (1) to provide reliable *in vivo* pharmacokinetic data over prolonged periods of time for the evaluation of multiple-dose antibiotic therapy.

A Finnigan MAT TSQ-70 equipped with a CF-FAB probe (Bioprobe) was used with a Carnegie Medicin microdialysis device. Briefly, the microdialysis device was inserted inside a 22 gauge needle cannula surgically placed in the carotid vein of a male rabbit. A Harvard microliter syringe pump was used to produce a flow of about 4 μ l/min of carrier solution through the microdialysis probe. The carrier solution consisted of 95% water and 5% glycerol. The output from this device was passed through a RP8 microbore HPLC column. The compounds trapped on the HPLC column were then back-flushed off by an elution fluid composed of 5:40:0.1:55 glycerol:acetonitrile:TFA:water at 5 μ l/min. The flow was directed via the CF-FAB interface into the mass spectrometer. Thus, the pharmacokinetic data was obtained through a series of flow injection analyses 10 min sampling time followed by a 10 min elution and analysis time, with a total cycle time of 20 min.

Initial studies were designed to identify analytical problems likely to be encountered in the use of the instrumentations for analyses of commonly-used antibiotics (2). For antibiotics such as penicillin G, which is easily microdialyzable and sensitive to the CF-FAB mass spectrometric analysis, we were able to use a 20 μ l sample loop on-line, without the use of a preconcentration column, to follow with easy the time course of the drug in the blood of a rat after a bolus of 15 mg/0.3 ml was injected intramuscularly (see Figure 1). Although piperacillin can also be measured sensitively by the CF-FAB mass spectrometry, the compound does not pass through the microdialysis membrane very efficiently. The recovery efficiency of the microdialysis device for this compound is approximately 4%, a factor of 4 times less than that of penicillin G. In order to follow the *in vivo* blood concentration, it became necessary to include a 2mm id x 2cm RP8 LC column on-line as a concentrating apparatus to ensure that there was sufficient amount of drug to measure with good accuracy.

This analytical procedure was used to follow the multiple-dose pharmacokinetics of piperacillin in live rabbits (see Figure 2). The first three IV doses were given via the ear vein at 25 min intervals, resulting in high blood piperacillin concentration. The blood level decreases rapidly. The next two doses were given at 70 min intervals. The blood concentration did not spike as before but the overall pattern of accumulative concentration changes from the time course curve is typical of multiple dosage pharmacokinetics.

Aminoglycoside antibiotics are a series of new potent but very toxic compounds. Our early studies with these compounds reveal analytical problems somewhat different to those cited above. The detection sensitivity for tobramycin by CF-FAB mass spectrometry, for example, is very much dependent on the composition of the mobile phase used to carry the compound to the ion source. The data show a 10-fold decrease in detection sensitivity for tobramycin when the compound is dissolved in a 5% glycerol and 95% water solution instead of in a 5% glycerol, 40% acetonitrile, 0.1% TFA and 55% water mixture. Thus, it appears that for these compounds, a different experimental protocol will be required to achieve maximum sensitivity.

In conclusion, for antibiotics to be effective in combating infectious microorganisms, the antibiotic should reach the target site in therapeutic concentrations. However, precise information on tissue and body fluid levels are not readily available, either because a suitable analytical method is lacking or because available methods are too time-consuming for *in vivo* measurements. Microdialysis combined with mass spectrometry offers some new approaches to such measurements. We have described some initial work in this area and have pointed to some problems for measurement of antibiotic concentrations *in vivo*. However, the fact that we are able to apply the technique to follow several antibiotics at therapeutic levels in the blood over prolonged periods of time shows that the technique is effective in such applications. The measurement and evaluation of these data are critically important for ensuring safe and beneficial administration of antibiotics and for the maintenance of therapeutical tissue and blood levels.

Figure 1.

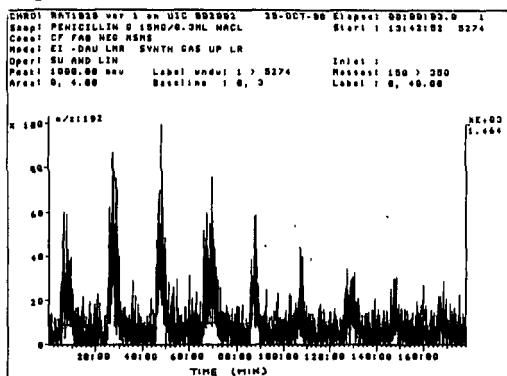
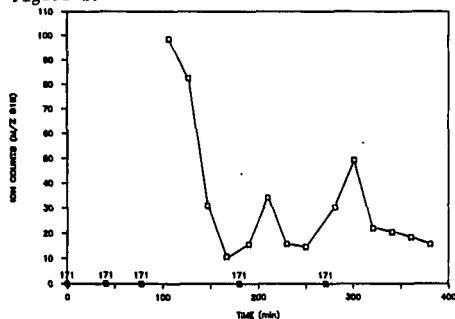


Figure 2.



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ANALYSIS OF URINARY ACYLCARNITINES BY DYNAMIC FIB - MS/MS

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L - Carnitine (3 - hydroxybutyrobetaine) is essential for the transport and metabolism of fatty acids. Its main function is to carry long chain acids across mitochondrial membranes for β - oxidation. An abnormal urinary excretion of L - carnitine and its acyl derivatives occurs in children with various inherited metabolic disorders (1). The excretion profile is generally disease specific. Acylcarnitines are not directly amenable to GC-MS. Millington and co workers (1,2) have analysed these compounds using static FAB-MS and FAB-MS/MS (daughter ion scanning) both neat and purified urine samples were analysed directly or after methylation to improve sensitivity.

Methods Urine samples were collected from patients with and without metabolic defects, on drug treatment and after L - carnitine administration. 200 μ l of unpurified urine was lyophilized and analysed directly or after methylation. (MeOH HCl, 50°C for 30 minutes). Dynamic FIB was carried out using a cesium gun under the operating conditions: mobile phase 70% H₂O, 20% MeOH, 10% glycerol; flowrate 6 μ l/min; sample volume 0.5 μ l; gun voltage 15kV Normal spectra were measured on a VG TRIBRID instrument, parent ion scans of m/z = 85 (non-methylated) and m/z = 99 (methylated) were carried out using collision cell 2 (MIKES cell); air admitted to 50% beam attenuation of m/z = 277.

Results and Discussion FIB mass spectra of both non-methylated and methylated acylcarnitines were dominated by pseudomolecular (M+H)⁺ ions (fig 1). Parent ion scans using the relevant characteristic daughter ions at m/z = 85 or 99 (2) gave molecular ions with excellent resolution. Such scans are only obtainable using an instrument of this geometry. Figure 2 shows the parent ion spectrum of a standard mixture of methylated C₄, C₅, C₆ and C₈ carnitines esters at m/z = 246, 260, 274 and 302 respectively. The resolution achievable makes the Spectra easy to assign.

Figure 3 shows the parent ion scans (m/z = 99) of a methylated urine sample from a neonate with diagnosed medium chain acyl-dehydrogenase deficiency (MCAD) following an L-carnitine load. The grossly elevated excretion of octanoyl-carnitine (m/z = 302 together with hexanoyl (m/z = 274) and octenoyl carnitine (m/z = 300) is characteristic of the condition. For comparison a normalised parent ion scan of urine from a normal neonate is shown in Figure 4. Other chemical studied by this technique included methylmalonic and propionic aciduria and valproate therapy.

Conclusions Dynamic and static FIB - MS/MS using high energy collisions and parent ion scans on a multi sector instrument enables the specific and rapid identification of acylcarnitines in complex mixtures.

References

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Fig.1 Normal FIB spectrum of Hexanoyl Carnitine.

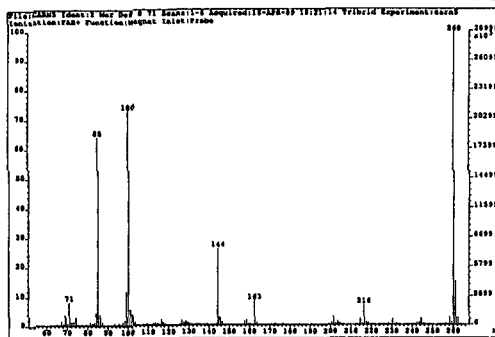
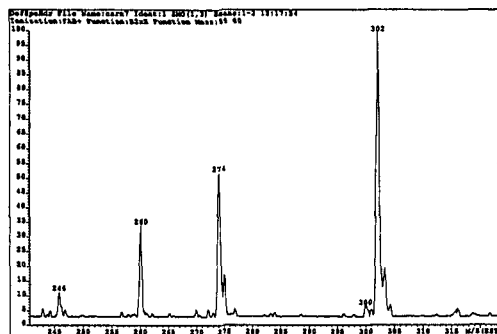


Fig. 2

Parents of m/z 99 methylated
 C_4 C_5 C_6 C_7 carnitine
esters

Fig.3 Parents of m/z 99 of unpurified urine from normal neonate.

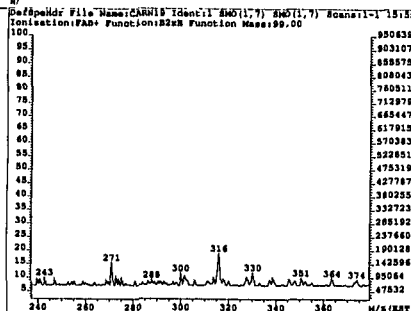
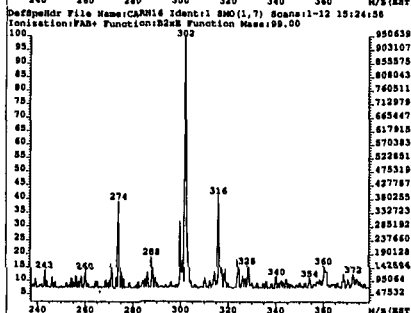


Fig.4 Parents of m/z99 from urine for a neonate with MCAD.



ANALYSIS OF PROTEOLYTIC DIGESTS AND PEPTIDE MIXTURES USING COAXIAL CONTINUOUS FLOW FAB MASS SPECTROMETRY

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Continuous flow FAB (CF-FAB) using microbore [1] and submicrobore [2] HPLC systems have been proven successful in the analysis of peptide mixtures and tryptic digests of proteins. This special interface also allows flow injection of aqueous samples into an effluent containing a low concentration of matrix (5% glycerol in H₂O). One of the distinct advantages of this technique over static FAB probe analysis is its improved sensitivity in the detection of peptides having different surface activities [3]. This phenomenon is thought to arise from the reluctance of hydrophilic peptides to migrate to the liquid interface. However the dynamic nature of the liquid surface under CF-FAB apparently minimizes this suppression effect and is of considerable advantage in the analysis of enzymic digests.

Most of the LC/CF-FAB interfaces reported thus far introduce the FAB matrix either in the mobile phase [1,2] or by addition via a post-column arrangement [4]. The main limitation of such system is that the addition of the matrix into the LC effluent not only changes the polarity and viscosity of the mobile phase but can also cause severe chromatographic peak-broadening. These inconveniences can be avoided by adding the FAB matrix independently to the FAB probe tip. Such an interface has been reported recently for conventional and tandem mass spectral analyses [5]. Briefly this arrangement consists of 2 coaxial capillaries, the inner one being used to deliver the LC effluent while the outer capillary is used to introduce the FAB matrix at the end of the probe tip. Figure 1 shows two LC/MS analyses of a simple peptide mixture using a 0.32 mm i.d. column, and compares the use of glycerol in the mobile phase with the coaxial arrangement. It is observed that under a similar gradient operation (flow rate, mobile phase composition of organic solvent) the incorporation of glycerol in the LC effluent increases significantly the diffusion of analytes in the column. Consequently individual peaks become broader and elute during a time period approximately two times longer than in the coaxial arrangement. A direct advantage of the narrower peak shape in coaxial CF-FAB is that a higher flux of analyte reaches the probe tip at a given time therefore allowing better sensitivity. This is illustrated in Figure 2 for the analysis of glucagon tryptic digest which contains approximately 100 pmols of each peptide. It is noteworthy that fragment 19-29 at m/z 1352 is clearly observed in the CF-FAB experiment although its signal was suppressed using static probe FAB analysis. This behavior is consistent with that reported for hydrophilic peptides [3,6].

In addition to the advantage described above, the coaxial CF-FAB interface permits operation with a gradient system containing up to 100% acetonitrile. This is not possible with glycerol in the mobile phase due to its limited solubility (approx. 5% glycerol in 60 % acetonitrile). Another application of this interface is illustrated in Figure 2 for the analysis of bovine growth hormone releasing factor 1-29. For purposes of clarity the selected ion traces are presented only for m/z: 843, 971, 1002 and 1130 corresponding to fragments 13-20, 12-20 and/or 13-21, 22-29 and 21-29 respectively. Because of the presence of arginyl-lysyl residues at position 11-12 and 20-21, the formation of m/z: 971 can arise from both 12-20 and 13-21 fragments. Due to the enzymic cleavage preferences and kinetics of this tryptic digestion it is observed that fragments 1-12 and 22-29 are not abundant. On the other hand this suggests that m/z: 971 would originate from the 12-20 fragment. This possibility was confirmed by recording MS/MS spectra of m/z: 971, using both an hybrid and 4 sector tandem mass spectrometers. The observed daughter ion masses were matched with those predicted from known peptide fragmentation schemes to validate the potential sequence candidate.

Better chromatographic resolution and sensitivity could be obtained using a 2 m micro packed column of 50 µm i.d. However the analysis time using this latter column was substantially longer (approximately 3 hours for the analysis of bovine growth hormone releasing factor compared to 30 mins with the 320 µm i.d. column).

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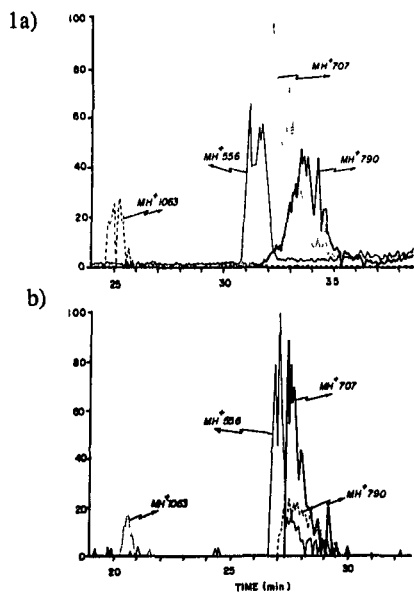


Figure 1: Chromatograms of selected ions from the analysis of a peptide mixture (YGGFL, KFLGLM, YFFPGPI VQAAIDYIN 70 ng of each) on a 0.32 mm C18 Alltech
a) Linear gradient from 15% A to 100% B in 40 min. (ACN/glyc./H₂O/TFA, A: 0/5/95/1, B: 60/5/35/1)
b) Coaxial LC separation, linear gradient from 15% A to 65% B in 40 mins (A: 0/0/100/1, B: 100/0/0/1)

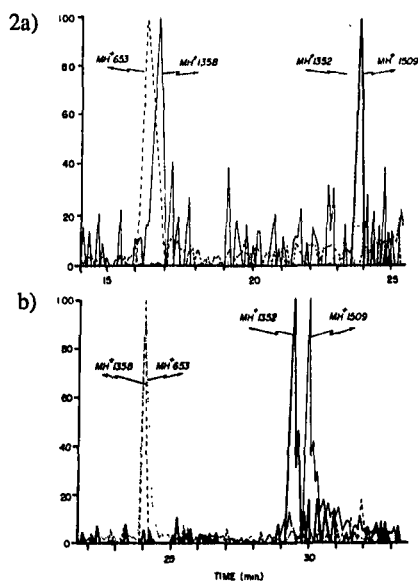


Figure 2: LC/CF-FAB analyses of a glucagon tryptic digest (106 pmoles) a) Linear gradient from 100% A to 100% B in 20 mins (solvents same as 1a)
b) Coaxial LC separation, linear gradient from 15% A to 65% B in 40 mins (solvents same as 1b)

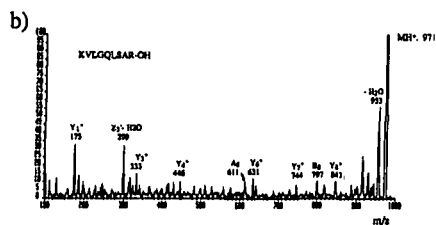
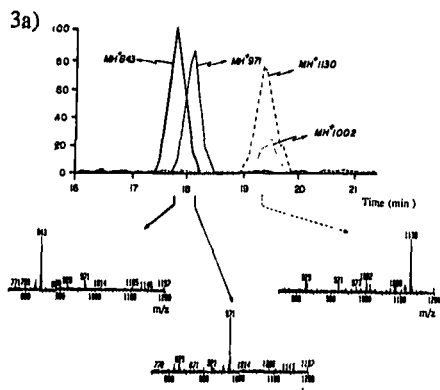


Figure 3 a) LC/CF-FAB analysis of a Bovine growth hormone releasing factor 1-29 tryptic digest (41 pmoles). Insets show narrow region (single scan) of the acquired mass spectra for the corresponding tryptic fragments.
b) Quadrupole daughter ion spectra of m/z: 971, 50 eV, 80% attn., Ar collision gas

**MICROBORE AND CAPILLARY HPLC ANALYSIS OF GLYCOPEPTIDE
ANTIBIOTICS USING CONTINUOUS FLOW FAB LC-MS**

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The use of continuous flow fast atom bombardment (CF-FAB) as an interface for liquid chromatography-mass spectrometry (LC-MS), especially in peptide analysis, has generated great interest in this technique. The constraints of this interface, including low flow rates (2-5 $\mu\text{L}/\text{min}$) and need for a non-volatile FAB matrix such as glycerol, have presented problems in the routine application of this technique. To date, most investigators have used effluent splitting along with isocratic systems containing glycerol in the mobile phase to sidestep these problems.

This work describes the adaptation of gradient high performance liquid chromatography (HPLC) systems using microbore (1mm) or capillary (320 μm) columns with post-column addition of matrix. The microbore column was a commercial column (1 mm x 250 mm Spheri-5 RP-18, ABI). The capillary column was slurry packed with 5 μ Spherisorb ODS using 320 μm i.d. fused silica to give a 250 mm long column. Teicoplanin was chosen as a model system because of the severe demands placed both on the chromatographic separation and the FAB mass spectrometry to obtain good results in the analysis of glycopeptide antibiotics.

For both systems gradients were formed by a conventional gradient HPLC system (Waters 600-MS) and the total flow was split before the injector (Rheodyne 7413 - 0.5 μL) by use of a dynamic balance column (4.6 mm x 250 mm Spherisorb ODS, 5 μ). In the case of the microbore LC system, the resulting flow was 75 $\mu\text{L}/\text{min}$ through the column. Matrix was added post-column and the combined effluent was split to allow 3 $\mu\text{L}/\text{min}$ (3%) to go to the mass spectrometer. For the capillary system, the resulting flow through the column was 2 $\mu\text{L}/\text{min}$, matrix was added post-column, and the entire amount was analyzed by MS. The capillary system is shown in Figure 1. For both LC systems good mass spectra of the main components were obtained (A2-1 through A2-5). The increased sensitivity of the capillary system allowed spectra to be obtained on components present at picomole concentrations. The UV chromatogram and TIC trace from a gradient separation of teicoplanin is shown in Figure 2. The mass spectrum of the major teicoplanin component (A2-2) is shown in Figure 3.

The increased sensitivity of the capillary system is primarily due to the fact that all material injected onto the LC column reaches the mass spectrometer. In addition the chromatographic resolution of the capillary system appears somewhat better than the microbore system due to the deletion of the extra column effects of a post-column splitter section in the interface. These advantages outweigh the problems involved in fabricating the packed capillary LC columns to make this the preferred system for CF-FAB. The use of gradients minimize the effect of large volume injections (0.5-1.0 μL) to make this a useful system for trace analysis with scanning detection limits in the low picomole range.

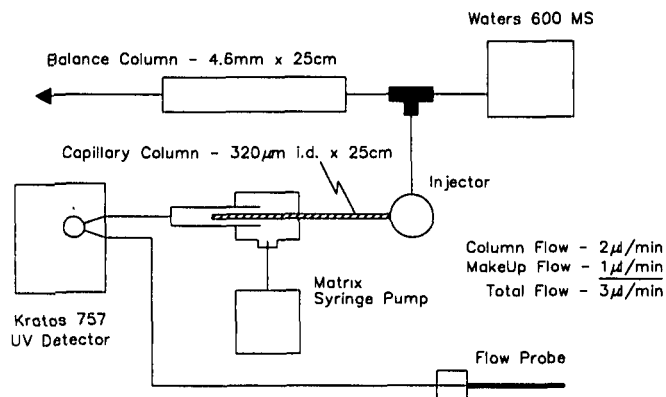


Figure 1. Diagram of capillary LC system for CF-FAB LC-MS.

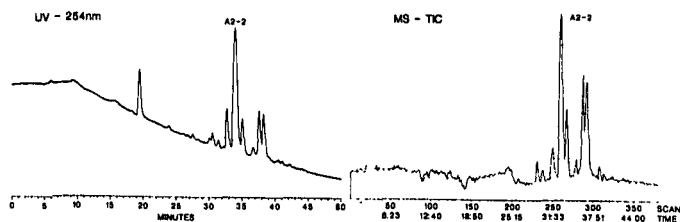


Figure 2. UV and Total Ion chromatograms for gradient separation of teicoplanin (1 nmole injected).

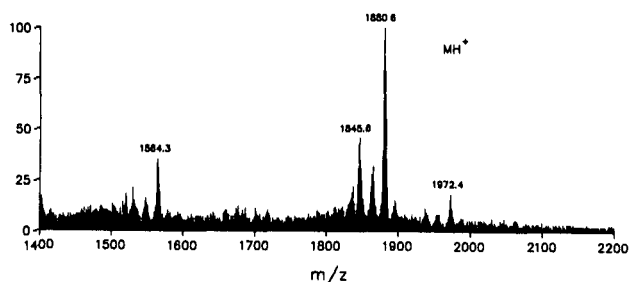


Figure 3. Mass spectrum of major teicoplanin component (A2-2).

**MICROCOLUMN LC/CONTINUOUS FLOW FAB MS
APPLICATIONS TO AGRICULTURAL CHEMICALS RESEARCH**

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Packed fused silica micro LC columns were interfaced with a magnetic mass spectrometer (Finnigan 8230) using a Continuous Flow FAB (CFF) probe designed and constructed in our laboratory to be interchangeable with a commercial moving belt interface. This gives us the capability to perform LC/MS-EI, CI and FAB, a powerful combination for the identification of unknowns (1,2,3). The low flow rates used with micro LC columns (1-2 μ L/min.) allow direct interfacing (no effluent splitting) of reversed phase LC columns using both the moving belt and CFF probe, which is very important in sample limited analyses. We routinely obtain good quality EI, CI or FAB mass spectra on injection of a 0.1 microgram sample into the micro LC.

The CFF target is heated by radiation from the ion source, and with a source temperature of 200°C the target temperature is 45-50°C. The FAB spectra obtained on thermally labile compounds under these conditions were identical to those obtained with the conventional FAB probe with the ion source at room temperature. We obtained a 10-fold increase in sensitivity with the CFF probe over that obtained with the conventional FAB probe, likely due to increased sample concentration in the glycerol matrix since a much thinner film of glycerol is obtained with the CFF probe. Addition of 10% glycerol to the mobile phase caused no problems with the chromatography. The glycerol ions are readily subtracted out of the sample spectra, allowing observation of low mass fragment ions which are very helpful for structure determination.

LC/MS-FAB has allowed us to obtain molecular weights of polar, thermally labile compounds that do not yield molecular ions using EI, CI or thermospray ionization. Figure 1 shows an LC/MS-FAB chromatogram obtained on a mixture of chlorsulfuron, a sulfonylurea herbicide, and two wheat metabolites, obtained by plotting a summation of the three protonated molecular ions. Figure 2 shows the background subtracted FAB mass spectrum obtained on the glucoside metabolite. This polar metabolite is extremely thermally labile, and does not give a molecular ion by EI, CI or thermospray ionization.

LC/MS-FAB has proven to be very useful for agricultural chemicals research. FAB mass spectra have provided unequivocal molecular weights and structurally useful fragment ions of polar, thermally labile metabolites and product impurities which are extremely helpful in their identification. These identifications are needed for environmental studies and product chemistry studies conducted on our products prior to and after market introduction.

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FIGURE 1

LC/MS-FAB Chromatogram of Chlorsulfuron and Wheat Metabolites

Column: 26 cm. x 0.25 mm ID, Spherisorb ODS-2, 3 μ
Mobile phase: 40% CH₃CN/50% H₂O (pH 3)/10% glycerol
Column pressure: 170 bar Injection volume: 0.1 μ L

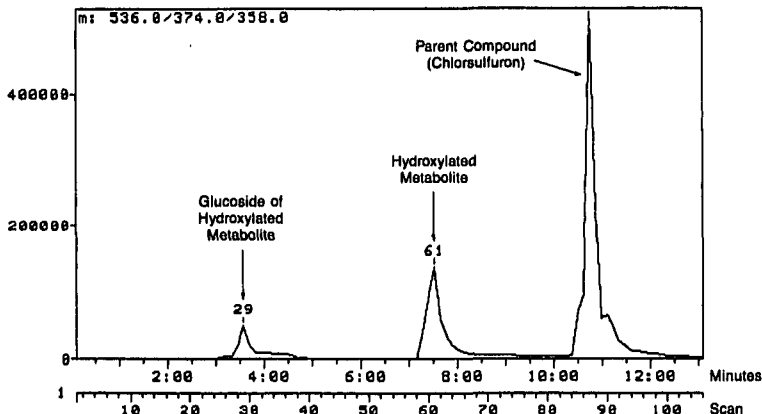
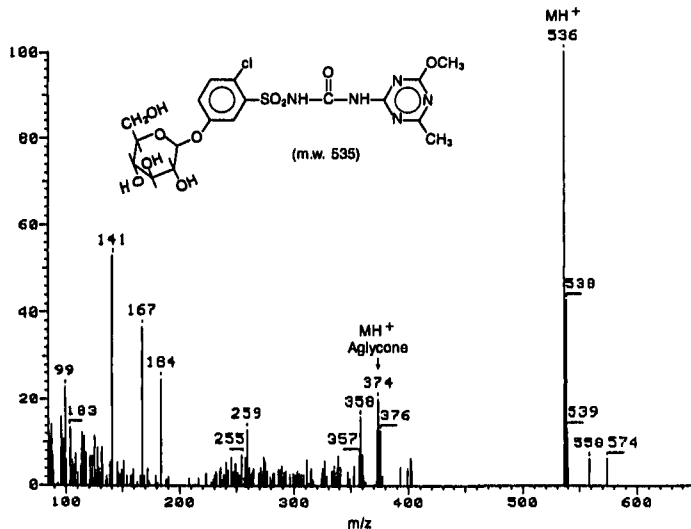


FIGURE 2 FAB Mass Spectrum of Glucose Conjugate of
Hydroxylated Chlorsulfuron Metabolite



Examples of the Use of a Particle Beam Interface for the HPLC/MS Analysis of Non-Gas Chromatographable Analytes

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Background. Recent developments in HPLC/MS interfaces have resulted in a commercially available particle beam interface which can be used with existing mass spectrometer systems. The present paper describes preliminary results showing the analysis of non-gas chromatographable analytes which was conducted on an HPLC/MS with such an interface. The goal of these studies was to determine the usefulness of this approach for the analysis of compounds which normally require derivatization or other multistep processes to obtain highly specific analysis results using a mass spectrometric detector system. No attempt was made to maximize sensitivity or improve HPLC chromatography.

Analysis Methods. The present study was conducted on an HP-1090 HPLC which was interfaced to the HP-5988 mass spectrometer system. Most studies involved 10 μ L direct injections of standard solutions in methanol, with no liquid chromatographic separation.

Selected standards were also analyzed using 20 μ L injected volumes and were separated using gradient elution from 50% acetonitrile/water to 100% acetonitrile. Analyte concentrations for most studies were approximately 1 μ g/ μ L. Comparative GC/MS analyses were conducted using a 30 meter DB-5 fused silica column interfaced to an HP-5985 (with 1 μ L injected volumes and 37.5 ng/ μ L concentrations).

Direct Liquid Injections. The total ion chromatograms and mass spectra of individual analytes using the HPLC/MS with particle beam interface indicated that the particle beam interface could be used to successfully analyze a number of non-gas chromatographable compounds. Table 1 summarizes the compounds and their properties used to evaluate this interface. When these components (at a concentration of 1 μ g/ μ L for each compound) were injected directly into the particle beam/mass spectrometer system, reserpine, 5,12-naphthacene dione, streptozotocin, and malonitrile dimer were detected with a total ion signal/

Table 1. Properties of Particle Beam Test Mixture Components

<u>Compound</u>	<u>Molecular Weight</u> (amu)	<u>Boiling Point</u> (°C)	<u>Melting Point</u> (°C)	<u>GC-able?</u>
Reserpine	608	----	26	no
5,12-Naphthacene dione	258	----	322	no
Trypan Blue	960	----	>300	no
Safrrole/iso-Safrrole	162	230	11	yes
Benzoic Acid	122	249	122	yes
2-Picoline	93	128	-67	yes
Streptozotocin	265	----	121	no
Malonitrile Dimer	132	(possibly decomposes)		monomer-yes
Resorcinol	110	176 ¹⁸	111	yes

noise ratio of 10 or greater. Benzoic acid, resorcinol and trypan blue produced responses with signal/noise ratios on the order of 10, and all other compounds could only be detected from their extracted ion profiles. Figure 1 shows total ion chromatograms from these direct liquid injections.

HPLC/MS Analyses. The sensitivity of this instrumentation was also evaluated using a 75 ng/ μ L test mixture of the test components shown in Table 1. Only

reserpine, 5,12-naphthacene dione, and streptozotocin could be detected from total ion chromatographic data, although several analytes could be detected using the extracted ion profiles of their most intense mass spectral peaks. All standards contained phthalate esters which are thought to be artifacts from the HPLC instrumentation.

Comparison with GC/MS Analysis Method. A number of standard compounds could be detected us-

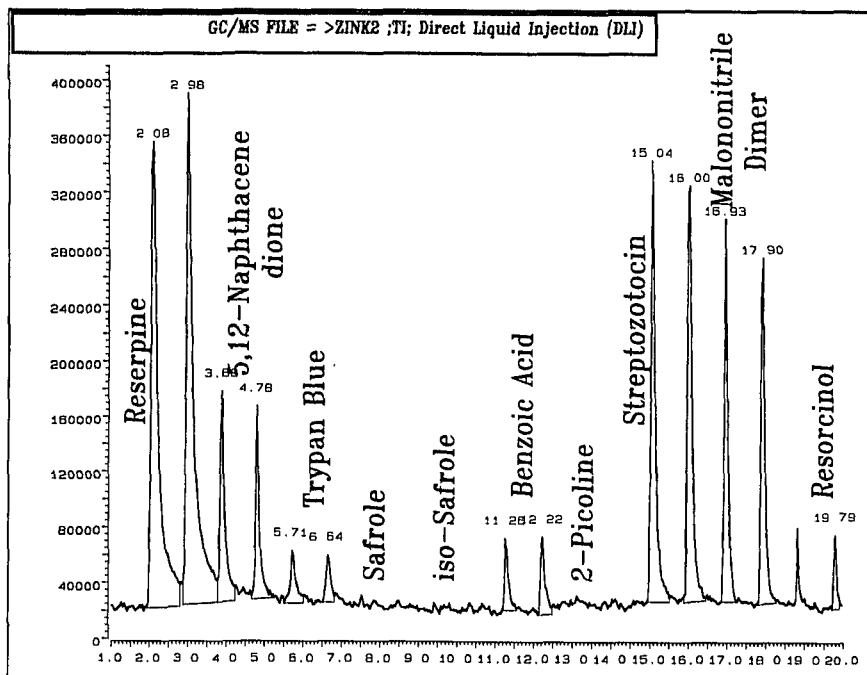


Figure 1. Total Ion Chromatogram of DLI Analyses of a Particle Beam Test Mixture.

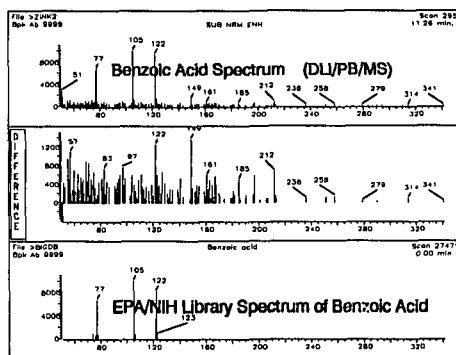


Figure 2. Comparison of DL/PB/MS Spectrum with EI Library Spectrum for Benzoic Acid

ing HPLC/MS and GC/MS techniques. However, poorer sensitivities were measured for the very volatile compounds, using HPLC/MS instrumentation. For moderately volatile compounds, the HPLC/MS method was from one to two orders of magnitude less sensitive than the GC/MS method, when the analyte could be detected in both systems. For very low volatility compounds, only the HPLC/MS instrumentation produced mass spectra.

Comparison with Electron Impact Libraries

Standard solutions of approximately 1 $\mu\text{g}/\mu\text{L}$ 2-picoline, safole and iso-safole, benzoic acid, and 5,12-naphthacene dione gave complete mass spectra which agreed with the EPA/NIH mass spectral library. Figure 2 compares a spectrum of benzoic acid obtained from the particle beam interface with a standard reference spectrum of benzoic acid.

Conclusions

The particle beam interface may prove very useful for the extension of analysis methods to non-gas chromatographable compounds. Its limitations include: 1) the requirement to use no or only volatile buffers, and 2) sensitivity on the order of 10 to 100 times less than normal GC/MS analyses.

LC/MS OF PROTEINS AND LARGE PEPTIDES, Jack Henion, T.R. Covey, W. Muck, and E. Huang, Drug Testing and Toxicology, Cornell University Ithaca, NY 14850.

INTRODUCTION

There is considerable interest in the separation and characterization of biopolymers today. Most biochemists resort to classical separation methods including gel electrophoresis, column chromatography, or other low resolution separation methods. Following this they use a variety of degradation reactions of established chemistries to characterize the "pieces" from the original biopolymer. When all these pieces are available, the challenge remains to put all this information together to determine the original structure.

HPLC separation of enzymatic digests with UV detection to produce a "map" has significantly eased the task of characterizing a tryptic digest, for example. In spite of the help provided by this analytical contribution to biochemical characterization, the technique requires knowing the retention time of each chromatographic peak with reference to analytical standards.

If HPLC with high molecular weight mass spectrometric detection(LC/MS) was used for the characterization of such samples the biochemist could have the separation and molecular weight determination of any unknown mixture with one analytical run of approximately 20-30 minutes! Molecular weights far beyond the mass range of the mass spectrometer may be determined by the multiply-charged ions produced by the ion spray(pneumatically-assisted electrospray) LC/MS interface(1). We have analyzed a variety of enzymatic digests and unknown samples to demonstrate this capability.

EXPERIMENTAL

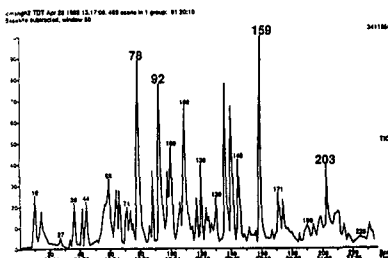
The system used for this work consisted of a Brownlee Labs Micro Pump for delivering gradient micro HPLC flow rates(10-40uL/min) through 1mm x 100mm 3 micron C8 reversed-phase packed columns to the ion spray LC/MS interface. The eluent typically was composed of methanol or acetonitrile mixed via a gradient of aqueous ammonium acetate or trifluoro acetic acid buffers(3-20mM). Ions generated at atmospheric pressure by the application of 3kV to the sprayer were sampled into a conical orifice of a Sciex TAGA 6000E tandem triple quadrupole mass spectrometer. The same ion spray LC/MS interface could be coupled to a capillary zone electrophoresis(CZE) column also. These liquid inlet systems provide modern, high efficiency separations for facile characterization by atmospheric pressure ionization mass spectrometry.

RESULTS AND DISCUSSION

Enzymatic digests of several proteins including hemoglobin, myoglobin, human growth hormone, and bovine serum albumin(BSA) were analyzed. The Figure below shows a representative ion spray LC/MS TIC for a 250 pmole injection of tryptic digest of met-human growth hormone. The molecular weights of all the peptides in the digests including undigested BSA, for example, which has a molecular weight approaching 68,000 daltons were determined by on-line LC/MS. In the LC/MS/MS mode structurally significant sequence information could also be obtained.

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ANALYSIS OF ENZYMATICALLY DIGESTED PROTEINS BY ON-LINE CAPILLARY HPLC/FABMS

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A JEOL mass spectrometer (MS) with a Frit-FAB probe has been linked to a packed capillary LC and utilized to obtain molecular ions for peptides of enzymatically digested proteins. The system provides the ability to completely mass analyze complicated peptide mixtures in the time frame of an LC separation. A tryptic digest of recombinant human Growth Hormone (rhGH) and an Asp-N endoprotease digest of Protein Carboxy Methyltransferase (PCMT) are used to evaluate LCMS performance. The low flow rates required for the packed capillary (5 μ L/min) permit the total column effluent to be introduced to the spectrometer. As a result, high sensitivity for molecular ions is demonstrated under mass spectral scanning conditions.

Our Frit-FAB LCMS system is shown schematically in figure 1. An Applied Biosystems, Inc. (ABI) microgradient HPLC system is equipped with a 0.32 x 15 cm packed capillary C-18 column (3 μ m packing, LC Packings, Switzerland) and an ABI Model 783 UV detector with an in-line capillary flow cell. A preinjector split (a Valco tee with one port connected to the appropriate length of 50 μ m ID capillary tubing to give an approximate 20:1 split) is used to generate a low flow rate thru the column and still maintain an efficient gradient delivery from the LC pump. The JEOL Frit-FAB probe is shown in more detail in figure 2. Capillary tubing exiting the LC is passed thru an insulated support and pressed up against a 0.25mm thick stainless steel frit. Liquid from the LC passes thru the frit and spreads across the surface exposed to the Xenon beam. Flow rates for all samples are 5 to 9 μ L per min. Water/glycerol/TFA 99:1:0.1 (Solvent A) and acetonitrile/glycerol/TFA 99:1:0.1 (Solvent B) is used in a gradient program of 2 to 60 percent B in 65 minutes with a hold time of 10 minutes for all LC runs. The small path length of the capillary flow cell combined with the concentrating effect of the column allow the UV detector to operate at 195nm wavelength. This gives an approximate five-fold increase in sensitivity over 214 nm.

Mass spectrometry was performed with a JEOL HX110/HX110 tandem mass spectrometer operated in the two sector mode. Samples were run at a resolution of 3000 and an accelerating voltage of 10kV. The Xe-FAB gun voltage was 6kV. Chromatographic runs were acquired at rate of 6 to 10 seconds per scan with a mass range of 600 to 4000 u.

The UV trace of a peptide mixture generated by 25 picomoles of an rhGH tryptic digest is shown in Figure 3. The peak separation is comparable to large column (4.6 mm) HPLC separations (1). The corresponding total ion current (TIC) trace (figure 4) shows little loss in peak separation caused by the MS or capillary transfer line. All of the tryptic peptides having masses within the scan range could be identified from the 25 picomole rhGH sample. Masses up to approximately 2500u molecular weight can be reliably detected at the 25 picomole level. The 0.5 picomole total injection amount spectra of the T1 rhGH tryptic, shown in figure 5, is indicative of Frit-FAB signal at the subpicomole level.

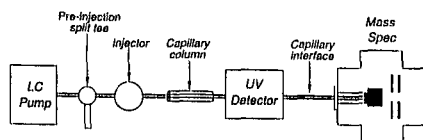


Figure 1. Schematic of the Frit-FAB LCMS.

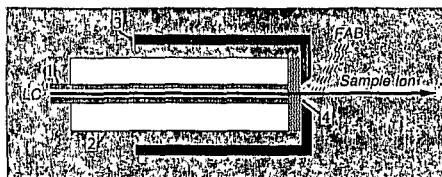


Figure 2. Detail of the Frit-FAB target.

1) Connection capillary 2) Guide 3) Cap 4) Frit.

Figure 3.

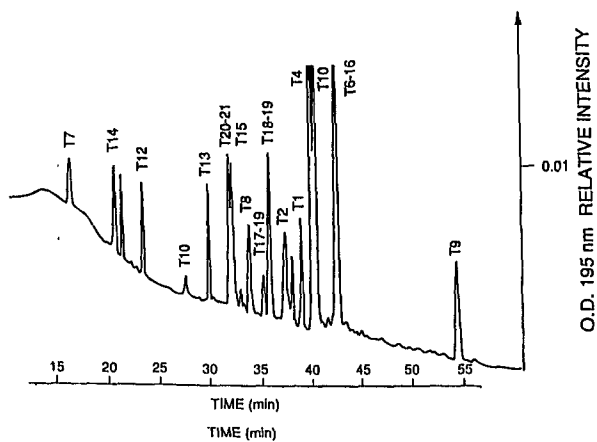


Figure 4.

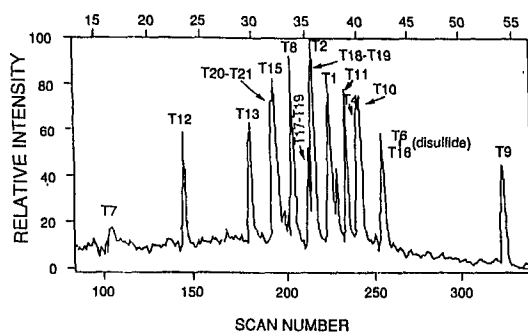
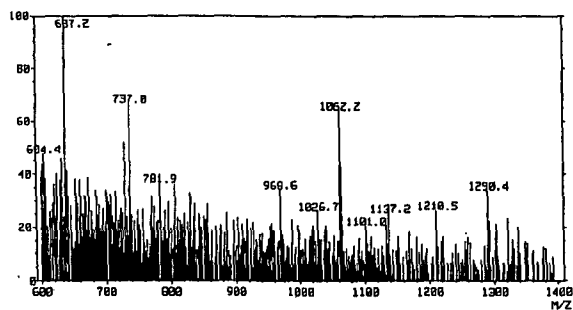


Figure 5.



A NEW INSTRUMENT FOR THE ANALYSIS OF HIGH MOLECULAR WEIGHT BIOMOLECULES
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We have developed an instrument for the analysis of high molecular weight biomolecules, incorporating an atmospheric pressure electrospray source and a VG 3000Da mass range spectrometer. Series of highly multiply charged ions are produced from which the molecular weights are deduced using simple algorithms. Data is usually acquired with the data system operating as a multichannel analyser (MCA), with 20 Channels/Da. Several scans are summed to give the final spectrum, giving total data acquisition times of between 1 and 10 minutes, depending on the ion current. Calibration is carried out by a separate introduction of a reference compound that gives a series of peaks in the required mass range (eg. myoglobin). Samples are usually introduced in a methanol/water solution at a typical flow rate of 5 μ l/min. Total sample consumption varies from a few picomoles to a few nanomoles, depending on the molecular weight of the sample.

Applications

1. Beta Lactoglobulins A and B. Figure 1 shows the peaks corresponding to the (M+14H)⁺ ion of beta lactoglobulin. The example shows the clear separation between the A and B variants of molecular weights 18363.3Da and 18277.2Da. Moreover, the resolution of ions containing 0,1 and 2 sodium atoms infers the potential to separate two proteins at this mass differing by only 22Da in molecular weight.

2. Molecular Weight Determination of Isopenicillin N Synthetase (IPNS). IPNS is a 338 amino acid enzyme containing one iron atom. A sample, produced by recombinant DNA technology, was suspected to have the N terminal methionine processed off ie, missing. Three analyses were made on 20 μ l aliquots of a 360 pmole/ μ l solution. Part of the spectrum from the third analysis, showing peaks corresponding to the molecule with between 30 and 47 charges, is shown in Figure 2. The results for the three analyses are summarised in the following table, and show a remarkable reproducibility from analysis to analysis of about 2Da.

Analysis No.	Measured MW	Std. Dev.
1	38364.1	$\pm$ 10.5
2	38364.2	$\pm$ 5.0
3	38362.0	$\pm$ 6.8
Mean	38363.4	
Std. Dev.	$\pm$ 1.2	

The measured molecular weight (38363.4) is consistent with the expected molecular weight of IPNS minus a methionine (38357.8), confirming that the N terminal methionine is indeed missing and the iron atom is present.

3. Bovine Albumin (Molecular Weight = 66kDa). Figure 3 shows a spectrum of bovine albumin taken over a mass range of 1400 to 1720Da. All the peaks show similar fine structure consistent with the samples containing several components or possibly the formation of adducts. Peaks are resolved which differ in mass by about 1 part in 500. Furthermore, mass measurements on the eight lowest mass peaks from 3 different albumins gave very reproducible values as summarised in the following table.

	Measured MW	Std. Dev.
Bovine Albumin	66444.1	$\pm$ 4.2
Porcine Albumin	66873.5	$\pm$ 6.7
Human Albumin	66456.4	$\pm$ 11.6

The measured value for human albumin is 19.5Da (0.03%) higher than the literature molecular weight (66436.9).

Acknowledgement The sample of IPNS was kindly provided by Professor J.E. Baldwin, C.J. Schofield, R.P. Aplin and Y. Fujishima, Dyson Perrins Lab, Oxford University.

FIGURE 1. BETA LACTOGLOBULINS A AND B

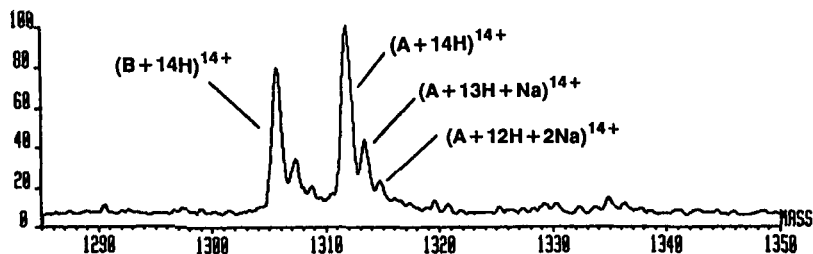


FIGURE 2A. ISOPENICILLIN N SYNTHETASE (IPNS)

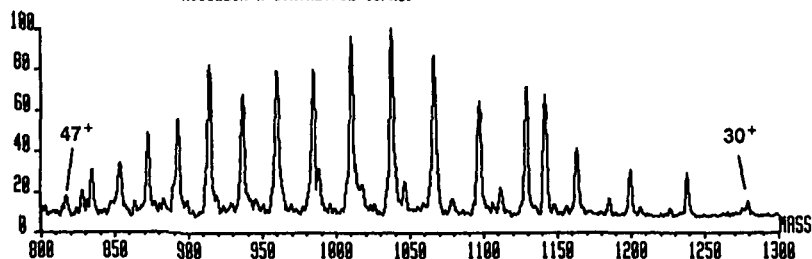


FIGURE 2B. ISOPENICILLIN N SYNTHETASE (IPNS)

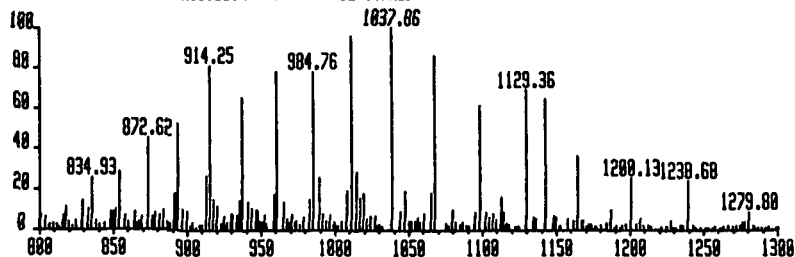
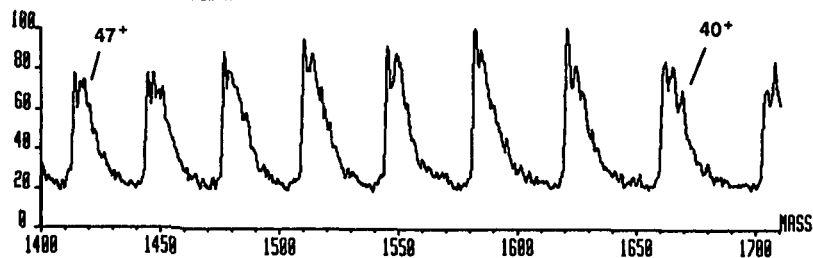


FIGURE 3. BOVINE ALBUMIN



DESIGN AND PERFORMANCE OF A NEW MAGNETIC SECTOR MASS SPECTROMETER WITH DIMIDIATE GEOMETRY

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Traditionally, double focussing sector mass spectrometers are either of forward (EB) or reverse (BE) geometry. Forward geometry instruments are usually characterized by large dispersion/magnification ratios (Dm/m) with relatively short path lengths, resulting in compact instruments with high sensitivity at high resolution. Under normal operating conditions, metastable peaks are present in the mass spectrum, and mass analyzed ion kinetic energy spectroscopy (MIKES) is not available. However, a forward geometry instrument allows linked B/E scans to be performed in order to monitor reactions occurring in the first field free (FF1) region of the mass spectrometer. In linked scans, at constant B/E, artefact peaks originating from the decomposition of higher mass species in FF1 can be observed¹. Reverse geometry instruments can also have large dispersion/magnification ratios but this is usually achieved at the expense of a longer path length resulting in larger instruments. Under normal operating conditions of a reverse geometry mass spectrometer, metastable peaks are rejected from the spectrum. However, a reverse geometry allows mass analyzed ion kinetic energy spectroscopy (MIKES) experiments to be easily conducted and linked B/E scans can also be performed. In both of these scan modes, artefact peaks are observed and they are caused by higher² and lower³ mass species undergoing decomposition in the first and second field free regions (FF1, FF2) respectively.

In order to combine the desirable characteristics of both forward and reverse geometries into a single instrument, a new double focussing magnetic sector mass spectrometer with dimidiolate geometry has been developed. The instrument consists of a magnetic sector ($r_m=381\text{mm}$) and a split electric sector arranged in an E1BE2 configuration as shown in figure 1. This geometry confers to the mass spectrometer the advantages of both conventional geometries and the preponderant character (forward or reverse) of the ion optics can be chosen by using an appropriate combination of asymmetrical dimensions for both parts (E1 and E2) of the split electric sector. The characteristics of forward geometry that can be found in the instrument described in figure 1, are revealed by its relatively small dimensions (width=1.5m) and its high dispersion/magnification ratio ($Dm/m = 819 \text{ mm}$) resulting in a compact geometry with high sensitivity at high resolution. Figure 2 shows a region of the mass spectrum of dibenzosuberone $C_{15}H_{12}O$ and as can be seen, the fragments $^{13}C_1C_{13}H_{11}$ and $C_{14}H_{12}$ are well separated at a mass resolution in excess of 80,000. Advantages related to reverse geometry instruments are also found in the instrument shown on figure 1. Metastable peaks are rejected from the normal mass spectra and (MIKES) experiments can be easily conducted with this dimidiolate geometry by scanning the second electric sector which is located after the collision cell in the third field free region (FF3). As with forward and reverse geometry mass spectrometers, the instrument of dimidiolate geometry offers B/E linked scanning facilities (E1/B, B/E2, E1/B/E2). However, in MIKES as in link scanning experiments most of the artefact peaks that are usually observed on conventional instruments are rejected.

In order to evaluate the ability of the instrument of dimidiolate geometry to eliminate most artefact peaks observed in MIKES or link scanning experiments compared with mass spectrometers of both reverse and forward geometries, several chemical systems known to produce artefact peaks have been studied^{1,2,3}. In the three systems that were chosen, the first has been reported to generate artefact peaks in linked scans at constant B/E conducted on forward geometry spectrometers¹ due to the decomposition of higher mass species in FF1. The second represents a classical example of artefact peaks in MIKES experiments² caused by the decomposition of higher mass species in FF1. The third has been reported to create artefact peak in linked scans at constant B/E on reverse geometry spectrometers³ due to lower mass species decomposing in FF2. The results obtained for the linked scans at constant B/E of the molecular ion of eicosane at m/z 282 on a ZAB-MS of reverse geometry and on an Autospec of dimidiolate geometry are compared on figure 3. The spectrum obtained on the reverse geometry spectrometer (figure 3a) shows the presence of intense artefact peaks at m/z 189 and 178 arising from the transmission of lower mass ions that decompose in FF2 immediately prior to the electric sector. The artefact peak at m/z 189 is due to the transmission by the magnetic sector of m/z 127 which decomposes to form m/z 85 in FF2. The results of the same experiment conducted on the instrument of dimidiolate geometry (E1/B/E2) are shown in figure 3b. As can be observed, the spectrum is free of the artefact peaks since the ions formed in FF3 have energies too low for

transmission by E2. The other systems studied have yielded similar results indicating the ability of instrument of dimidiatic geometry to eliminate artefact peaks from linked scans or MIKES spectra.

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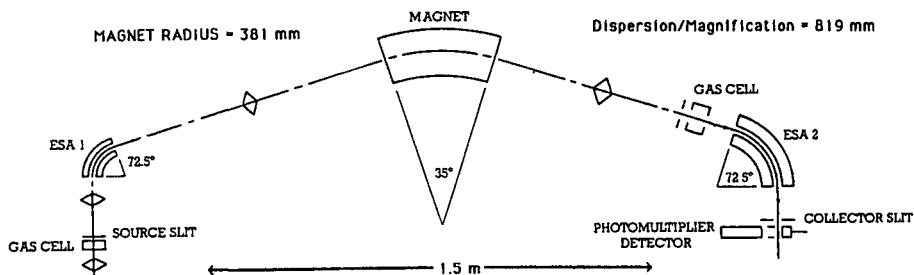


FIGURE 1 MASS SPECTROMETER WITH DIMIDIATIC LUC GEOMETRY

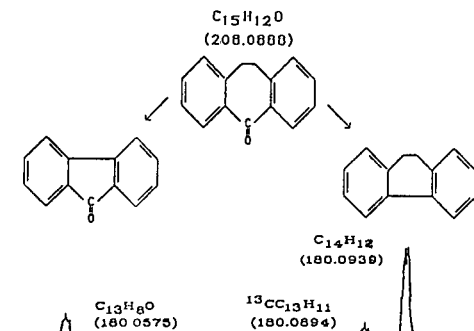


FIGURE 2 $R = M/\Delta M > 80,000$

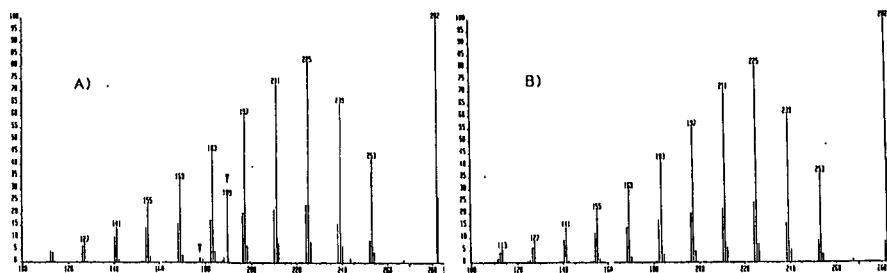


FIGURE 3- LINKED SCAN OF THE MOLECULAR ION OF EICOSANE
A) B/E, VG-ZAB-HS, B) E1/B/E2, VG-AUTOSPEC

A HIGH SENSITIVITY/HIGH RESOLUTION TANDEM MASS SPECTROMETER FOR HIGH MASS BIOMOLECULES

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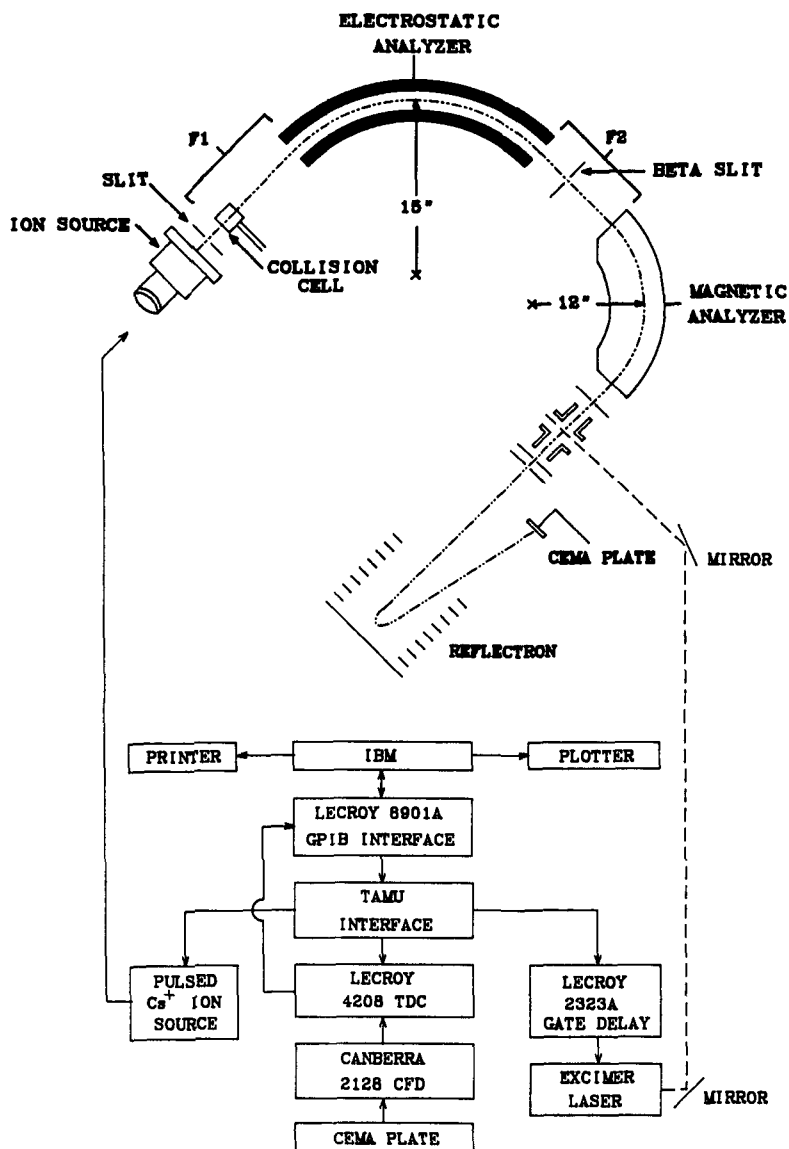
Tandem mass spectrometry is a versatile method for studies of the chemical and physical properties of gas phase ions, and the method is useful for structural characterization of complex organic molecules, *e.g.*, the primary structure of peptides, nucleotides, oligosaccharides, and *etc.* Although there are clear indications that tandem mass spectrometry can be used for the analysis of complex molecules, especially peptides and small proteins, the development of tandem mass spectrometry for structural characterization of biomolecules is hampered by sensitivity and mass resolution of the second mass analyzer. Two factors which limit the sensitivity of MS-II are: (i) independent of the ionization method, the "molecular ion" yield decreases as the sample molecular weight increases, and (ii) the efficiency for dissociating the mass selected ion decreases as the size of the molecule increases. Consequently, improvements in tandem mass spectrometry can be made by increasing the ion source performance and/or by improving the performance of MS-II. One approach to improving the performance of MS-II has been to couple together two high resolution sector instruments.¹ The biggest drawback to this approach is the cost of two high performance sector instruments and the added complexity of tuning of the instruments. Such instruments are designed primarily for collision-induced dissociation to provide the structural information; a process with low efficiency ($1/10^6$ or less for ions above m/z 1000) and the collisional activation process gives rise to energy loss and increases the translational energy spread of the ion beam---both factors degrade the performance of MS-II. I feel other alternatives can be designed which can yield higher performance than the four-sector instruments and the cost of these alternatives are less. To demonstrate the feasibility of the approach I designed a hybrid high resolution (EB) magnetic sector/reflectron time-of-flight (TOF) instrument with capabilities for high sensitivity and high resolution tandem mass spectrometry experiments (see Figure 1). The instrument utilizes pulsed Cs^+ ion liquid SIMS ionization (sensitivity increased by 30-300 over CW liquid SIMS),² laser photodissociation using a pulsed excimer laser system (photodissociation efficiency increased by 10^3 - 10^4 over CW photodissociation), and time-of-flight detection of both the photofragment ion and neutral. In addition, because of the high peak laser power photodissociation can be performed by either single or multiphoton processes.

Although pulsed experiments are not a natural combination with scanning magnetic sector instruments, the first mass analyzer (MS-I) for tandem mass spectrometry is usually operated in the static mode, *i.e.*, MS-I is tuned to mass select the $[\text{M}+\text{H}]^+$ ion of the analyte, and the MS-MS spectrum is obtained by scanning MS-II. Thus, pulsed ionization and pulsed laser photodissociation can be used if MS-II is a time-array detector (TOF) or an ion trap. The EB-TOF instrument is designed around a Kratos MS-50 instrument, thus MS-I is a high sensitivity/high resolution analyzer. The instrument has been modified for pulsed liquid SIMS ionization. The TOF analyzer is a home-built gridless-reflectron system which uses off-the-shelf LeCroy electronics and an IBM-AT computer system. In the MS-MS mode, ions are produced by a short duration Cs^+ ion pulse (4-10 ns), the ions are accelerated and focussed using a high sensitivity SIMS source, and the ions are then mass analyzed by the EB instrument. Prior to photoexcitation, the mass-selected ions are decelerated (from 8 keV to ca. 10 eV to 4 keV; the drift velocity of the ions can be varied continuously over this energy range) and following photodissociation the ions drift through a field-free region (drift times range from ca. 900 ns to several microseconds) and are then re-accelerated to 6-10 keV for TOF mass analysis. Following photodissociation of the mass selected ions, the photofragment ions can be deflected off-axis and the photofragment neutrals can be ionized (multiphoton ionization) by a delayed laser pulse; efficiencies for MPI of the photofragment neutrals approach unity. The TOF mass analyzer has high collection efficiency (approaching unity) and high resolution (upto ca. 10,000). We anticipate that the EB-TOF instrument will be capable of detecting less than 100 femtomole levels of peptides of molecular weights greater than 5000.

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Tandem MS-TOF Instrument



A TANDEM QUADRUPOLE MASS SPECTROMETER OF NOVEL GEOMETRY OPTIMIZED FOR INTELLIGENT DATA ACQUISITION

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Triple quadrupole mass spectrometers have become one of the most widely used instrument types for tandem mass spectrometry (MS-MS) due to their low cost, high level of automation and above all their ease of use. We have designed a tandem quadrupole mass spectrometer of novel geometry optimised for intelligent data acquisition that incorporates (Fig.1) an intermediate ion detection device that is situated off-axis, between MS1 (the first mass filter) and the collision cell. A second detector is fitted in the conventional position, after MS2.

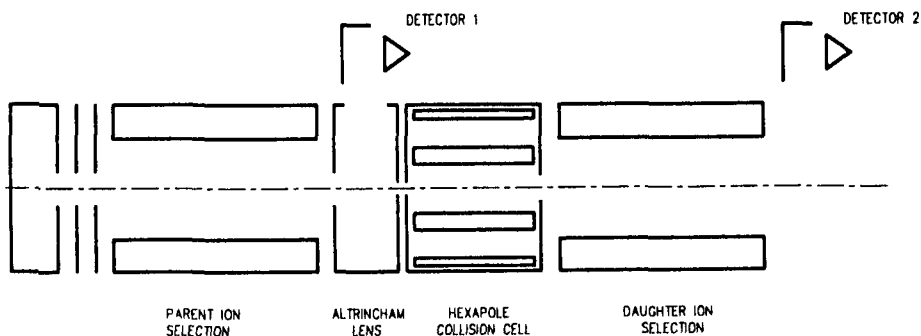


Figure 1. Schematic diagram of the dual detector geometry of the VG QUATTRO tandem quadrupole mass spectrometer.

The intermediate detector permits conventional MS spectra to be recorded whilst maintaining the collision cell conditions at an optimum for MS-MS analyses. Switching (under microprocessor control) between MS and MS-MS scanning is achievable in less than 50 ms and is enabled by electrostatically focussing the ion beam emerging from MS1 either into the intermediate detector (for MS spectra) or into the entrance aperture of the collision cell (for MS-MS spectra).

"Intelligent" or "expert" systems that utilize rule-driven control software to operate tandem instruments provide unprecedented scope for automated analysis. Our dual detector geometry is designed to expedite the implementation of data dependent decisions made by such rule-driven control software.

An overview of the digital architecture is shown (Fig.2). The core of the system is an unbounded local area network (or array) of 32-bit RISC architecture transputers. The operating system within the transputer array (OCCAM) is designed to facilitate high speed (20 MIPS) concurrent processing. The unbounded design of the array structure together with the concurrency of OCCAM allows the possibility of expanding the array to provide enhanced processing power.

The transputer array performs all aspects of real-time instrument control. Specific transputers within the array may be used to carry out targeted tasks for example:-

- "Expert" system control.
- Fast library search (from RAM memory)
- Intelligent peripheral device control
- High speed communications (file conversion)

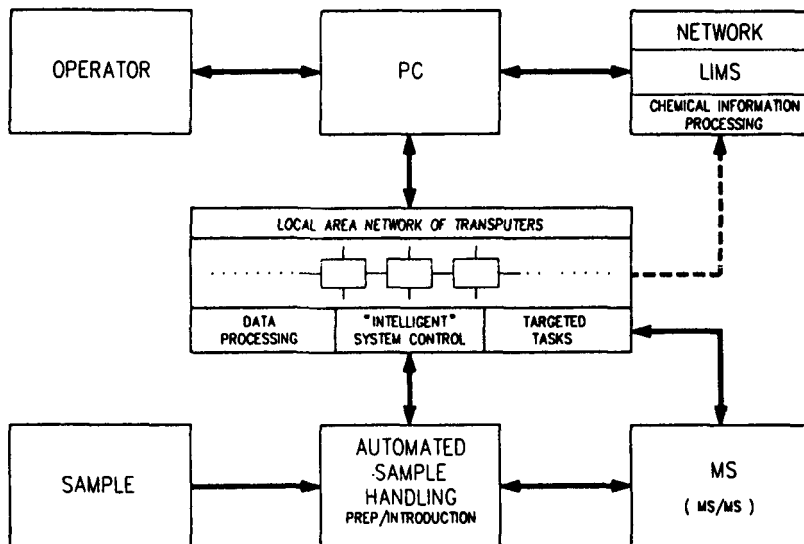


Figure 2. Schematic overview of the digital architecture of the VG QUATTRO tandem quadrupole mass spectrometer.

An "expert" system running within a high speed concurrent processing architecture is capable of making sophisticated data dependent decisions in real-time. Our dual detector geometry enables decisions of this type (involving complex changes in scan functions MS/MS-MS) to be implemented within 50ms. Thus both optimum MS and MS-MS data can be obtained from even narrow chromatographic peaks as they elute in real-time. A consequent saving in analysis time is observed compared to less responsive systems that require multiple chromatographic runs to acquire the same rich diversity of data.

Acknowledgement

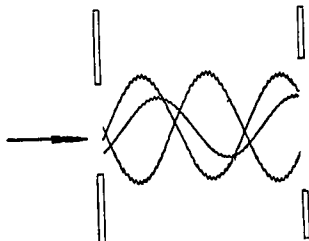
We are indebted to D.A. Dahl and J.E. Delmore of the Idaho National Engineering Laboratory for providing the copy of SIMION (version 4.0) used in this study.

ION TRANSMISSION CONSIDERATIONS IN TANDEM QUADRUPOLE MS-MS

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The quadrupole filter has been established as a means of containing ions, and it was a logical step to use a small quadrupole as the collision cell for MS-MS applications.

Figure 1.



The daughter spectra obtained albeit reproducible, are very dependent on collision cell conditions. A study of the ion trajectories gives a clear explanation of the transmission characteristics which are most apparent to an operator. A transmission characteristic has been generated from the model and shows very good agreement with the observed characteristic.

Figure 2.

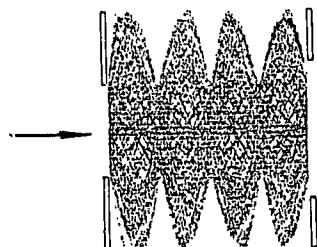
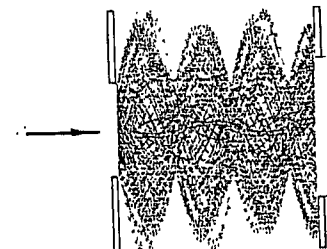


Figure 1 shows three ions traversing the quadrupole in RF only mode. Small excursions from the path at the applied frequency can be seen, but more importantly, there are longer cyclic excursions from the axis. These excursions are of similar length for all ion starting conditions, and therefore nodes and antinodes are formed along the path. This is seen in Figure 2, where one hundred ions have been injected with different starting conditions. The maximum excursions in the RF cycle are plotted as points.

Figure 3.



The trajectories in the quadrupole are stable, but the entrance and exit slits are restricted in order to maintain a higher pressure in the gas cell than in the analyser enclosure by a factor of at least 100. If therefore a maximum excursion occurs at the exit slit, some ions will not be transmitted, as illustrated in Figure 3. Thus at the higher collision energy in the condition of Figure 3 than those in Figure 4, there will be a drop in transmission observed in the parent ion between those two points.

This is exactly what is observed in practice. The figures 4 and 5 show the transmission of mass 502 over two different ranges of collision energy. The continuous curve is derived from the model, and the points from observations. The point marked A corresponds to Figure 1 and points B to Figure 2.

Figure 4. (Quadrupole)

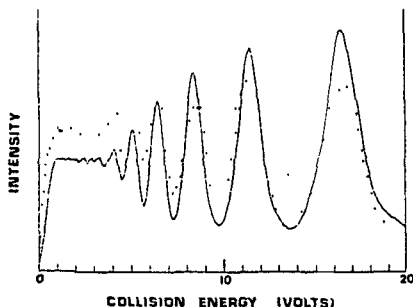


Figure 5. (Quadrupole)

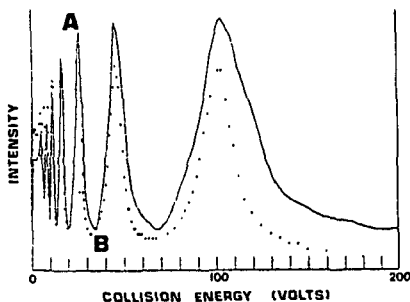
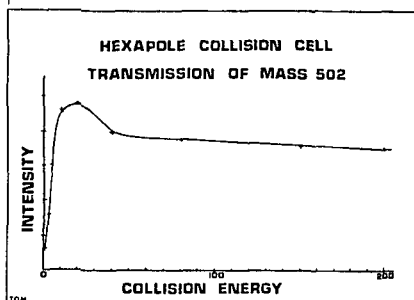


Figure 6.

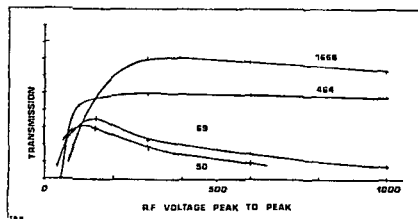


It is only a parent ion which shows this cyclic pattern, because of the defined entrance image. Daughter ions will have the same kind of trajectories, but ions start at collision, and the cyclic pattern will be smoothed.

The hexapole in RF only mode also will contain ions. It does not have the same focussing characteristics as the quadrupole and does not show the nodes and antinodes characteristic of the quadrupole.

The hexapole therefore shows a smooth characteristic with collision energy. This makes the setting of collision cell conditions much simpler since collision energy can be increased without an oscillating daughter parent ratio occurring. The equivalent characteristic is shown in Figure 6.

Figure 7.



Another characteristic of interest is the response with RF amplitude. The transmission with RF is mass dependent, and therefore the RF amplitude has to be varied with mass to obtain the best transmission. This is shown in Figure 7. It can be seen that more than a factor of 10 in mass between parent and daughter can be accommodated by scanning the RF amplitude with mass.

In conclusion, it is clear that the hexapole has characteristics which are less complex than the quadrupole, and therefore reproducible results can be achieved more readily.

A PENTAQUADRUPOLE MASS SPECTROMETER: INSTRUMENTATION,
SYSTEM CONTROL, AND APPLICATIONS TO MS/MS/MS SCANS

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The high transmission, reaction efficiency, and scan speeds, as well as facile computer control, make a pentaquadrupole mass spectrometer^{1,2} ideally suited for implementing new and established MS/MS/MS³ scan types and for demonstrating their applications. A pentaquadrupole instrument (QqQqQ) which utilizes three mass-analyzing quadrupole mass filters and two rf-only quadrupole collision cells has been constructed and is shown in Figure 1. A simple and efficient data system was designed and assembled consisting of a Keithley series 500 measurement and control system, Zenith Z-200 PC, and software written in "C" using the Turbo C programming environment. This inexpensive data system has very good characteristics including 16 bit DAC, 14 bit ADCs, and overall average acquisition speed (while scanning) in excess of 20 kHz.

The entire set of known MS/MS/MS scan modes⁴ can be readily implemented using this system and it has allowed new scans including ones which produce multidimensional data, to be developed. Figure 2 shows an example of a new scan called the selective neutral loss scan. This example utilizes n-decane and scans for all ions which lose (or gain) a particular neutral mass between the first (Q1) and last (Q5) stages of analysis, but which also do so via a particular intermediate ion selected by Q3. The selective neutral loss scan is a highly specific scan combining compound class recognition and particular fragment ion specification. As such, the applications of this scan are limited, but its existence is quite significant as this scan completes the set of the 2ⁿ zero and one-dimensional MS/MS/MS scan types which are shown in Figure 3.

Scans which are two dimensional in mass, i.e. require two mass axes for the display of the data, also exist in the complete set of MS/MS/MS scan types. Just as conventional MS/MS scans such as parent, daughter, and neutral loss scans are subsets of the entire MS/MS data domain, these two-dimensional spectra are subsets of the entire MS/MS/MS data domain. An example of a two-dimensional scan, the familial scan, is shown in Figure 4 for n-decane. This scan completely characterizes intermediate fragment ions of a particular mass for a pure compound or a mixture of compounds by scanning all parent ions and subsequent daughter ions for the chosen intermediate mass. Potential applications of this scan include isomeric and isobaric ion separation with simultaneous identification.

MS/MS/MS has been applied to study ion chemistry important in polyisocyanate characterization by mass spectrometry. Figure 5 shows the application of the reaction intermediate scan to help characterize polymers of n-hexyl-isocyanate. Upon pyrolysis and subsequent electron impact, this compound produces an abundant protonated trimer at m/z 382. This ion fragments by successive alkene losses which can be specifically observed by scanning for all intermediates between the protonated trimer and the completely dealkylated odd-electron daughter ion at m/z 130.

Various MS/MS/MS scans can be used to simplify sequencing of peptides by mass spectrometry. The QqQqQ has been extensively used for these studies⁵ and one example is shown in Figure 6. The reaction intermediate scan is utilized to exclusively observe only certain types of fragment ions, in this case for a particular pentapeptide with molecular weight 579 in a mixture of peptides. By setting Q1 on the M+H⁺ ion and setting Q5 on the fragment ion at m/z 173, a simple spectrum results which readily yields the sequence of the peptide.

Both collision activated dissociation or ion/molecule reactions in either or both collision regions can be utilized in a pentaquadrupole¹. MS/MS/MS under these conditions produces interesting results. When using 1,3-butadiene as both the sample and the neutral reagent gas in both reaction regions, MS/MS/MS scans produce data on gas-phase Diels-Alder and retro-Diels-Alder reactions and related chemistry. Figure 7 shows sequential daughter and reaction intermediate scans for this chemical system demonstrating the various processes which take place.

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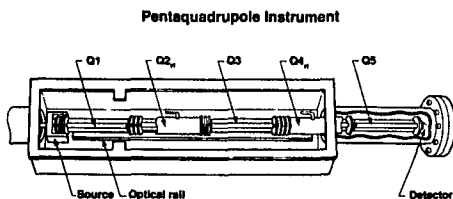


Figure 1

**ALL MS/MS/MS SCANS HAVING
ZERO OR ONE MASS DIMENSION**

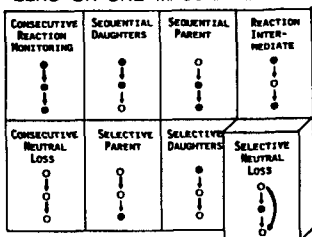


Figure 3

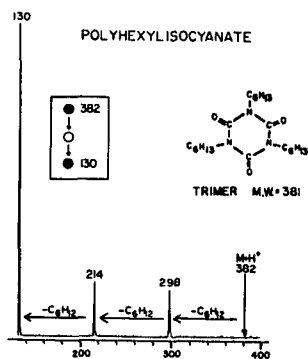
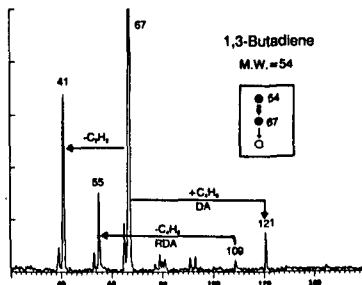


Figure 5



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Figure 7

SELECTIVE NEUTRAL LOSS SCAN

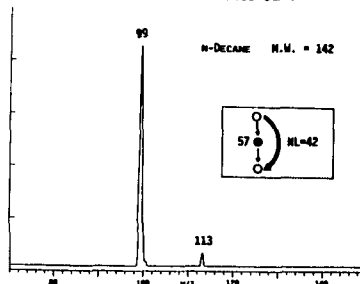


Figure 2

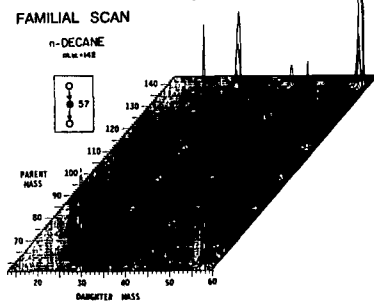


Figure 4

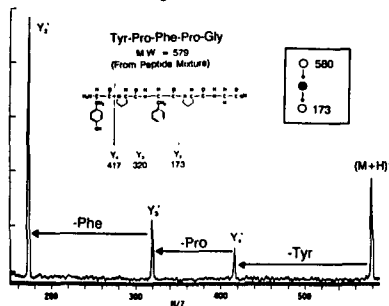
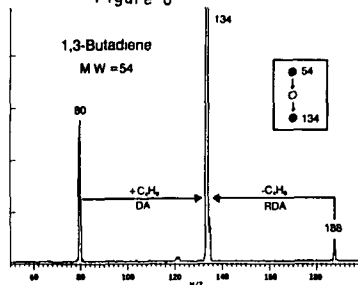


Figure 6



b

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

HIGH RESOLUTION DAUGHTER ION ANALYSIS APPLICATIONS IN HYBRID MASS SPECTROMETRY

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While many different geometries of instruments have been used for MS/MS, most have a limited daughter ion mass resolution. Of the beam type instruments only four sector, some three sector, and selected hybrid instruments have the capability of high daughter ion resolution. Among hybrid instruments, only the QEB instrument in our laboratory has the ability to perform high resolution daughter ion mass measurements. One of the features of hybrid instruments is the possibility of doing MS/MS experiments in which the daughter ions are formed from parent ions with low translational energies (1-100 eV) or high translational energies (3-10 keV). The QEB is thus the only MS/MS instrument in which both collision energy regimes can readily be accessed. This paper compares the sensitivity/resolution of the two operating modes on the QEB and also demonstrates other experiments that take advantage of the high daughter ion resolution capabilities of the instrument.

The QEB has been described in detail previously.¹ Briefly, the Q portion of the instrument is a UTI 100C quadrupole mass filter. The EB portion of the instrument is an AEI MS50. Ions exiting the quadrupole are focussed into a collision cell (reaction region 1, RR1) where low energy collision-induced dissociation (CID) can be performed. Ions exiting the collision cell (daughter or parent) are subsequently accelerated into the MS50. High energy CID can be performed in a collision region (RR2) at the source slit of the MS50. Data were acquired using a Nicolet 4094 digital oscilloscope.

Of particular interest in this study was the relative sensitivity/resolution that could be obtained via high energy CID in RR2 versus low energy CID in RR1. Figure 1 shows the m/z 43 region in the daughter ion spectra of the molecular ion of 3-methyl-2-butanone obtained using 9 keV CID (top) and 55 eV CID bottom. The two peaks are due to isobaric daughter ions $C_3H_7^+$ and $C_2H_3O^+$. The high energy CID spectrum was the best obtainable after considerable effort was made to maximize the intensity of the signal. The low energy CID spectrum is readily achievable and with some effort the resolution of this spectrum can be increased by a factor of two to three. Thus, in this particular instrument we find that the low energy CID provides up to an order of magnitude better sensitivity/resolution (i.e. at a given signal level the resolution is up to ten times better for low energy CID).

The ability to select daughter ions at high resolution allows the QEB instrument to perform high resolution parent ion scans. An example of this, using the same compound as above, is shown in Figure 2. In the upper part of this Figure the $C_2H_3O^+$ m/z 43 ion was selected and it was observed that the parent ions of this were the molecular ion, the protonated molecule (formed by self protonation in the ion source) and an ion at m/z 129, which is assumed to be an ion/molecule reaction product formed in the ion source. When the $C_3H_7^+$ m/z is selected as the daughter ion, the parent ions observed are m/z 71 ($M-15$)⁺ along with the molecular ion and protonated molecule.

A unique variant on the high resolution parent ion scan is the targeted daughter ion mode of operation². In this experiment, instead of scanning the quadrupole it is set in the rf-only mode to pass ions above a desired cut-off mass. By having the quadrupole in the rf-only mode, the transmission of parent ions can be increased by up to and order of magnitude or more providing increased sensitivity, albeit with reduced specificity. To demonstrate this mode of operation the magnet was scanned over the m/z 43 region at rf cut off levels of 70 and 100 amu. The results are shown in Figure 3. At the lower cut-off level, both ions at m/z 43 are observed, as expected since the quadrupole is passing both the molecular ion and protonated molecule. At the higher cut-off level only the $C_2H_3O^+$ ion is observed as the quadrupole is not passing the molecular ion or protonated molecule. The signal is due to the parent ion at m/z 129 and the MS/MS spectrum of this ion is shown on the bottom of this Figure, confirming that it only fragments to the $C_2H_3O^+$ ion.

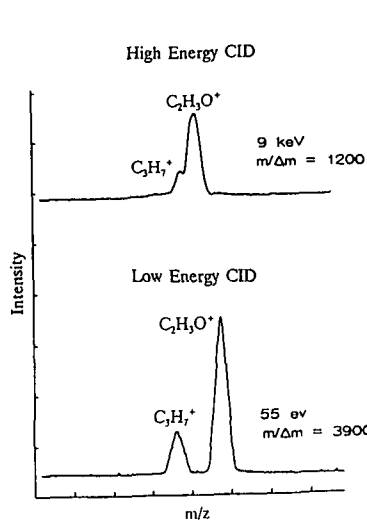


Figure 1

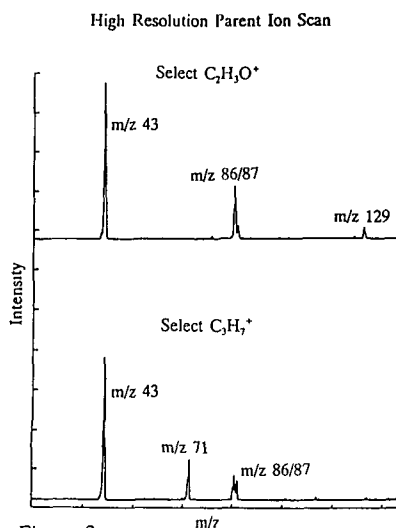


Figure 2

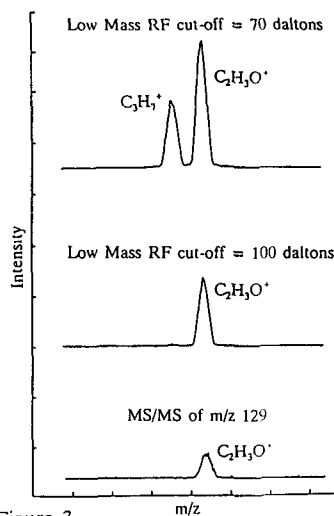


Figure 3

1. G.L. Glish, S.A. McLuckey, E.H. McBay, L.K. Bertram, *Int. J. Mass Spectrom. Ion Processes*, **70**, 321 (1986).
2. S.A. McLuckey, G.L. Glish, B.C. Grant, *Anal. Chem.*, submitted.

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RESIDUAL GAS ANALYZERS AS INEXPENSIVE SOURCES OF MASS-SELECTED ION BEAMS

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The ease with which gas phase ionic species can be manipulated continues to stimulate dynamic and spectroscopic studies of charged molecules and atoms. Frequently, a beam of mass-selected ions is required. If the ionic species desired is simple, e.g., Ar^+ , an ion beam can easily be generated - using an electron ionization (EI) or discharge source, for example. However, if polyatomic ions are required, a mass-selection step will probably be required. Thus, "ion beam" experiments frequently become cost-prohibitive, since a mass spectrometer is required. In our present work, an attempt is being made to "freeze" mass-selected ions into an argon matrix at 10 Kelvins for subsequent spectroscopic analysis. This experiment requires two mass spectrometers, since a mass selected beam of counter ions (anions) must also be directed towards the 10 Kelvin target. The decision was made to evaluate residual gas analyzers (RGA's) as the basis for mass-selected ion sources. An RGA is commonly a small quadrupole mass filter, with an open EI ion source, that is mounted on a small flange. It is usually a component of a vacuum system that analyzes background gases. Ions are formed, mass analyzed, and directed towards a collector on the flange.

Our design goals were to 1) redesign the ion source into a closed configuration, which would allow for a substantial pressure differential between the source and the rest of the experiment, 2) turn the assembly around, to produce a directed ion beam without degradation of resolution, and 3) add focusing and deflection optics for conditioning and directing the beam. We have successfully completed these design goals with two RGA's, one of which has a mass range of 1-200 Daltons.

Two tools have been very important in the successful implementation of this work. The first is the ion optics modeling program SIMION (1), which was used to evaluate the effects of source modifications, and for the design of lenses and deflection assemblies after mass-selection. The second is a channel electron multiplier (CEMA) with a phosphor screen, used as a "beam visualizer". This was used for measuring beam divergence out of the quadrupole, which is necessary information for proper modeling, and for performance evaluation of the system when constructed. The CEMA also provided useful insights into how the shape of the "beam" out of the quadrupole varied with m/z .

Figure 1 shows the completed mass-selected ion beam source that was constructed from a Dycor M200 residual gas analyzer. The quadrupole and EI source are the original Dycor components. The device was mounted on a Conflat flange. The ion source was enclosed in a "gas tight" housing, such that high pressures could be maintained within the source (which is required for high ion fluxes) with minimal increase in pressure in the vacuum system. The figure shows a port for gas introduction directly into the ion source. Gas flow is controlled by an external leak valve. Source voltages are introduced through the top flange, while other voltages are introduced through two additional multi-pin feedthroughs. The focusing and deflection optics that were designed using SIMION are mounted on the end of the mass filter. The original Dycor electronics were used to operate the instrument, with minimal retuning of the quadrupole circuits required due to wiring modifications. The design modifications have been met, and mass-selected ion beams (m/z 1 to 200) can be formed from this device, without degradation of the resolution of the original RGA.

1. Developed by D.A. Dahl and J.E. Delmore at Idaho National Engineering Laboratories.

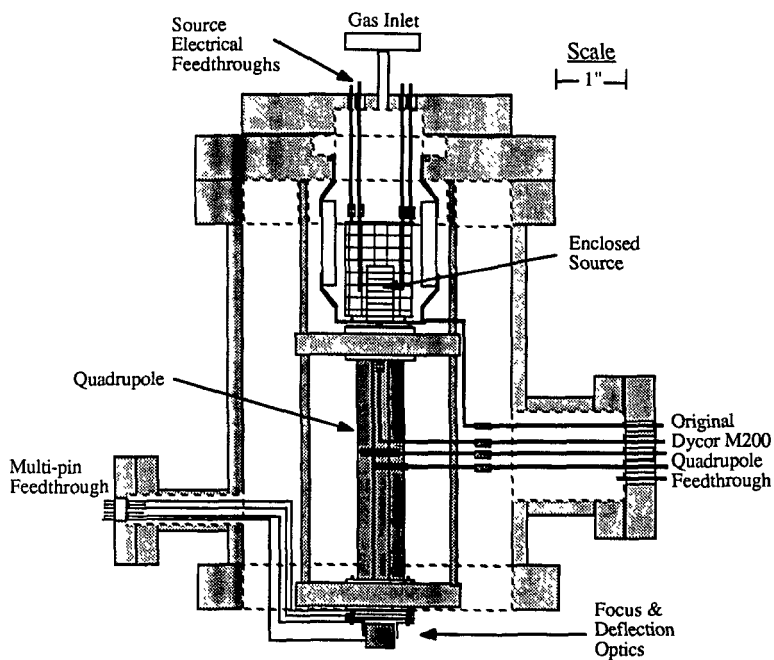


Figure 1. Completed mass-selected ion beam source

NEW B-Q TANDEM MASS SPECTROMETER

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Laboratory work centering around the exact isotopic analysis of uranium, thorium, lead and certain recent applications in geological age dating (U/Th disequilibrium) require the measurement of a minor isotope in the presence of an adjacent major isotope. However, the ratios of interest between 1: 50,000 to 1: 400,000 challenge the capabilities of state-of-the-art magnetic sector mass spectrometers due to the fraction of 1-2 ppm of scattered ions of the major isotope falsifying the measured intensity of the minor isotopes. The common technique for the elimination of scattered undesired ions is the improvement of the abundance sensitivity by employing a second magnetic sector field followed by an electric sector in a so called three-stage (BBE) instrument.

We have designed a new type of a three-stage equivalent isotope ratio mass spectrometer. The system is constructed as an addition to the MAT 262 mass spectrometer equipped with a multicollector array and an ion counter. A quadrupole mass filter is employed as a second stage to eliminate the elastically scattered undesired ions of incorrect mass. Ions of the major isotope travel from the ion source through the magnetic sector field into a Faraday-cup of the multicollector (see Fig.1).

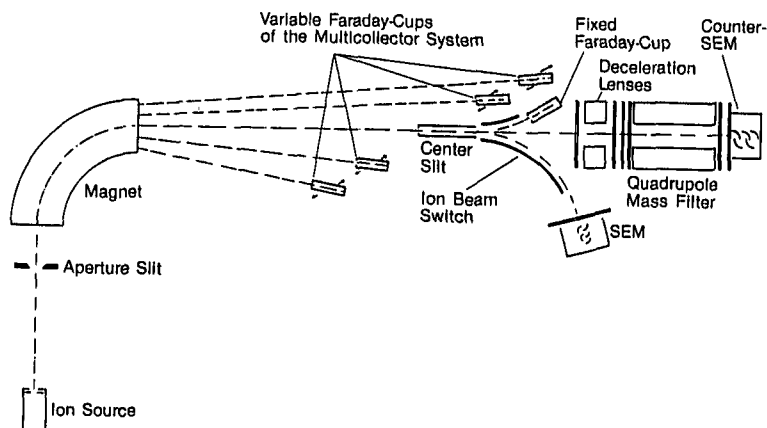


Fig. 1

Ions of the minor isotope pass through the center slit of the multicollector. They enter the decelerating lens system to be focussed into the mass filter quadrupole, which is floated to nearly ion source potential (10 kV). These ions are detected behind the mass filter quadrupole in an ion counting system.

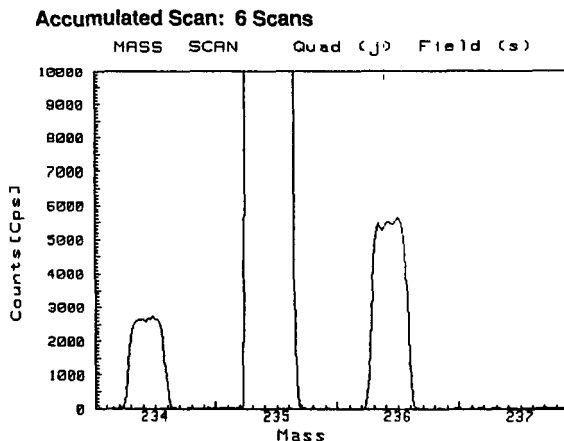


Fig. 2

The extraordinary abundance sensitivity is demonstrated in a mass scan using the standard U005. The U236/U238 ratio of this standard is 0.0000468. Hence, there is a minor isotope to be measured in the close neighbourhood of a major isotope. The magnet and the quadrupole filter is repeatedly scanned from mass 233.6 to mass 237.4 in an accumulated scan. Obviously, the long peaktail produced by the scattered ions of mass 238 disappeared (see Fig. 2).

The abundance sensitivity was measured with a stable ion beam of U005 by tuning the magnet and the quadrupole filter to mass 237. During the integration time of 600 seconds, 503 events were detected at the counter. When the system was tuned to mass 236 more than 807,000 ions were counted. (Abundance sensitivity: 0.03 ppm) With a different set-up of the ion counter, tested on an other mass spectrometer, the dark count noise will be reduced to 90 events during 600 seconds. (Abundance sensitivity : 0.01 ppm).

Performance Optimization of a Kratos MS80RFAQ Mass Spectrometer for High Mass Operation
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The role of mass spectrometry in pharmaceutical research has until recently been concerned with the analysis of small molecules, with molecular weights less than 1,500 daltons. The majority of mass spectrometers purchased during the early to mid 1980's were ideally suited for this type of analysis, generally possessing a mass range of 1,500 - 2,000 daltons, fast scanning magnets for GCMS, and equipped with EI, CI, and an early version of FAB ionization. Sensitivity for ions above 2,000 daltons was not an overriding concern.

Mass spectrometers designed specifically for high mass analysis with optimized FAB or liquid SIMS ion sources, high mass magnets, and array detection are of course now available. An alternative to the high cost of the purchase of a new system is the optimization of an existing instrument. We wish to report such an optimization of a 6 year old MS80RFAQ hybrid mass spectrometer. Four goals were set in order to meet our objective:

1. Improve MS80 FAB sensitivity
2. Increase intermediate detector sensitivity for MS1 detection of ions > 2,000 daltons
3. Increase final detector sensitivity for MS2 detection of daughter ions
4. Maintain daughter ion resolution and peak shape in MS2 at high collision energy (> 100eV)

I. FAB Source Optimization

The original MS80 source configuration incorporated a standard Ion-Tech FAB11NF ion gun mounted on the top of the source housing. The exit of the ion gun measured 10 cm. from the sample probe (this distance is the maximum limit specified by Ion-Tech). This gun was removed and replaced by a standard Kratos insertion lock. A Phrasor Scientific Capillatron ion gun mounted in a direct insertion probe (DIP-gun) was purchased and introduced through this insertion lock. The sample tip normally mounted on this probe was removed. The distance between the exit of the Capillatron gun and the sample can be varied within the range of 2.5 - 10 cm. by inserting or retracting the Capillatron probe. The gun controller allows operation up to 10KV with independent control of emission current between 10 - 100 μ A.

The standard MS80 FAB source is essentially an open block held at accelerating potential - no extraction lenses are used. This source was replaced by an MS50/890 FAB source which contains additional ion extraction optics. Sample introduction is accomplished through an insertion lock mounted co-axially with the ion exit.

II. Intermediate Detector

The fixed output 8KV power supply for the post acceleration detector (PAD) was replaced by a variable 0 - 30 KV Spellman high voltage DC supply. The existing high voltage feedthroughs were not modified; continuous stable operation is possible with up to 18 KV applied to the PAD electrode. Fig.1 & 2 demonstrate the observation of MH⁺ ions for bovine insulin and the oxidized β -chain of insulin. These observations were not possible prior to these modifications. The total gain in sensitivity at high mass (m/z 3510) realized through these modifications is approx. 100 fold (table 1).

III. Final Detector

Installation of the 30 KV variable output high voltage supply in place of the standard 8 KV supply at the final MS 2 detector had a measurable effect on daughter ion sensitivity. Fig. 3 illustrates the 4 - 5 fold improvement in the intensity of daughter ions obtained in the 150 eV CAD spectrum of m/z 1172, $(Cs(CsI)_4)^+$. Additionally, an improvement in daughter ion peak shape was observed, particularly at higher collision energy (> 100 eV). This may be attributable to a more efficient collection of daughter ions with high axial kinetic energy or the higher PAD voltage may correct for minor misalignment of the PAD dynode.

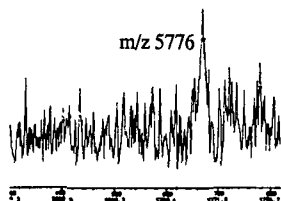


Fig. 1- MH^+ , Bovine Insulin

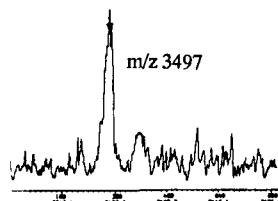


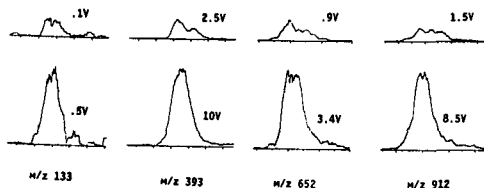
Fig. 2- MH^+ , Oxidized β -chain, Bovine Insulin

TABLE 1. SENSITIVITY ENHANCEMENTS - MS80RFAQ

INSTRUMENT PARAMETERS	OBSERVED ION ABUNDANCE	
	m/z 912	m/z 3510
original MS80RFAQ*	1.0 V	.02 V
10 KV FAB gun	2.0 V	.04 V
18 KV PAD voltage	2.5 V	0.1 V
3 cm. gun - sample dist.	14.0 V	0.25 V
optimized MS80RFAQ**	70.0 V	2.1 V

* 8KV FAB gun voltage, 7.5KV PAD voltage, 10.0 cm gun - sample dist.
** 10KV FAB gun voltage, 18KV PAD voltage, 3.0 cm gun - sample dist

150eV DAUGHTERS OF m/z 1172, CsI
7KV PAD (TOP) VS. 12KV (BOTTOM)



An Electrostatic Quadrupole Deflector for Mass Spectrometer Applications

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INTRODUCTION

Certain mass spectrometer applications may require the interface of two or three ion sources to a common mass filter. In SIMS (secondary ion mass spectrometer) or FAB (fast atom bombardment) experiments, for example, an additional electron impact ionization source may be used to measure residual gas composition.

Electrostatic ion selection from one of two or three sources can be made with the use of a 2-dimensional quadrupole deflector.^{1,2} The energy dispersive properties of this device will be of additional benefit for high mass resolution quadrupole SIMS applications where it is necessary to tailor the emittance of a source to the acceptance of the mass filter.

A quadrupole deflector has been designed for use in a mass spectrometer under development for a NASA mission. The instrument includes two gas phase electron impact ionization sources as well as a SIMS source.³ The quadrupole deflector allows a sequential introduction of ions from each source to the mass filter.

Ion trajectory calculations for various entrance aperture geometries allow fringe field effects to be studied. The ion transmission characteristics of the device developed were also experimentally studied over a 100 eV range of ion energies.

COMPUTATIONAL METHOD

Several configurations of the quadrupole deflector were studied using the electrostatic lens analysis software SIMION developed by D.A. Dahl.⁴ A 120x120 grid density was used.

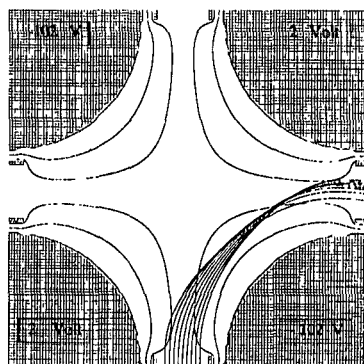
In the calculation the selected symmetry is planar, and the entrance apertures are modeled as slits parallel to the deflector rod axis. In our experimental implementation, however, these apertures are circular.

The quadrupole assembly chosen to approximate the hyperbolic field consists of 90 degree circular rod sectors where $R_{cyl} = 1.16 R_0$. R_{cyl} is the rod radius and R_0 is the distance from the center of the quadrupole assembly to each rod.

FRINGE FIELD EFFECTS

The trajectories obtained by ray tracing with no entrance and exit electrodes were found to be very close to the trajectories described analytically by Zeman¹. Addition of simple entrance and exit aperture electrodes, however, creates fringe fields which considerably change the characteristics of the quadrupole deflector. In particular, the potential difference between adjacent rods required for 90° deflection is now considerably higher.

The figure below shows trajectories in a configuration where the electrodes associated with the entrance



and exit apertures extend approximately 0.7 mm into the 2.43 x 2.43 cm box defined by the centers of the four deflector rods. The potential on the aperture electrodes is set midway between the two adjacent rod electrodes and the initial ion energy is 50 eV.

There is an optimal extension of the entrance and exit electrodes into the deflector assembly. As these electrodes are extended into the assembly, the average beam deflection angle approaches and then becomes greater than 90°.

EXPERIMENTAL TESTS OF THE DEFLECTOR ASSEMBLY

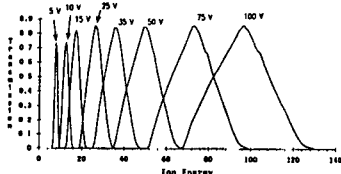
A deflector assembly was fabricated for experimental evaluation. The distance between the rod centers was 2.43 cm in this device and the circular entrance and exit aperture diameters were 0.3 cm.

The transmission of the deflector assembly was measured using a Cesium thermionic emitter mounted several cm from the entrance aperture, and ion collector plates mounted at each exit aperture. Ion transmission could readily be determined by measuring the total current entering the device with the rod voltages set to zero, then setting appropriate deflection voltages and measuring the current on one of the 90° collector plates.

All voltages were controlled by microcomputer and could be repeatedly set to better than 0.1 V. In the experiments described below, the deflection voltages were symmetric around zero, with equal voltages on opposite plates. The entrance and exit apertures were kept at zero volts for convenience in measuring ion currents.

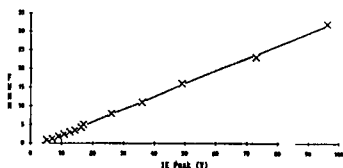
DEFLECTOR TRANSMISSION CHARACTERISTICS

The characteristics of the fabricated device are demonstrated below. The family of transmission curves are obtained by setting the rod voltages and then



ramping the ion energy.

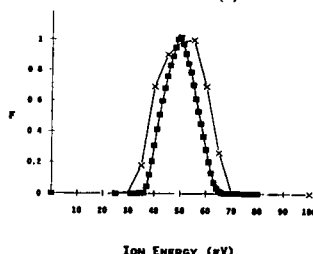
Also shown below is the variation in the peak width (FWHM) with the energy of the peak maximum.



COMPARISON OF CALCULATED AND MEASURED TRANSMISSION

It is apparent from the trajectory studies that some of the ions may enter the final lens at an extreme angle. These ions are not expected to cause anomalous behavior in the experimental transmission, as the length of the electrode selected for the exit aperture will result in the neutralization of most of these extreme angle ions.

In fact, for the geometry chosen, the transmission variation with ion energy can be calculated and compared with experiment. The results of this ray tracing for a circular entrance area uniformly filled with ions is shown below. The vertical axis (F) is the transmis-



sion divided by the maximum transmission with a fixed 100 V between adjacent rods. The experimental results (solid square points) are compared to the calculation (x symbols). The approximations involved in modeling the cylindrical entrance and exit apertures in 2 dimensions is presumed to account for much of the difference from experiment.

SUMMARY

An electrostatic quadrupole deflector has been developed and shows promise for use in interfacing multiple mass spectrometer ion sources. The presence of fringe fields associated with the entrance and exit apertures, causes the device characteristics to deviate considerably from those of an ideal hyperbolic deflector.

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DESIGN OF A FOCUSED CESIUM ION SOURCE FOR CONTINUOUS FLOW LIQUID SIMS

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Continuous flow liquid SIMS (CF/L-SIMS) has offered a method which requires minimal sample preparation and chemical derivatization for quantitative analyses of polar compounds (1). However, these advantages are currently obscured by the rapid consumption of each cesium charge (100 hrs operation), and a higher detection limit than anticipated from static L-SIMS. For the above reasons, a focusing lens system (2) has been designed and built using the existing unfocused cesium ion source (Phrasor Scientific, Inc.) on a Finnigan Mat TSQ 70. The advantages of using a defined focused ion beam for L-SIMS studies have been reported by Stoll et al. (3). The advantages obtainable when such a focused beam is applied to CF/L-SIMS system where the surface of the sample is continuously refreshed is an other objective of this study.

Our goal was to produce a lens system which will project the ions generated from any position of a cesium source surface with any angular deviations from the mean ion axis onto a small (less than 1 mm diameter area) final image where the sample is located. Additionally, to efficiently project the ions, the design should confine the ion paths through the lens system to a space which is close to the mean ion axis. The simulation processes to define ion focusing conditions were performed using the MacSimion[®] computer program (4). The lens system design was evaluated by simulating the ion trajectories of cesium ions with kinetic energy of 0.1 eV emitted from the center of the source surface -60°, 0° and 60° from the mean ion axis and emitted from the extrema of the source surface -60° and 60° from the mean ion axis. It was found that a lens system consisting of an accelerating lens and an Einzel lens (Fig. 1) is able to focus the beam to an area with diameter less than 0.8 mm at the probe tip. The image was approximately the same for these two sets of initial conditions. This image would be observed for any conditions between these limits when the same focusing environments are applied. As shown in Fig. 1, the accelerating lens consists of the cesium pellet assembly, an extraction, a first focusing, and a first grounded element. The Einzel lens consists of the first grounded, a second focusing, and a second grounded element. Also given in Fig. 1 are the dimensions of these elements and an example of their corresponding voltages which were found to produce a defined focused beam at the sample location.

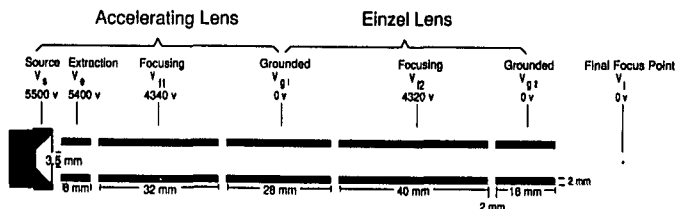


Figure 1 Dimensions and voltages for each cylindrically symmetrical lens element in the focus lens system.

It is important to note that as a result of hardware requirements (e.g. high voltage feedthroughs, steering mechanism, etc.), the cesium ion source has been positioned further from the probe tip target (15.7 cm from the target as compared with 6.8 cm from the target with original cesium ion gun). However, when there exists a long distance between the ion source and the final focus point or the target, it is impossible to achieve focus without having a large image. In this design, an Einzel lens has been added to circumvent that phenomenon such that the ion beam can be projected onto the target without producing an image enlargement. The object image at the first crossover was projected onto the final image plane. One can make the size of the image bigger by adjusting the voltages: V_e , V_{f1} , and V_{f2} ; however, the minimum image size is determined by the size of the source aperture.

As presented in Fig. 2 these lens elements were mounted on a stainless steel cylinder, which is attached to an extended flange by Vespel[®] spacers. The final grounded element was 2 mm away from the source block. The beam steering capability was provided mechanically through a Huntington Laboratories, Inc. 10° Acu-Port (model VF-175-10). The 4 to 6 KeV accelerating voltage was provided from the original Phrasor 10 keV PID power supply; other lens element voltages were obtained by directing this source voltage through a voltage divider with three adjustable voltage outputs.

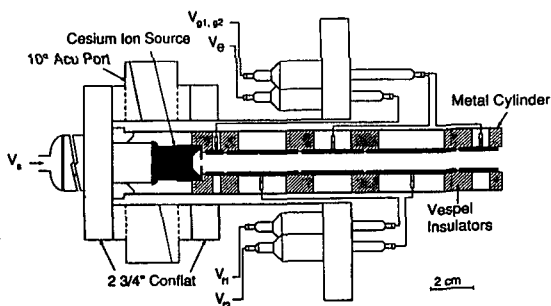


Figure 2. Mechanical assembly cross-section of the cesium gun and focusing lens system.

The characteristics of the focusing lens system was first studied by removing the source block and placing a NaI doped filter paper or tantalum pentoxide refractory film at the target plane. The size of the focused beam could then be optimized by adjusting V_e , V_{f1} and V_{f2} while looking at the yellow ion beam image as ions irradiated the NaI doped filter paper through the glass view window of TSQ 70. At optimal conditions, the voltages applied were in good agreement with theoretical predictions. A permanent image was obtained when tantalum pentoxide refractory film was used instead of NaI. The image produced when the unfocused gun at its original position is compared with the image from the same ion gun equipped with the lens system and displaced 8.9 cm in. A 20 to 100 fold reduction in image area size (as opposed to 200 fold reduction predicted from theoretical calculations) at the target plane has been achieved with this lens system. As a result, significant extension in the cesium charge life time and a consequent reduction in the maintenance cost is anticipated.

Fig. 3 (a) and (b) compares the spectra obtained without and with focusing effects when approximately the same cesium ion currents were used. Both spectra were produced from 5 ng MHPG sulfate (3-Methoxy-4-sulfatoxyphenylethyleneglycol) injected into a 6-7 $\mu\text{L}/\text{min}$ flow stream contained 20% glycerol/ H_2O . Obviously, a defined and focused beam with a higher current density gives 30 times increase in signal-to-noise ratio when compared with the signal from the unfocused ion beam. Furthermore, a smaller region of secondary ion generation resulting from a focused primary ion beam produces narrower and less tailing CF/L-SIMS signals. Further optimization of the beam irradiation position and beam size on the probe tip through the steering mechanism is in progress.

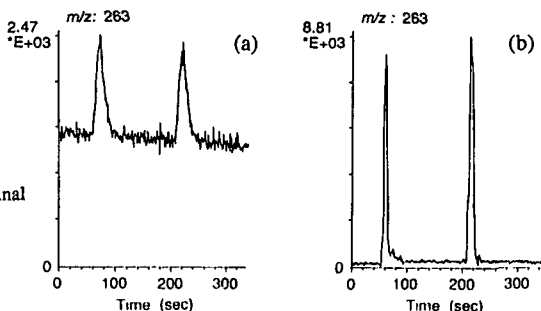


Figure 3. Spectra obtained when 5 ng MHPG sulfate is injected: (a) with original unfocused cesium ion gun and (b) with focusing lens system on the same gun.

ACKNOWLEDGEMENT

We thank P. J. Todd, Oak Ridge National Laboratory, for his valuable suggestions for the lens design and its applications. We also thank B. L. Bentz, David Sarnoff Research Center, for providing the tantalum pentoxide refractory films and William Frasure, Analytical Specialties Co., for power supply modifications.

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MASS SPECTROMETRIC DETECTION OF THIN LAYER CHROMATOGRAPHY USING PULSED LASER
EVAPORATION WITH UV LASER IONIZATION

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Pulsed laser desorption/evaporation is used as a means of volatilizing thermally labile analytes directly from thin layer chromatography plates. The neutral plume is then entrained in a supersonic jet expansion where it undergoes rapid adiabatic cooling and subsequent UV laser ionization at 266 nm in a time-of-flight mass spectrometer. Using this method, we have been able to detect labile molecules such as catecholamines, indoleamines, aromatic amino acids, dipeptides and neuroleptic drugs vaporized directly from TLC plates with observation of only the molecular ion, M^+ , in a time-of-flight mass spectrometer (TOFMS). The detection limit ranges down to the subnanogram level and the quantitation curve is linear over 4 orders of magnitude. The laser ionization is spectroscopically selective against the TLC stationary phase which is also vaporized in the desorption process as opposed to other desorption methods such as SIMS and LDMS where this may appear as a strong background in the mass spectrum. In addition, using laser ionization we can detect laser desorbed species from TLC such as amino acids and peptides which are not easily detected in laser desorption from the TLC plates using CI detection. The unique feature demonstrated here is the ability to obtain sharp spectra as a function of wavelength for the analyte desorbed and entrained in the jet expansion for specific identification using R2PI spectroscopy. We have used this method to uniquely identify various indoleamines and catecholamines based on their fingerprint cold spectra. In addition, using jet cooled ionization spectroscopy we have been able to optically separate and identify compounds such as indoleacetic acid and tryptophol in the TOFMS, although these compounds were not separated in the TLC plate.

The idea of selective spectroscopic detection is illustrated in Figure 1. These spectra were obtained by placing a 2 μ l sample solution containing 10 μ g each of indole-3-acetic acid and tryptophol onto a TLC plate with a microliter syringe and developing the plate with 50% EtOH/50% ethyl acetate. The unresolved TLC spot is then prepared by cutting out the spot and attaching it to an injection probe for introduction into the mass spectrometer system. In these experiments, ~ 5-10 μ l of glycerol is coated onto the spot. The glycerol dissolves the spot and allows for repetitive desorption for performing spectroscopy. By monitoring the molecular ion intensity produced by R2PI in the TOFMS as a function of wavelength using a gated integrator, we obtain sharp wavelength spectra of tryptophol (Figure 1a) and indole-3-acetic acid (Figure 1b). These spectra can unambiguously identify these compounds in combination with mass spectrometry. The corresponding TOF mass spectra are shown in figure 2a and b.

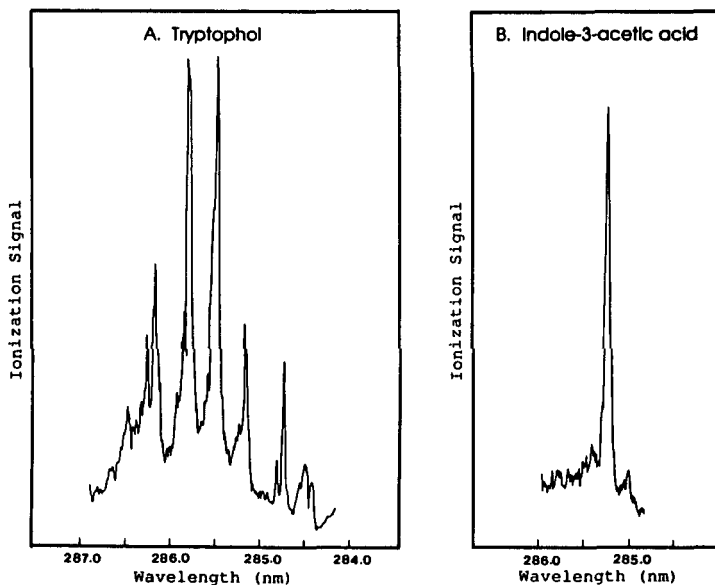


Figure 1. R2PI jet-cooled spectra of (A) Tryptophol, (B) Indole-3-acetic acid desorbed from a TLC plate.

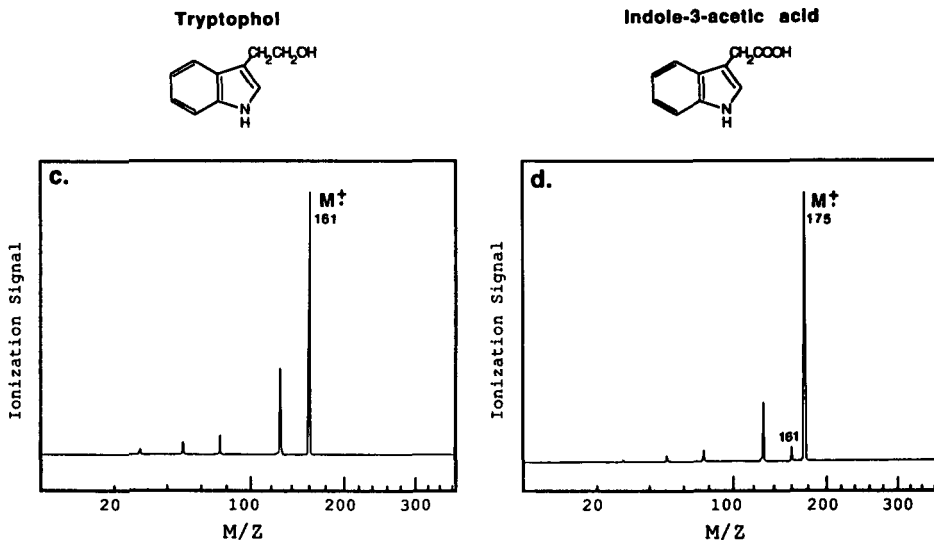


Figure 2. R2PI/MPI mass spectra of tryptamine analogs after TLC separation for: (1) Tryptophol. This mass spectrum was obtained by selective laser ionization at 285.75 nm ($P = 5 \times 10^6 \text{ W/cm}^2$). (2) Indole-3-acetic acid. This spectrum was obtained by selective laser ionization at 285.30 nm ($P = 5 \times 10^6 \text{ W/cm}^2$).

Direct FAB-MS From Empore™ TLC Sheets

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Thin-layer chromatography is a fast, simple, and effective technique for the separation of mixtures. In addition, it is a highly versatile technique which can be applied to a wide range of analytes. A significant shortcoming of TLC, however, is the limited variety of "in-situ" detection methods available, that allow the unambiguous identification of the separated components.

Mass spectrometry could be ideally suited for TLC detection due to its sensitivity and specificity. Its combination with other separation techniques such as GC and LC has yielded powerful methods of analysis for multicomponent samples. Early attempts at TLC-MS involved scraping the separated component with the silica substrate from the TLC plate and analyzing it by electron ionization using a direct-insertion probe.^{1,2} More recently, techniques have been reported where the TLC plate is scanned past a source of thermal desorption energy, after which a chemical-ionization reagent gas transports the desorbed material into the ion source.^{3,4} Since many materials separated by TLC are relatively nonvolatile, fast-atom bombardment (FAB) is well suited for direct analysis of TLC spots. Chang, et. al.⁵ and Bare, et. al.⁶ accomplished this by covering the FAB probe tip with double-faced adhesive tape and pressing the probe tip against the TLC spot to transfer the material to the probe for analysis.

Our approach to TLC-FABMS utilizes the unique properties of the recently developed Empore™ TLC sheets. Each Empore™ TLC sheet is comprised of a chemically modified 8-micrometer silica, or C-8 bonded silica, particles which are bound tightly in a dense "web" of micro-polytetrafluoroethylene fibrils.⁷ This web removes the need for both a binder and a glass or plastic support. As a result, the spots on Empore™ TLC sheets can easily be cut out and analyzed without flaking off the stationary phase. After development in a suitable mobile phase, the spots were cut out with an X-acto™ knife and mounted on a copper FAB probe tip with double-faced adhesive tape. The FAB matrix liquid, thioglycerol + 1% oxalic acid, was applied around the outer edges of the cut-out spot to wash the analyte toward the center. Better results were obtained if the matrix was diluted 1:2 with methanol, since the solution was then less viscous and more readily absorbed into the TLC sheet.

Good results were obtained for a separation of the ionic dyes Rhodamine B, Fluorescein, and Fast Green on an Empore™ C-8 bonded silica TLC sheet. (Fig. 1) Intense pseudomolecular ion peaks are indicated for each of these three compounds, which were present in amounts of approximately 15 micrograms per spot. The highly polar or ionic nature of these compounds requires FAB ionization. Positive results were also obtained for a mixture of the alkaloids haemanthidine and haemanthamine separated on an Empore™ silica TLC sheet. (Fig. 2) Although these alkaloids could be analyzed by conventional ionization methods, the direct FAB-MS method offers a straightforward approach to molecular weight confirmation.

Caprioli, et. al.⁸ demonstrated the use of a sample introduction probe for FAB-MS that permits a continuous flow of solution to be brought directly into the ion source. Preliminary experiments in our laboratory indicate that continuous-flow FAB (FFAB) could also be a useful TLC detection method. A standard KRATOS flow probe with a 50-micrometer capillary was used. About 4 or 5 microliters of a 30-35% glycerol in methanol matrix solution were added externally to the sample spot on the TLC sheet. The tightly bound nature of the stationary phase caused the matrix liquid to bead up on the TLC sheet, thus extracting a portion of the sample spot into the matrix. Introduction of the sample was accomplished by dipping the capillary into this "bead" of matrix and sample for 10 seconds. Figure 3 shows the spectra obtained for Rhodamine B and Fluorescein from "dips" off the TLC sheet. Attempts to carry out this experiment using conventional HPTLC plates were not successful, however. The less tightly packed stationary phase of the HPTLC plates absorbs the matrix liquid rapidly, spreading out the sample spot. As a result, it was difficult to draw any of the solution into the source with the capillary.

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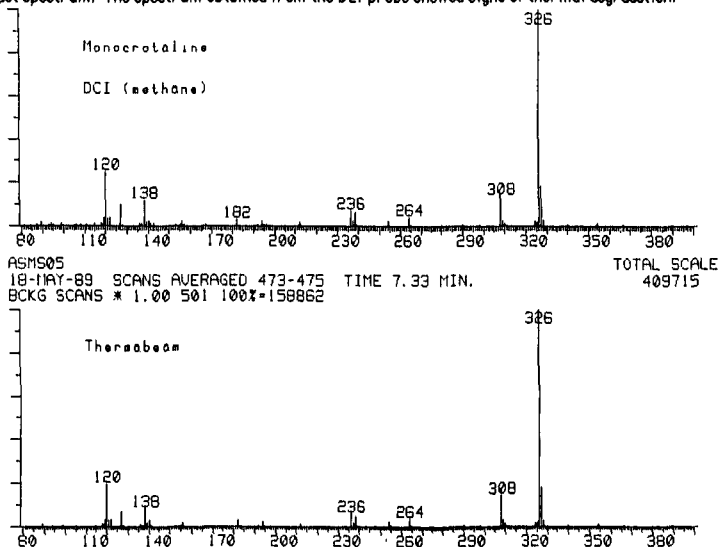
COMPARISON OF LC/MS PARTICLE BEAM SPECTRA WITH DCI AND DEI PROBE SPECTRA. Matthew J. Dilts, and Thomas Marecic, Extrel Corp., 240 Alpha Drive, Pittsburgh PA 15238

INTRODUCTION: This study compares EI and CI spectra obtained from particle beam introduction to spectra obtained from DCI probe introduction. A wide variety of compounds of environmental, pharmaceutical, and industrial interest have been analyzed successfully. In most instances, the spectra obtained with the particle beam interface have demonstrated enhanced volatilization over those obtained from the DCI probe. Mass spectra have also been obtained for higher molecular weight compounds with the particle beam interface. For example, polystyrene (MW 2000) has shown the $n = 14$ oligomer under standard electron impact conditions (1).

EXPERIMENTAL: All worked described was performed on an EXTREL ELQ 400-1d differentially pumped mass spectrometer equipped with EI/CI switchable source interfaced with a Varian 9010 liquid chromatographic system by means of the ThermoBeam LC/MS Interface. The DCI data was aquired using a Vacumetrics DCI probe and controller. Data was acquired under full scan conditions from 80 to 1500 AMU at 1000 AMU/sec.

OBJECTIVES: Demonstrate the ability of the particle beam interface to generate EI and CI spectra from flow injections. To compare EI and CI spectra obtained from a DCI probe with those obtained from the particle beam interface. Also to determine if there are differences in the spectra due to enhanced volatilization or thermal degradation.

RESULTS: Both techniques gave comparable EI and CI spectra (see figure). With the particle beam interface the analysis was faster and easier than with the DCI probe. Some of the compounds, for example, Cyclophosphamide fragmented differently with the particle beam interface. The spectrum obtained with the particle beam was more representative of an electron impact spectrum. The spectrum obtained from the DEI probe showed signs of thermal degradation.



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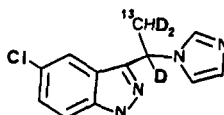
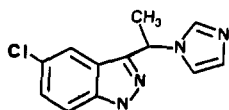
CONCLUSION: The particle beam interface and the DCI probe gave comparable spectra for both Chemical and Electron Impact Ionization. For the compounds studied only subtle differences were noted in the spectra. Of the two techniques the particle beam interface was less cumbersome to operate and appeared to be more amenable to automation

(1) Jones, G.G., McCoy, R.W. and Pauls, R.E. (1988). Electron Impact Ionization Mass Spectra of Polystyrene by ThermoBeam LC/MS. In "Proceedings of the 36th ASMS Conference on Mass Spectrometry and Allied Topics", pp. 464-465.

Comparison of GC/MS and GC/MS/MS for the Analysis of CGS-18416A

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CGS-18416A (Fig 1) is a benzoxazole derivative that has been shown to be an effective antagonist of electroshock-induced seizures. It has potential applications for the treatment of grand mal epilepsy¹. The structures of the drug and its internal standard are shown below.



During the course of method development it was determined that the compound produced structurally diagnostic fragment ions under MS/MS conditions. We therefore examined the utility of this methodology, both as a potential quantitative approach and for its application to future metabolism studies.

Extraction - The extraction procedure consisted of an acidic wash step followed by extraction at pH 11 into 25% ethyl acetate in pentane. The extract was then concentrated and analyzed directly. The extraction recovery from human plasma was 90%.

Chromatography - Due to the strongly basic character of this compound, chromatography on conventional 0.25 μ m columns gave severely tailed peaks and reduced column lifetimes. The use of a short column with a 3 μ m film thickness solved these problems and kept the analysis times reasonable (5 min). GC was done with a 10m methyl silicone column (3 μ m film). The injector and transfer line were at 290°C. Temperature programming was done from 100°C (1 min) to 270°C at 40°C/min.

Ionization - A variety of ionization methods were examined in order to obtain the optimum sensitivity. These included EI, positive and negative ammonia, methane, and nitrous oxide CI. Positive ammonia at 0.2 torr was significantly more sensitive than the others. The $[M+H]^+$ ions were monitored at m/z 248 and m/z 252 for the compound and internal standard respectively.

GC/MS - The method was initially developed using GC/MS. With the experimental conditions described above, the method was validated for sensitivity accuracy, and reproducibility. The GC/MS method resulted in a limit of quantitation of 200 pg/ml of plasma with a corresponding CV of 8.6%. Figure 1a shows a chromatogram of a plasma extract at the limit of quantitation. Table 1 shows the quality control summary from three consecutive runs.

GC/MS/MS - In the GC/MS/MS work, all analytical conditions up to and including the ionization method were the same as for GC/MS. In order to obtain the maximum sensitivity, the collision cell conditions were optimized for daughter ion intensity. The nature of the collision gas, the pressure of the gas and the collision energy were all examined.

The optimum CID conditions were found to be argon at 0.1 torr with a collision energy of -5V. Under these conditions, the CID spectrum resembles the EI spectrum. They both show the molecular ion as well as fragments at 69 (imidazole ring) and 180 (benzoxazole ring).

Quantitation was done using the m/z 69 daughter ion for both the compound and the internal standard. This gave a limit of quantitation of 10 ng/ml and a detection limit of 2 ng/ml (S/N 3:1). Figure 1b shows a chromatogram of a plasma extract at the limit of quantitation (10 ng/ml).

Table 1

Quality Control Summary for
CGS 18416A GC/MS Method Validation

Added (ng/ml)	% Found	C.V.	n
0.2	99.5	8.6	16
1.0	98.3	8.8	16
5.0	101.5	5.4	16
50.0	107.3	3.7	16
overall	101.7	7.6	64

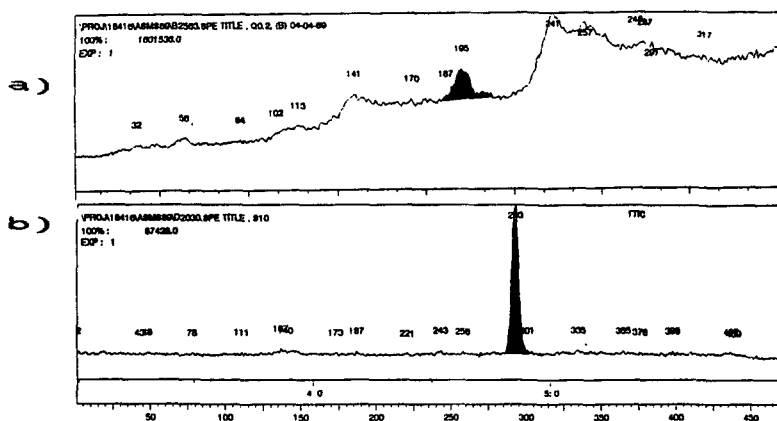


Figure 1. Selected ion chromatograms of CGS 18416A plasma extracts spiked at the limit of quantitation for two different techniques a) GC/MS, concentration 0.2 ng/ml b) GC/MS/MS, concentration 10 ng/ml.

Conclusions - In applications requiring quantitative analysis, the GC/MS approach offers significantly greater sensitivity over GC/MS/MS. There are no interferences that would require the significantly greater selectivity afforded by GC/MS/MS. The limit of quantitation is 200 pg/ml with excellent accuracy (99.5%) and reproducibility (CV 8.6%) at that concentration.

For qualitative studies such as *in-vivo* or *in-vitro* metabolism, the MS/MS provides structurally diagnostic daughter ions that can aid in the discovery and structural analysis of metabolites. The above MS/MS data provide the optimum experimental conditions for conducting such studies using either daughter or parent ion techniques. It also demonstrates that quantitation can be directly and reliably performed in such studies at concentrations as low as 10 ng/ml.

Reference: Bernard, P., R. Bowman, M. Williams, J. Lehmann, J. J. Kapeghian, J. Leibman and R. Robeson, "CGS 18416A : Pharmacology and Neurochemical Profile of a Novel Anticonvulsant", Princeton Drug Research Symp., Princeton NJ, May 1989

**DIRECTLY COUPLED SUPERCRITICAL FLUID EXTRACTION-GAS
CHROMATOGRAPHIC/MASS SPECTROMETRIC ANALYSIS OF ORGANIC COMPOUNDS
FROM SOLID SAMPLES**

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The extraction and concentration of organic analytes from sample matrices is often the most time consuming and error prone step in an analytical protocol. Conventional techniques such as Soxhlet extraction and sonication require large volumes of pure solvents and extraction times of hours to days. The resulting extract must then be concentrated prior to analysis, which can lead to sample loss and/or degradation. Supercritical fluids have solvent strengths approaching those of liquid solvents. The solvent strength of a supercritical fluid is directly related to its density and can be modified by changing the extraction pressure or temperature. Mass transfer is enhanced by the lower viscosities and higher solute diffusivities of supercritical fluids. Common supercritical fluids (CO_2 and N_2O) are gases at room temperature, which makes sample concentration relatively simple and allows supercritical fluid extraction to be directly coupled to Gas Chromatography/Mass Spectrometry (SFE-GC/MS).

All GC/MS analyses were performed using a Hewlett-Packard Model 5985B equipped with a model 5890 GC on-column injector. Electron impact ionization spectra were acquired at 70 eV with a scan range of 50-350 amu. Capillary fused-silica DB-5 columns supplied by J & W Scientific were used.

All supercritical fluid extractions (SFE) were performed using an ISCO Model $\mu\text{LC}-500$ syringe pump. The extraction cells (Figure 1) were constructed from Parker brand 316 stainless steel fittings and had sample volumes of approximately 0.1 to 0.5 mL. Supercritical pressures were maintained inside the extraction cells by using 15-cm lengths of small inside diameter (15 to 30 μm) fused-silica tubing (F in Figure 1). Temperature was maintained at 45 $^\circ\text{C}$ by inserting the extraction cell into a thermostated tube heater.

Directly coupling the SFE to the GC column was accomplished by inserting the SFE outlet restrictors into the GC column through the on-column injection port. The GC oven was cooled during the extraction to trap the extracted analytes inside the GC column at the outlet of the SFE restrictor. At the conclusion of the extraction step, the restrictor was removed from the column and the supercritical fluid was allowed to flush from the GC column for 2 to 3 minutes prior to the initiation of a conventional GC/MS analysis.

The ability of coupled SFE-GC to yield chromatographic peak shapes comparable to those obtained using conventional on-column injections of a liquid solvent extract (methylene chloride) is shown in Figure 2 by the analysis of the spice, basil. SFE-GC analysis yields a chromatogram that compares favorably with one from an on-column injection of the unfractionated methylene chloride extract. The SFE-GC technique required only 35 minutes to extract, concentrate, and perform the GC separation, while the methylene chloride extract required ca. 8 hours to extract, concentrate, and analyze. The ability of supercritical fluid extractions to yield quantitative recovery is demonstrated by the lack of significant peaks in the second 10 minute SFE-GC extraction and analysis of the sample.

SFE-GC/MS analysis of a variety of sample matrices including environmental solids (cigarette smoke particulates, contaminated soil, and soot), sorbent resins (wood smoke and roofing tar volatiles from polyurethane foam sorbent plugs), and food products (chewing gum, orange peel, and sage) demonstrates the ability of supercritical fluid extraction coupled with gas chromatography/mass spectrometry to extract, concentrate, and obtain GC/MS data in a fraction of the time (< 1 hour) that is required for conventional liquid solvent extractions. Maximum sensitivity is also achieved since all of the extracted analytes are quantitatively transferred into the GC column, resulting in part-per-billion sensitivities with milligram sample sizes. Replicate analyses show good reproducibility and quantitative values obtained from the analysis of spiked samples and a NIST certified standard (urban dust, SRM 1649) show excellent agreement with known values.

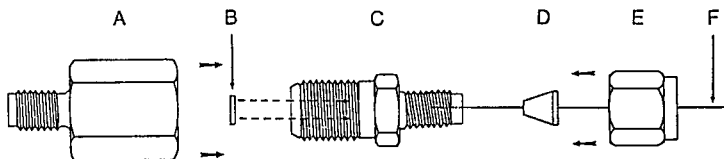


Figure 1. Supercritical fluid extraction cell for coupled SFE-GC/MS analysis. The components of the cell are (A) 1/16 in. female NPT x 1/16 in. tubing union, (B) $\emptyset$.5 μ m x 1/8 in. o.d. stainless steel frit, (C) 1/16 in. male NPT x 1/16 in. tubing union, (D) 15% graphite/85%polyimide ferrule, (E) 1/16 in. nut, and (F) fused-silica capillary restrictor. All fittings were stainless steel Parker brand.

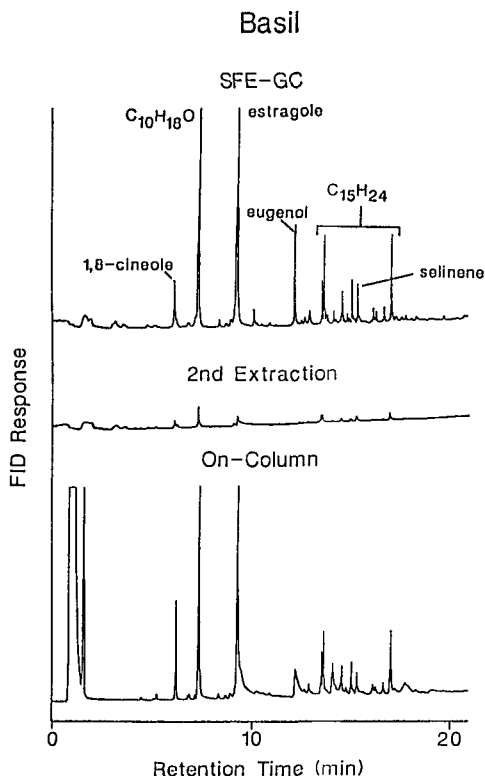


Figure 2. Comparison of chromatographic peak shapes resulting from SFE-GC/FID analysis (300 atm CO_2 for 10 minutes) and conventional on-column injections of a methylene chloride extract of 1 mg of basil. The middle chromatogram shows the second SFE-GC/FID extraction and analysis for the sample. Tentative identifications are based on SFE-GC/MS analysis of replicate samples.

COUPLING OF SUPERCRITICAL FLUID CHROMATOGRAPHY AND SUPERCRITICAL
FLUID EXTRACTION WITH SECONDARY ION MASS SPECTROMETRY

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Supercritical Fluid Chromatography (SFC) and Supercritical Fluid Extraction (SFE) with carbon dioxide as mobile phase have been described in a number of papers [1-3]. This paper describes the first results of SFC-SFE coupled on-line and off-line with secondary ion mass spectrometry (SIMS).

The on-line interface and the modifications on the SIMS instrument were designed to allow a fast exchange of restrictors, whilst maintaining the required low pressure in the SIMS. The inlet system for supercritical CO₂ was used to deposit different analytes on a silver target contained in a UHV quadrupole secondary ion mass spectrometer. The target was continuously exposed to the SFC-eluate and simultaneously bombarded with primary ion current densities ranging between 10⁻⁵ and 10⁻⁹ A cm⁻², corresponding to sputter rates between 1 and 10⁻⁴ monolayers s⁻¹. At high current densities the target could be cleaned within seconds (e.g. between two SFC peaks) at low current densities the analyte accumulates on the substrate and complete spectra can be obtained. Analytes studied include PAH's (pyrene, chrysene etc. fig.1) and some di-alkane-phthalate esters (fig.2). Without optimization low nanomol quantities could be easily detected. The analytes could be identified due to mass fragments similar as in EI/MS. SIMS showed additional cationized (argentated) fragments. For the SFE-SIMS experiments the extract was either directly deposited onto the silver target or collected in a sample vial with chloroform and an aliquot of the solution was pipetted onto the target.

Experimental:

SIMS: UHV mass spectrometer, quadrupole mass filter, pressure during measurements 4 x 10⁻⁵ hPa, turbo pump (plus cryo pump for on-line experiments).

PI: 3 keV [Xe]⁺ or [Ar]⁺, ion current densities 3 x 10⁻⁹ A cm⁻².

Target material: Silver

Scanning time: 0.3 s amu⁻¹

Column: Scientific Glass Engineering, 0.1 mm ID, 1m.

Valve: Valco C14W 0.2 ul

Mobile Phase: CO₂, 9.8 MPa, 40 C

Results:

- a) On line coupling of SFC / SIMS is possible. The necessary low pressure for SI conditions can be maintained with properly adjusted flow rates.
- b) Off line coupling is also possible for SFC and SFE. The sample can be deposited either directly out of the restrictor onto a target or pipetted after collection in a solvent.

Literature

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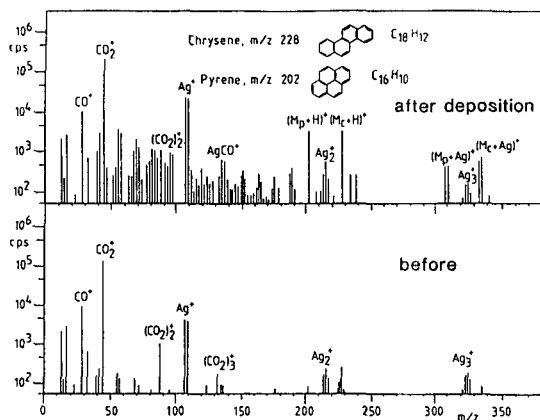


Fig. 1 SI mass spectra of pyrene and chrysene before and after deposition through the interface

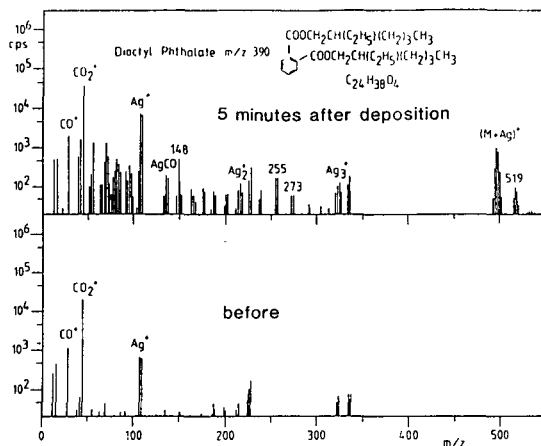


Fig. 2 SI mass spectra of dioctylphthalate-ester before and 5 minutes after deposition on the target

AN OPTICAL CCD CAMERA INTEGRATED WITH A SECONDARY ION MASS
SPECTROMETER FOR ANALYSIS OF PLANAR CHROMATOGRAMS

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Two-dimensional imaging analysis of organic and bio-organic thin-layer chromatograms and electropherograms by secondary ion mass spectrometry has been described in a number of fundamental and application papers from our group over the past few years. For complex mixtures, and larger chromatograms, the time required to locate component spots and bring them to the point of analysis can become quite lengthy. Practically, all chromatograms are pre-screened visually before placement with the mass spectrometer.

It seems obvious that an optically-based imaging system should be integrated with the SIMS analysis of chromatograms. This arrangement would allow the user to more easily locate component spots on a TLC plate.

The design of our second generation chromatography/SIMS instrument included the option of placing other detector modules in the z-axis normally occupied by the quadrupole mass analyzer. We describe here the replacement of the quadrupole with a charge-coupled device (CCD) camera assembly. Figure 1 shows the chromatography/SIMS instrument with the quadrupole installed and subsequently replaced with the CCD assembly. This arrangement allows for component spots on a TLC plate to be located by visual light, UV radiation, or by reversible general and specific color-developing reagents.

We have determined that three detection schemes are sufficient to detect all compounds. We first exploit the fact that we are using TLC plates coated with a fluorescent coating. Illuminating the plate with 254 nm UV light yields a bright plate with dark spots corresponding to the component spots. Figure 2 shows a plot of light intensity versus pixel number for one particular row of the pixel array that intersects the component spots of a plate containing differing amounts of testosterone, using UV illumination. (This type of plot will herein be referred to as a row-plot). If a particular compound does not occlude the fluorescence of the plate, and hence is not detectable by this method, the compound may iodine stain. Exposing the plate to iodine vapors turns the component spots brown. Visible light can now be used for the detection. Figure 3 depicts the row-plot of four "onium" salts detected using this iodine staining technique. And finally, if iodine staining fails, a specific color-developing reagent can be used. For instance, reacting polycyclic aromatic hydrocarbons with 2,4,7-trinitro-9-fluorenone yields purplish spots.

The CCD camera also gives us data complementary to the mass spectrometry data. For instance, some compounds, such as some polysulfonated dyes, are not particularly amenable to SIMS analysis, but are able to be detected visually using the CCD. Conversely, a compound may not be detected optically, but may yield a usable mass spectrum.

The inherent high sensitivity of the charge-coupled device lends to significantly lower detection limits in the analysis. Figure 4 shows a row-plot of approximately 390 pg of oxazine 4 perchlorate, a laser dye, detected using 366 nm UV radiation. This signal to noise ratio, along with the fact that this row-plot only intersects 1/25th of the total sample, corresponds to a detection limit of about 7 pg (24 femtomoles!).

CCD/Chromatography/SIMS Instrument

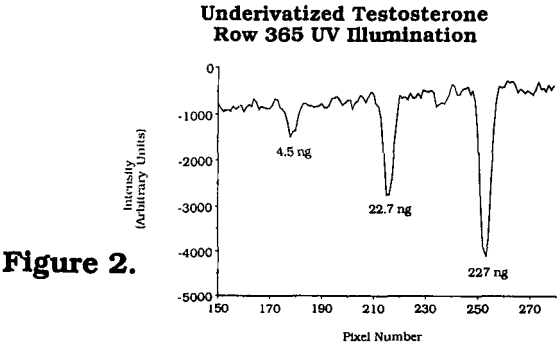
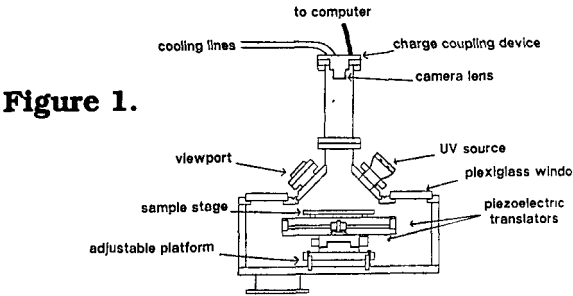


Figure 2.

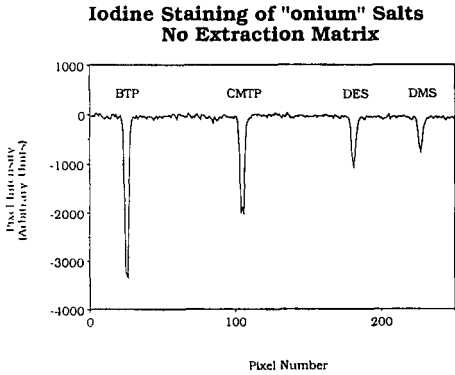


Figure 3.

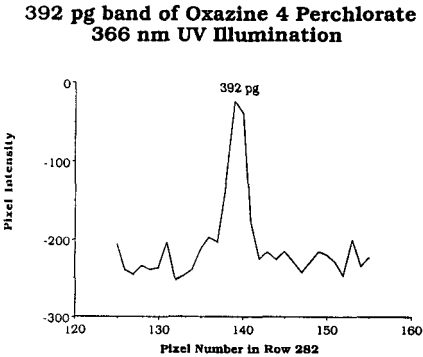


Figure 4.

Evaluation and Optimization of a CCD Detector for SIMS

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In an effort to improve the integrity of the final mass-filtered image in ion microscopy, our laboratory has developed a charge-coupled device (CCD) detection system for SIMS (1). The system replaces the microchannel plate/ fluorescent screen currently in use with a single, back-side illuminated CCD. The direct detection of keV ions by a CCD demonstrates their potential utility in mass spectrometric applications.

Back-side illuminated devices are CCDs which have been thinned to the p-Si/ p⁺-Si interface. Incoming radiation strikes the back-side and does not have to penetrate the thick gate structure. It is such a device which has been shown to be sensitive to keV ions.

The sensitivity of CCDs to low energy ions can be examined in two parts: signal electron formation, and signal electron detection. The formation of electrons derives from the inelastic loss of energy as the ion traverses the device. The location and amount of electrons produced depends strongly on the nature of the ion. Much theoretical attention has been given to these ion-solid interactions and our model of the CCD can incorporate many of the contemporary theories. Other factors which can affect the total inelastic loss are dead layers of native oxide, and ion reflection at the surface. Detection of signal electrons is independent of their origin and is controlled by the internal parameters of the CCD. The ability of the CCD to capture and store charge is its charge collection efficiency (CCE). The CCE is depth dependent and drops dramatically at the back-side. By incorporating these factors into our model we can understand the CCD's performance and design improvements.

Under ion bombardment the detector displays the superior qualities inherent to CCDs (1). Detector linearity is excellent and spans a broad dynamic range (Fig. 1). The noise behavior of the device is easily characterized and understood. Three simple domains exist: read noise, shot noise, and fixed pattern noise caused by pixel inhomogeneities. The CCD has a distinct mass dependence which agrees very well with calculations from our performance model. Its greatest weakness is sensitivity. The CCE at ion penetration depths is on the order of 1% or less. Increasing the CCE near the back-side is the key to sensitivity enhancement.

The potential well at the native SiO₂/Si interface at the back-side drains signal electrons and is responsible for the low CCE. Increasing the CCE is possible by altering the structure of the CCD's surface (2). Enhancement is a two step process: 1) a quality oxide roughly 30 Å thick must be grown; 2) the oxide is 'charged', flipping the interface potential. Charging can be achieved temporarily by a UV light flood (2) or permanently with the deposition of a thin layer of Pt (3). Termed a 'flash gate' the 15 Å of metal can boost the CCE to 100%. We are currently refining oxide growth techniques in an effort to enhance CCDs via the flash gate procedure.

Oxide growth must be carried out in a contaminant-free environment; and the procedure must not damage the delicate CCD. Our procedure employs 200°C steam under an Ar atmosphere. Test structures are being constructed to assess the method. In addition, these structures can be exposed to the ion beam to explore possible damage to the flash gate.

CCDs have strong potential as detectors in ion microscopy, and in mass spectrometry in general. The devices retain their positive attributes under keV ion bombardment; sensitivity being the one exception. Enhancement to sensitivity is possible and this avenue is being explored. The basic architecture of CCDs is also being refined and redeveloped. Progress in this area may lead to devices which do not require enhancement at all.

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- (3) J.R. Janesick, T. Elliot, T. Daud, and D. Campbell, *Instrumentation in Astronomy VI 1986*, (SPIE, Bellingham, 1986), SPIE Vol. 627, p.543.

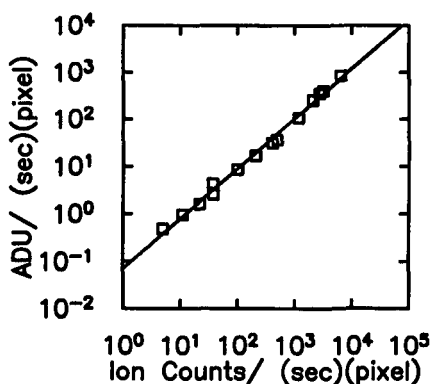


Figure 1: CCD calibration line for $^{27}\text{Al}^+$

DIRECT INTERFACE OF A RUSKA THERMA-CHROM WITH AN HP 5985B GC/MS

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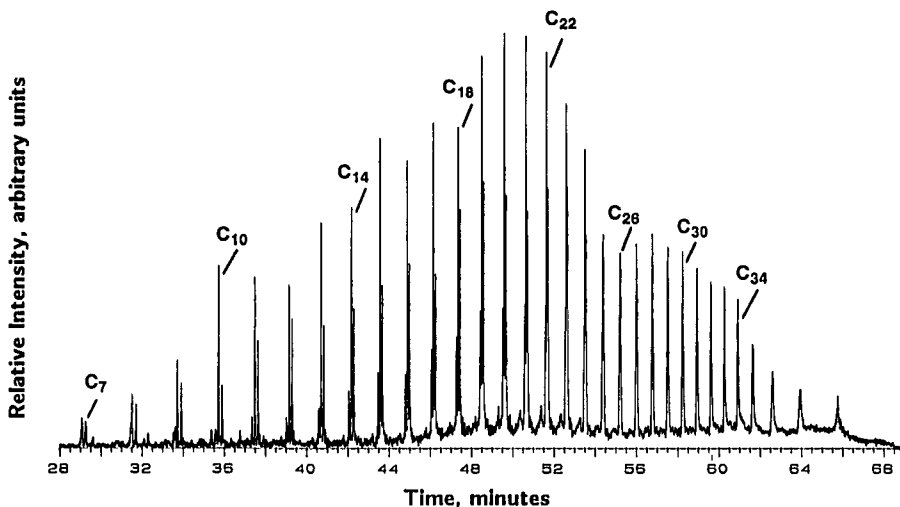
and

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Thermal Chromatography (TC) is a unique method for disassembling materials using accurate and reproducible heating programs. The all-fused-quartz systems for TC/FID and TC/MS that comprise the Ruska Thermo-Chrom and Thermo-Chrom Spectrometer Interface (TCI) respectively eliminate sample contact with metal surfaces and enable rapid independent heating and cryogenic cooling of all zones. The trap, splitter, and gas chromatograph between the sample heating chamber and the mass spectrometer allow concentration and separation of species evolved during TC/MS. Under computer control, temperature programming on the TCI can be divided into any number of steps or "thermal slices," allowing identification of species evolved within specified temperature ranges.

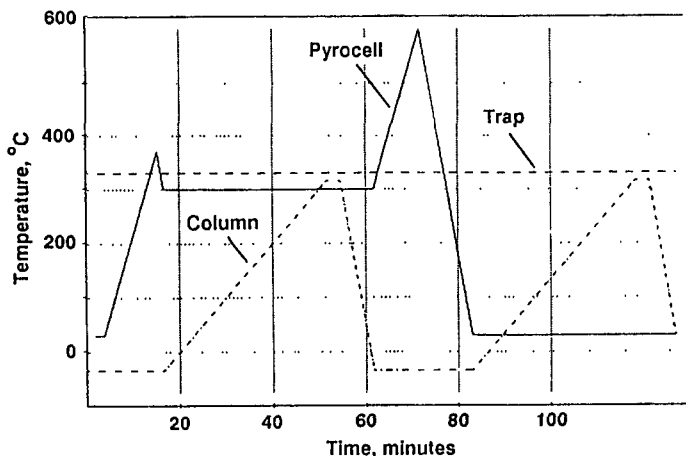
Using thermally shielded ~85 inch long quarter-inch stainless steel tubing to house the capillary gas chromatographic (GC) column, we have directly connected a Ruska Thermo-Chrom Spectrometer Interface to a Hewlett-Packard (HP) 5985B quadrupole mass spectrometer. The column emerges from the Ruska oven inside the transfer line, passes through the HP GC interface oven, and enters the mass spectrometer vacuum manifold through one of the ports in the flange that forms the HP GC/MS connection. The two GC columns enter the mass spectrometer ion source in parallel, and switching between TC/MS and GC/MS is simply a matter of turning down the helium flow in one column and turning it up in the other. No degradation in performance of either technique has been noted with this configuration. As shown in the figure below, cold spots have been eliminated from the transfer lines, and we are able to see hydrocarbon peaks from the TC/MS analysis of polyethylene out to C_{38} .

**TC/MS ANALYSIS OF POLYETHYLENE
DEMONSTRATING EFFICIENCY OF TRANSFER LINE**

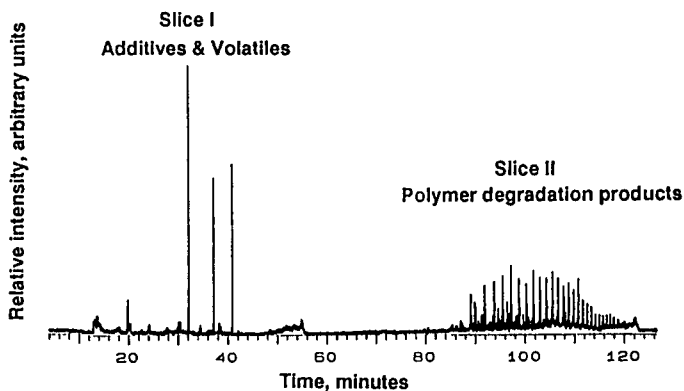


We have used thermal chromatography to study differences in the outgassing characteristics of PVC insulation of wire used in electrical circuits that develop high potentials. Thermal profiles of insulation taken from wire supplied by several vendors were obtained used TC/FID. These profiles, different in shape for the different vendors' materials, showed that the insulation degrades in two steps: a small but significant amount of material is evolved between 30° and 300°; between 450° and 550° C the bulk evolves. Differences in shapes of the curves at low temperature indicate differences in the composition of semivolatiles of the different vendors' polymer formulations. To study the compositional differences, a two slice TC/MS method was developed, shown in the upper half of the figure below: Slice I is the range 30° - 370° C; Slice II 300° - 625° C. While the sample was being heated, the GC column was held at -35° C to trap evolved species. At the completion of each sample heating, the column was heated, yielding chromatograms like those shown in the bottom half of the figure. As expected, semivolatile additives such as flame retardants, lubricants, and antioxidants were found in the Slice I chromatogram, while in Slice II, species related to PVC degradation were observed. The species identified from the spectra obtained in Slice I for each sample were different, confirming the TC/FID indication of compositional differences among the samples.

TEMPERATURE PROGRAM FOR "TWO SLICE" TC/MS METHOD



"TWO SLICE" TC/MS ANALYSIS OF PVC WIRE INSULATION



A DATA SYSTEM FOR DAUGHTER-ION MEASUREMENTS WITH A REFLECTING TIME-OF-FLIGHT SPECTROMETER:

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Introduction:

Recent improvements in time-of-flight mass spectrometry have placed increasing demands on the required data systems. A high range spectrum with moderate resolution may require a few hundred thousand channels (usually at 4 bytes per channel). Thus a spectrum can easily require 1 Mbyte of memory; in our case a typical size is 1/2 Mbyte. The measurements of daughter ions described here require a time digitizer (TDC) with inputs for 2 separate detectors, rapid processing to detect correlations and large memory to store multiple daughter ion spectra. Development of electronics has, of course, also been rapid and we present an inexpensive system that satisfies the criteria mentioned.

Daughter ion mass spectra from a selected parent ion are normally obtained with a tandem instrument where one parent is analyzed at a time and the daughter ion spectrum is obtained by scanning, unless an array detector is used. A time-of-flight spectrometer with an ion mirror provides an alternative method of obtaining daughter ion spectra, i.e. it acts as a tandem spectrometer [1].

Fig. 1 shows a schematic diagram of the spectrometer (Manitoba TOF II). Mass resolution up to $m/\Delta m = 10,000$ (FWHM) at m/z 650 has been achieved; typical resolution for organic molecules up to m/z 3000 is about 5000 (FWHM). Of central importance to the daughter ion measurements is the grid at the back of the mirror that allows neutral daughters to be detected in detector 1.

The neutral daughters from metastable decay between the target and the mirror retain the velocity of the parent so their flight time (measured in detector 1) identifies the parent ion. Any ions detected in detector 2 in the same event (primary pulse) are assumed to result from the identified parent. (This assumption is accurate if the primary ion current is low). Since the daughter ions have only a fraction of the parent energy, the mirror - an energy selector - separates them on the basis of their mass. A daughter ion spectrum for a given parent may then be recorded by considering only those events in which a neutral is detected at the selected parent flight time. An important difference from normal tandem mass spectrometry is that any number of such daughter ion spectra may be recorded simultaneously.

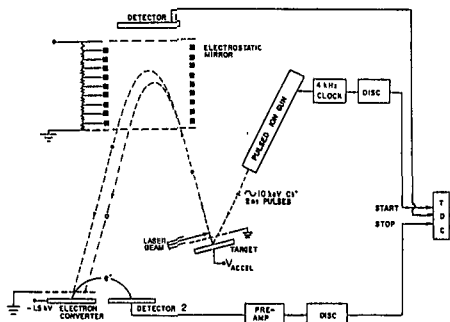


FIG 1: Schematic of TOF II

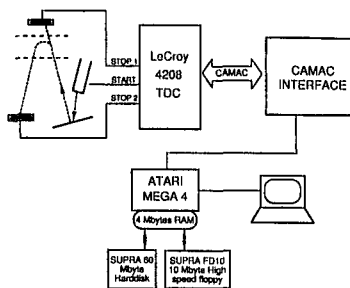


FIG 2: Data System

The Data System:

The data system is shown in Fig. 2. It is based on the Atari Mega 4 computer (based on a 68000 processor running at 8 MHz). To handle the large spectra, it was important to select a processor with 32 bit addressing. The 4 Mbytes of RAM on the Atari are all directly addressable.

The LeCroy 4208 TDC has a common start and up to 8 separate stop inputs. For daughter ion measurements it is configured to use only 2 inputs, each of which can accept up to 4 stops for each start. At present we use an INCAA CAPRO 68K CAMAC controller to read the TDC and transfer the data to the Atari through a Kinetic Systems 3063 IGOR (input-gate output-register). This arrangement was convenient because the CAPRO was available. However, we intend to implement a direct Atari-to-CAMAC interface that has recently become available.

For data storage we use a Supra FD-10, 10 Mbyte extended high density 5 1/4" floppy drive. This has a speed comparable with that of hard disks and is suitable for both archiving spectra and for logging direct event-by-event data (see below).

Real Time Correlations:

Up to now we have recorded daughter ion spectra in real time. That is, after each primary ion pulse, the computer looks for neutrals in preselected time windows and sorts the flight times of the ions accordingly. At 2 kHz repetition rate, there is enough time between primary ion pulses for any number of correlations to be checked; the only limitation is the available memory for the daughter ion spectra. Fig. 3 shows the daughter ion spectra for two components of tyrothricin (a natural mixture of 9 cyclic decapeptides), recorded at the same time as the parent spectrum.

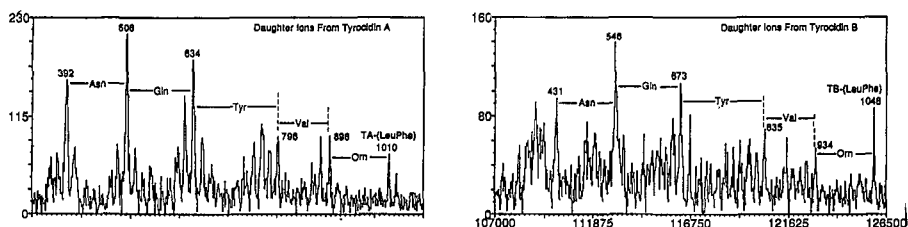


FIG 3: Daughter ion spectra of tyrothricin

Event logging:

An alternative to detecting coincidences as they occur is to log each event on disk as a group of neutral and ion flight times. Then the experiment may be "replayed" at a later time and any daughter ion spectra may be extracted. An event-by-event data log thus contains information for a complete MS/MS map from a single experiment. The code for this procedure is now being tested. Preliminary measurements have confirmed the feasibility of using the 10 Mbyte floppies for this purpose; each floppy can store about 20 minutes of data.

Alternate spectra:

Controlling an experiment largely from software allows considerable flexibility. We have begun laser dissociation of desorbed secondary ions. The laser is pulsed on every second primary ion pulse and the trigger pulse is used by the computer to identify the laser-on event. Thus normal SIMS and laser dissociation spectra may be taken on alternate pulses under otherwise identical conditions [2].

Resolution of Daughter Ions:

As another example of the flexibility of our system, we are now developing a software method of improving the resolution of the daughter ions. Part of the broadening of daughter ion peaks is caused by the kinetic energy released in the break-up. This gives one fragment slightly higher velocity than the center-of-mass, and the other slightly less. By observing the position of the neutral fragment in a defined window, a correction can be made to the flight time of the correlated ion.

In summary, a powerful data acquisition system for a reflecting time-of-flight mass spectrometer has been assembled using an inexpensive computer and commercially available components.

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1. X. Tang, et. al., *Anal. Chem.* **60** (1988) 1791.
2. F. Mayer, et. al., these proceedings.

A NEW TIME-OF-FLIGHT SECONDARY ION MICROSCOPE

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We have developed a new stigmatic imaging time-of-flight (TOF) secondary ion microscope. Unlike most TOF instruments built to date, this mass spectrometer is capable of transporting a magnified stigmatic image of the secondary ion emission pattern of the sample to an imaging ion detector, simultaneous with the acquisition of secondary ion mass spectra. Secondary ion mass spectra and images may be recorded at primary ion doses of less than 10^{12}cm^{-2} . Under these analysis conditions only about 0.1% of the sample surface are bombarded by the primary ion beam. Low primary ion doses are essential for true surface analysis and for the analysis of organic materials. For organic surface analysis, e.g. biological surfaces, the additional information of the lateral ion distribution can help to identify correlated ion species as they would arise from molecular fragmentation.

A schematic diagram of the instrument is shown in Figure 1. The ion optical system of the primary ion gun focuses a pulsed (approx. 1.4ns f.w.h.m.) beam of Cs^+ ions onto a sample area of 50-1000 μm diameter. Secondary ions are electrostatically accelerated to 4.5keV and imaged by the immersion lens. This image is projected by either one of the following transfer lenses (STL2 or STL3) into a system of three hemispherical 90° electrostatic analyzers (ESA), which transports the final secondary ion image onto the detector. A "contrast diaphragm" (CD) and an energy slit are placed into the secondary ion crossover points to provide radial (perpendicular to the spectrometer axis) and axial ion energy filtering - if desired. A field aperture is placed close to the entrance of ESA1 to limit the imaged sample area. This spectrometer system provides first order time (energy) and lateral focusing with small residual second order aberrations.

A dual microchannel plate (DMCP) detector is used in conjunction with a resistive anode encoder (RAE) as position sensitive detector. The timing signal for ion arrival is extracted from the DMCP and digitized by a time-to-digital converter (TDC) of 156ps resolution. The corresponding position information is obtained from the RAE and processed with a position computer. Both position and time information are stored by a computer system and made available for evaluation. Mass spectra and several mass separated secondary ion images can be simultaneously recorded.

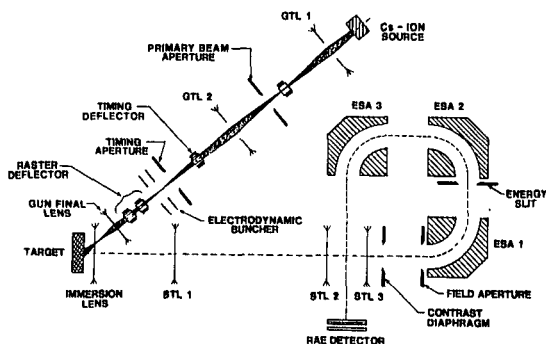


Figure 1: Schematic diagram of the TOF secondary ion microscope.

When the first transfer lens (STL2) is activated in conjunction with the immersion lens, a 200 μm diameter area of the sample is imaged onto the detector. This corresponds to a spectrometer magnification of $m=60\times$. Mass resolutions of $m/\Delta m=3100$ (Figure 2) have been obtained with this configuration at mass 28 (^{28}Si), still limited by the duration of the primary ion pulse. In this operation secondary ion image resolutions of approx. 3 μm

can be achieved with ion transmission losses of some 30% (filtering with CD) to reduce aberrations caused by the imaging lenses. Axial energy filtering (energy slit) is usually not employed. When STL3 is activated together with the immersion lens, sample areas of $60\mu\text{m}$ diameter ($m=270\times$) are imaged to the detector and image resolutions of some $1\mu\text{m}$ can be obtained with transmission losses of the order of 60%. Mass resolutions of $m/\Delta m=1800$ (at ^{28}Si) are obtained in this configuration. For both configurations the mass resolution of the secondary ion images is identical with the mass spectral resolution.

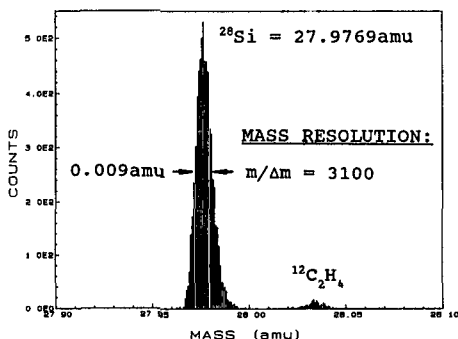


Figure 2: Section of a mass spectrum taken from a silicon wafer surface to demonstrate the mass resolution. The analyzed sample area was $200\mu\text{m}$ in diameter.

Figure 3 shows examples of mass resolved positive secondary ion images of an etched Al pattern on a SiO_2 substrate. The image fields are $200\mu\text{m}$ in diameter. The images were recorded using charge compensation by a low energy (20eV) pulsed electron gun and pulsing the accelerating potential⁽¹⁾. Figure 3a shows the clearly recognizable pattern of Al on the surface. Al can be clearly separated in the ion images from the C_2H_3 ion. Figure 3b demonstrates the deterioration of the image contrast when both Al and C_2H_3 are included. This loss of contrast occurs no matter how good the lateral resolution of the image may be, and demonstrates one advantage of high mass resolution imaging. This capability becomes important when atomic contaminants in e.g. polymers are to be analyzed. Figure 3c shows as expected that C_2H_3 is homogeneously distributed across the sample surface. It should be mentioned that in this application the total secondary ion images (the sum image of all ions emitted) did not exhibit any recognizable surface structure.

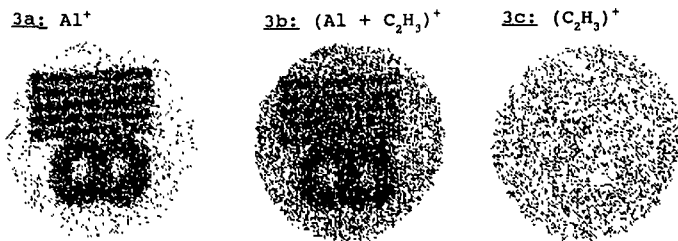


Figure 3: Mass resolved ion images taken from an Al pattern on SiO_2 using low energy (20eV) electron charge compensation. The image fields are $200\mu\text{m}$ diameter. Darker regions correspond to higher ion intensity.

Reference:

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IMPLEMENTATION AND ASSESSMENT OF BEAM DEFLECTION TIME-OF-FLIGHT MASS SPECTROMETRY FOR TIME ARRAY DETECTION IN GC-MS APPLICATIONS

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A conventional time-of-flight (TOF) mass spectrometer has been modified for beam deflection, as opposed to ion source pulsing, for use with an integrating transient recorder (ITR) to accomplish time array detection. In time array detection (TAD), it is essential that all ions from each extraction pulse of the ion source reach the detector in optimal focus. Such mass-independent focusing is required so that all of the ions in each transient mass spectrum can be detected with adequate resolving power.

The beam deflection TOF instrument described here consists of a continuous ion source which was originally designed for a magnetic mass spectrometer. Ion current continuously passes through the slits of the ion source into an electrode assembly which deflects the beam creating time-resolved ion packets which enter the field-free drift region of a two meter flight tube. At the end of the flight tube the ions strike a flat CEMA channel plate detector. The output from the detector is fed to an eight-bit ADC which operates at 200 MHz as the front end of the ITR that has been developed in the MSU Mass Spectrometry Facility. A schematic diagram of this instrument configuration is shown in Figure 1. The resolving power achieved with this instrument is in excess of 1000 as computed by the peak width at half height formula of mass spectral peaks in the analog mass spectrum of xenon. The mass spectral peak shapes are symmetrical at low and high mass in the mass spectrum of perfluorotributylamine as ionized by electron impact, indicating that the ion focus is adequate across the mass range. It is important to realize that these data were collected from a time-of-flight mass spectrometer with a gas phase ion source and without the use of time-lag focusing.

A major goal of the Mass Spectrometry Facility at MSU is to exploit the rapid spectrum generation capacity of a TOF instrument for GC-MS. As has been recognized for the last 25 years, it is necessary to acquire the mass spectrum of a gas chromatographic component as quickly as possible to minimize distortions among the mass spectral peak intensities due to changes in the partial pressure of the analyte in the ion source during the period required to record the mass spectrum. The problem of a changing partial pressure of the analyte in the ion source during GC-MS has become more and more severe as improvements have been made in capillary column GLC. As the peak widths in a capillary chromatogram approach one second, many mass analyzers are not capable of scanning the mass axis quickly enough to capture an unskewed mass spectrum, at least not without incurring problems with ion statistics.

In TAD, the ITR, designed and developed at MSU, acquires and sums consecutive transient spectra from the mass spectrometer detector continuously. The ITR is capable of generating as many as 1000 summed mass spectra per second. The design goals for the particular application to GC-MS are to provide 20 summed spectra per second which should be more than adequate to accommodate changes in sample concentration in the ion source during the emergence of a component from the capillary gas chromatograph. Each of the summed spectra in this case are the sum of 250 transient mass spectra which reach the detector following each extraction pulse of the TOF ion source.

In a preliminary evaluation of the beam deflection TOF instrument for GC-MS applications, 200 ng of methyl palmitate was applied to a 30 meter DB-1 0.32 mm id capillary column at 180° with programming to 250°C at a rate of 16° per minute. During the emergence of the methyl palmitate from the capillary column, approximately 50 mass spectra were recorded. As shown in Figure 2a, a reconstructed total ion current chromatogram illustrates the chromatographic profile of the methyl palmitate entering the TOF ion source. As can be seen in Figure 2a, the methyl palmitate emerged from the column during the acquisition of scans 70 through 120. The peak for methyl palmitate in this reconstructed chromatographic profile is approximately two and one half seconds in width. Figure 2b is the mass spectrum which was acquired during scan #95 which occurs near the apex of the elution profile. Scan #95 consists of the sum of 250 transient mass spectra. The mass spectrum in Figure 2b is recognizable as that of methyl palmitate in showing a molecular ion peak at m/z 270 with peaks corresponding to the loss of a methoxyl group at m/z 239 and loss of C_2H_5 at m/z 241. Also discernible is a significant peak at m/z 143, m/z 87 and, of course, the base peak at m/z 74. Mass spectra in the other 54 scans which were compiled during the emergence of methyl palmitate are similar in appearance although the S/N is not so good as that in Figure 2b. An important aspect,

however, is that the relative peak intensities in each of the other 50 or so mass spectra are not skewed in their relative intensities even though they were acquired during periods where the change in partial pressure of the analyte was greater than that in scan 95.

In conclusion, these preliminary data are encouraging in that they show that the instrumental configuration for beam deflection TOF-MS provides good quality mass spectral information which is focused properly in a time frame suitable for TAD. These results also indicate that the perceived benefits from TAD in GC-MS have been achieved. The success of the beam deflection approach to TOF-MS indicates that existing TOF instruments can be modified in this way to take advantage of the benefits of TAD in GC-MS applications. The beam deflection approach to TOF-MS also offers the possibility of extending the TAD benefits to other continuous forms of ionization such as CI or FAB-MS which would benefit from the ion statistical and temporal advantages of TAD.

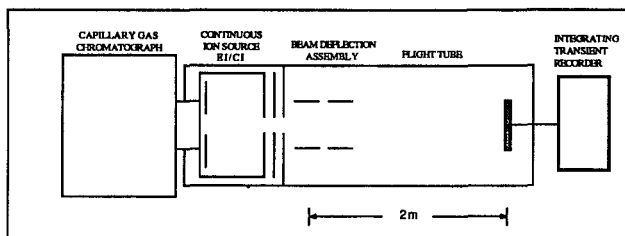


Figure 1. Schematic diagram of beam-deflection TOF GC-MS ITR system

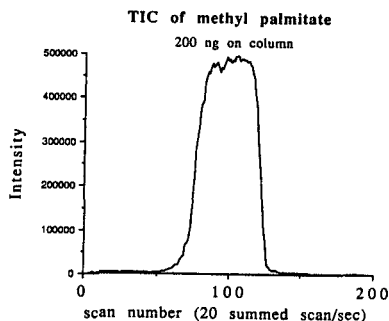


Figure 2a. Reconstructed Total Ion Current chromatogram resulting from 200 ng methyl palmitate injected on-column.

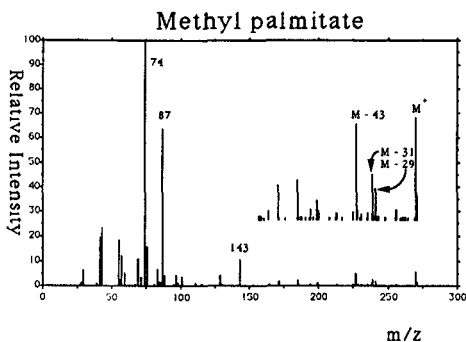


Figure 2b. Mass spectrum corresponding to scan #95 in Figure 2a.

**ION KINETIC ENERGY RELEASE MEASUREMENT BY POST-
SECTOR BEAM DEFLECTION IN TIME-RESOLVED ION
MOMENTUM SPECTROMETRY WITH TIME ARRAY DETECTION**

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Time-resolved ion momentum spectrometry (TRIMS) is a tandem mass spectrometry technique where mass analysis is produced by simultaneously measuring the momentum and velocity of an ion. In this hybrid instrument the ion beam leaving the magnetic sector is time-encoded by means of electrostatically sweeping the beam modulation assembly. Using time array detection (TAD), all the information produced by each sweep is captured for each increment of the magnetic field strength. Thus the full B-t data field can be collected much more quickly with better signal-to-noise ratios than with conventional time slice systems.

As fragment ions are produced either by metastable or collisionally-induced decomposition, the kinetic energy released due to isotropic effects manifests itself in the TRIMS experiment along lines of constant B times t. By measuring the peak width along this line for a daughter ion, the kinetic energy released (T) can be calculated.(1) A TRIMS instrument with a pulsed source has been successfully used to measure kinetic energy release as a function of ion lifetime.(2) The values obtained using TRIMS/TAD with post-sector beam deflection are comparable to those obtained by other methods such as IKES and MIKES.(3)

All experiments were performed on an LKB-9000 magnetic sector instrument modified for post-sector beam deflection with a flight path length of 1.6 meters.(4) All data collection was done in TAD mode using an integrating transient recorder (ITR) developed in this laboratory. The pertinent data is extracted from the B-t field and downloaded to a PC-AT compatible computer for further processing.

An acceleration energy of 3500 V, a source temperature of 200C and an emission current of 60 mA was used for all experiments. The magnetic field was DAC-controlled by the ITR and the field strength recorded using a Hall probe. For each DAC step 1000 transients were summed for optimum signal-to-noise ratios. After extracting energy vs. intensity pairs for each daughter ion peak, a 15 point Savitsky-Golay algorithm was used to facilitate measurement of the peak width.

In TRIMS the energy of an ion (E) is a function of B/t and is given by the following expression:

$$E = 1/2 B r d^2 / t \quad \text{or} \quad (1)$$

$$E = B/t (k) \quad (2)$$

where r is the radius of the magnetic field and d is the flight path length. Thus by calculating k for the stable ions at energies of 3500 eV, the energies for all daughter ions can be obtained. Along the lines of constant B times t, the intensities of ions on these isomass lines represent the spread in energies. This is in contrast to pulsed-source TRIMS experiments where the energy spread is along lines of constant B over t.

The energy released (T) in the fragmentation processes of a stable ion is obtained from the following equation:

$$T = (m_1^2 eV / 16 m_2 m_3) (\Delta E / E)^2 \quad (3)$$

where $\Delta E = \Delta E_{\text{obs}} - (m_2/m_1) \Delta E_{\text{stable}}$ (4)

and m_1 , m_2 and m_3 are the parent, daughter and neutral loss masses, respectively, eV is the energy of the parent ion leaving the source, E is the energy of the stable ion and ΔE is the width of the daughter ion energy profile measured at half-height and corrected for the energy distribution of the parent ion.

Kinetic energy release measurements were made for the decomposition of several stable ions with T values reported in the literature. Shown in Figures 1 and 2 are the energy profiles of the parent and daughter, respectively, of the unimolecular loss of chlorine from 2,4 dichlorotoluene. The energy release for this reaction was calculated to be 28.0 ± 2.0 meV where the uncertainty was due to the error in measuring the peak height of the daughter profile which resulted in a spread in the width at half-height. This value compares well with that reported in the literature (28.6 meV).⁽³⁾

TRIMS has been shown to be a useful technique for making kinetic energy release measurements. By using the TRIMS/TAD system the energy distributions can be both accurately and rapidly determined.

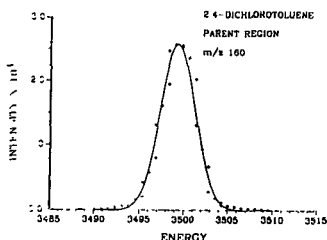


Figure 1

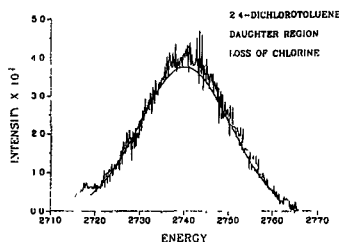


Figure 2

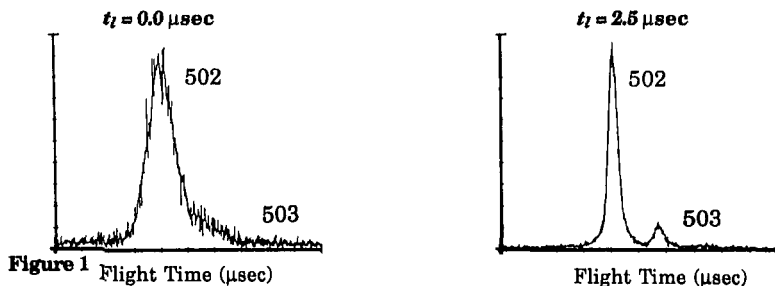
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Mass Dependence of Focusing Parameters in Time-of-Flight Mass Spectrometry

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In conventional time-of-flight mass spectrometry (TOFMS), temporal focusing of isomass ions having different energies is obtained by the use of a delay time between the formation and extraction of ions from the source, known as time-lag focusing [1], and by the adjustment of extraction grid potentials. Time-lag focusing relies on the movement of the ions as a consequence of their initial kinetic energies, hence its effects are mass-dependent. This mass-dependence is clearly demonstrated in Fig. 1 where the m/z 502 and 503 ions of perfluorotributylamine are shown with the time lag adjusted for two different regions of the spectrum.



The mass-dependence of the time-lag focusing technique is inconsequential when scanning detection schemes are used where only one m/z value is monitored for each extraction of ions from the source. In these cases, focusing parameters can be changed with successive extraction pulses to obtain optimum focusing for each m/z being monitored. Mass-dependent focusing is not adequate for time-array detection schemes, however, where many ions are monitored after each ion extraction pulse [2]. While the search continues for mass-independent methods of focusing [3-6], we have been examining an alternative in which different values of time lag and other instrumental parameters are used for successive ion extraction pulses, each of which gives acceptable levels of signal quality over a portion of the relevant range of m/z values. The total spectrum can then be reconstructed using the focused regions from each transient.

The size of these focused regions and the parameters which affect them have been investigated through the use of a digital simulation which models a CVC 2000 TOFMS instrument. This simulation employs normal (Gaussian) distributions of initial ion positions and velocities in the source to calculate probabilities of various arrival times at the detector. These probabilities represent ion intensities measured on the actual instrument. Probabilities at the peak maximum and the valley between adjacent m/z values are used to identify m/z ranges which achieve unit mass resolution at an acceptable valley height. Losses of ion intensity from migration out of the ionization region during the time lag results in a skew of the peak shape. The characterization of useful mass regions therefore includes the minimization of this form of signal degradation as well.

Identification of optimal mass regions is illustrated in Fig. 2. All ions to the left of the resolution curve have at least unit mass resolution with a 10% valley definition. All ions between the two intensity curves have no more than a 10% loss in peak intensity. The overlap of these two regions defines the useful m/z regions for each value of time lag. With the extraction potentials used to generate Fig. 2, as few as three time-lag adjustments are needed to cover the m/z range from 50 to 700. This figure also reveals the maximum m/z that will have acceptable signal quality with these parameters.

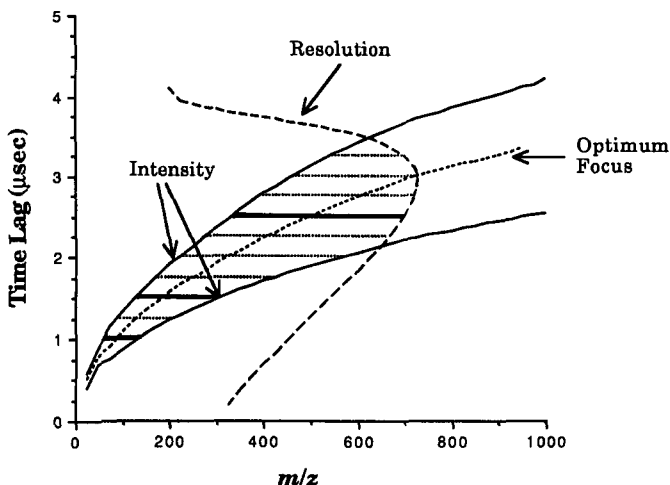


Figure 2.

The effects of ion extraction potentials and non-ideal extraction pulse shapes on intensity and resolving power have been examined. Changing the grid potentials alters the shape of the overlap region (Fig. 2) and thus affects the size of the useful m/z regions as well as the overall m/z range. Resolving powers obtained using this simulation match values collected using the commercial instrument within experimental error.

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POST-SOURCE PULSE FOCUSING: A SIMPLE METHOD TO ACHIEVE IMPROVED
RESOLUTION IN A TIME-OF-FLIGHT MASS SPECTROMETER: G.R. Kinsel and M.V. Johnston;
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Post-Source Pulse Focusing (PSPF) is a simple variation to time-lag focusing in which the focusing voltage pulse is applied to a short field free region after the ions have been accelerated from the source region by a conventional static electric field. No major modifications of the mass spectrometer source region or electronics are necessary. Mass resolution of 1000 should be readily attainable and for certain applications much higher resolutions are predicted. The two major drawbacks of time-lag focusing are eliminated. Since the ions are accelerated using a conventional static electric field, no extraneous ion loss occurs due to a time delay. Also, all ions which have exited the acceleration and pulsing region will show equivalent resolution to a conventional static field (SF) TOFMS. All ions within the pulsing region will show substantial resolution improvement. The total mass region affected may be adjusted by increasing or decreasing the length of the pulsing region.

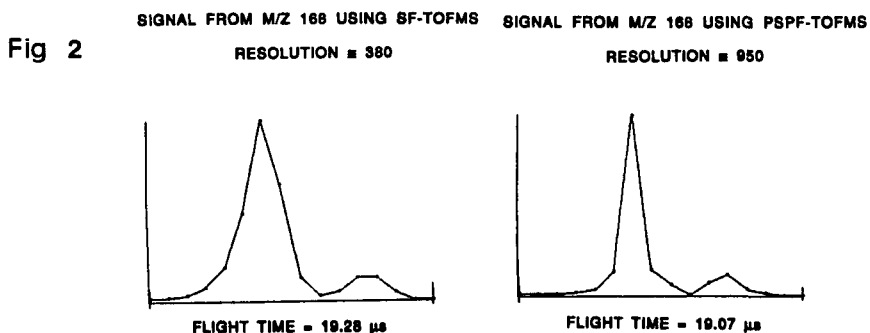
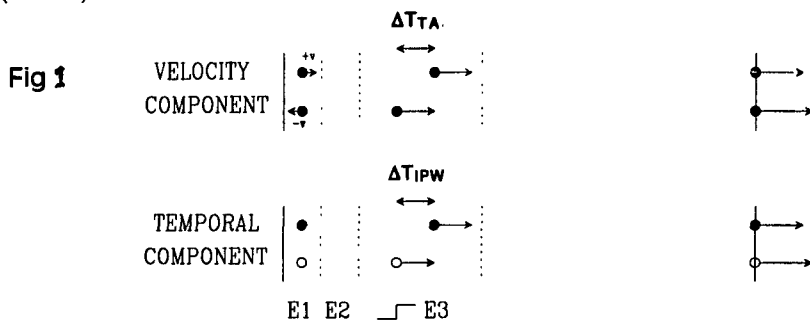
The method by which PSPF resolution improvement is achieved can be visualized in the following manner (See Figure 1). Once all ions of an isomass packet have entered the field-free drift tube of the TOFMS, both the initial velocity and temporal contributions to mass resolution can be viewed in terms of spatial distortions of individual ion pairs within an isomass packet. In other words, an ion having an initial velocity towards the detector, $+v$, and an ion having an equal initial velocity away from the detector, $-v$, enter the drift tube with exactly the same velocity, merely separated by the amount of time required for the $-v$ ion to initially turn around and return to its original starting point, ΔT_{TA} . If these two ions are viewed in the drift tube from a frame of reference moving at the same velocity as the ions they appear to be spatially defocused. An analogous argument can be made for the temporal contribution to resolution. If an accelerating voltage is now applied to the pair of ions, the trailing ion will be accelerated and achieve a slightly higher velocity than the leading ion. This additional velocity will cause the trailing ion to eventually catch up to the leading ion. Through proper choice of the voltage pulse the point at which these two ions become coincident can be adjusted to coincide with the plane of the detector. Since PSPF is designed to primarily compensate for the velocity and temporal components of resolution it will be particularly well suited for applications where σ_{spatial} is small (surface desorption/ionization) or where σ_{velocity} is large (GC-MS and thermal desorption MS), or where longer ionizing pulses are desired.

Computer modeling has been used to investigate the effects of varying different parameters while employing the PSPF technique.¹ It has been shown that using available electronic equipment and a standard TOFMS design, for typical conditions a resolution of ca. 1100 can be achieved using a focusing pulse of 136 V. If σ_{velocity} is increased to values typical for GC-MS, the resolution achievable remains relatively unaffected at ca. 1000. If σ_{spatial} is reduced to values typical for

surface / desorption ionization resolutions of 10,000 or greater are expected. Further studies have shown that the temporal component of resolution are indeed compensated for using PSPF, even to the extent of making the width of the detected ion signal narrower than the width of the ionizing pulse. In addition, methods for focusing up to 80% of the mass spectrum have been demonstrated. Variations of other parameters have been discussed which can be used to achieve even higher resolutions using PSPF. These results have all been contrasted to resolutions using static field TOFMS.

Experimental support of the theoretical basis for PSPF-TOFMS has been achieved (See Figure 2). These spectra show the parent ion of naphthyl-acetate at m/z 168 using SF and PSPF TOFMS. The PSPF spectrum was generated using an extremely unstable pulsed voltage supply and it is expected that even better resolution can be achieved. Even under these less than ideal conditions however, the resolution improvement using PSPF is clearly evident.

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ORGANIC SECONDARY ION TIME-OF-FLIGHT MASS SPECTROMETRY WITH A FOCUSED LIQUID METAL ION PRIMARY BEAM

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We have designed and constructed a pulsed secondary ion time-of-flight (TOF) mass spectrometer to investigate the physical and chemical processes governing the ejection of organic secondary ions from liquid and solid matrices bombarded by fast atoms or ions. In order to examine emission and ionization mechanisms under a variety of well defined dynamical conditions, we desired that the energy, flux density, particle mass, and particle type (atomic or molecular) of the primary beam be variable as broadly as possible. Furthermore, we wanted our mass analysis to extend at least to M , 10,000, to be highly sensitive, and to be capable of detecting metastable ions. We decided to couple a focusing liquid metal ion (LMI) column to a Wien filter to meet our requirements for the primary beam and to use a TOF tube with an electrostatic mirror to meet our requirements for mass analysis.

The LMI column, a commercial (FEI Company) single-lens unit, is capable of delivering a positive ion beam of 0.1-10 nA into a spot on the sample-target $\leq 10 \mu\text{m}$ in diameter; under continuous bombardment this corresponds to current densities in the range of 1-70 mA cm^{-2} . The column can accelerate its beam through potentials up to 25 kV. The LMI beam can be rastered; hence, the system can be operated in a scanning ion microscope mode by using the TOF-analyzer's channel plates to detect secondary electrons as shown in Figure 1. This feature facilitates tuning the LMI column and aiming at very small samples. A LMI beam is comprised of atomic, molecular, and cluster ions of various charge states. In order to conduct experiments with a single, known primary species, the LMI beam is passed through a small Wien filter (Colutron Model 600), which disperses the beam into its component parts. Figure 2 shows a spectrum of a tin-LMI beam produced on the system; the rich variety of particles available for mechanistic studies is evident.

Pulsed secondary ionization is normally accomplished by rapidly sweeping an ion beam across a slit at regular intervals, Figure 3a. A novel feature of our instrument is the use of the sample zone itself to determine the duration of secondary ionization event. This is accomplished by sweeping the finely focused LMI beam across the sample which is deposited on a small diameter ($\sim 10 \mu\text{m}$) wire or narrow edge ($\sim 10 \mu\text{m}$), Figure 3b. If it were used on instruments designed for analytical purposes, this approach could eliminate the need for the complicated and expensive mass and energy filters used to compensate for the spreading that occurs in primary ion packets formed by conventional ion pulsing techniques, and because the secondary ions emerge from virtually a point, it should be easier to achieve mass resolution near the electronic limit. Moreover, it should be possible to achieve secondary ion emission rates comparable to plasma desorption and conventional pulsed secondary ion TOF from sample areas 2-3 orders of magnitude smaller than required for those methods.¹

The time of flight mass analyzer is modeled after the Manitoba design.² Our flight tube is 34 cm long and has a 90° electrostatic mirror to permit study of metastable ions. Flight times are measured with a TDC (LeCroy Model 4208) and analyzed on an Atari Mega 4 computer system with software provided by the Manitoba group.³ We have just begun testing the instrument and do not as yet have any performance data. Nonetheless, the performance characteristics of the mass analyzer are reasonably predictable: unit resolution should be achievable up to m/z 500 without and up to m/z 1,000 with the aid of the ion mirror; errors in mass assignment should be ≤ 0.01 mass unit; and compounds with molecular weights up to $\sim 10,000$ daltons should be detectable.

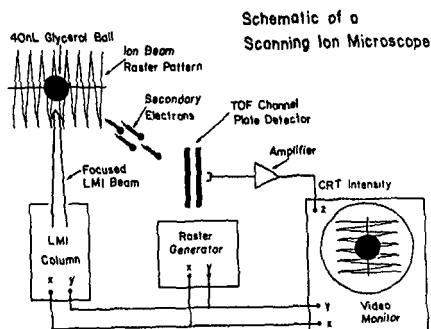


Figure 1

Figure 2

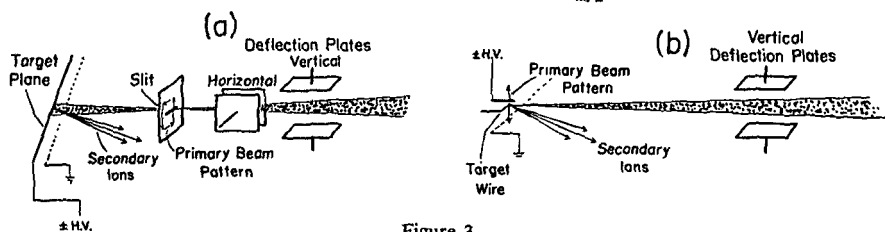
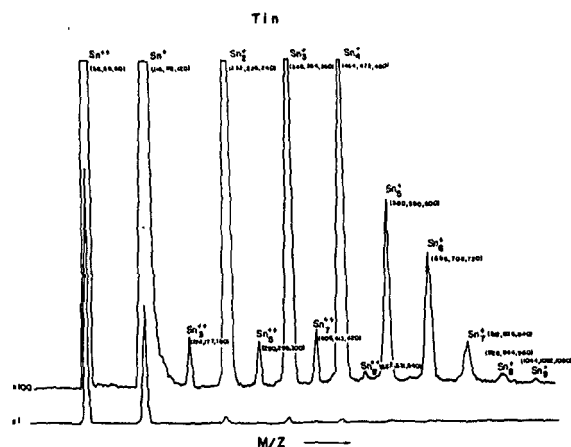


Figure 3

Acknowledgement

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EXTENDED LIFETIME ELECTRON MULTIPLIERS

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INTRODUCTION

Continuous dynode electron multipliers (CDEMs) have been used in mass spectrometers and surface instruments since the early 1970s. At the March '89 Pittsburgh Conference, Detector Technology, (DeTech™) introduced the Extended Life CDEM Series. This includes the Segmented CDEM⁽¹⁾ and the EverLast™ CDEM⁽²⁾ which incorporate the only major design innovations in CDEMs since their original development by the Bendix Research Labs in the early 1960s. Both of these inventions dramatically increase the life potential for continuous dynode electron multipliers. Each detector design has undergone extensive in-house testing and evaluation. Field tests and lifetime qualifications are currently being performed in a number of instruments by both manufacturers and users. Results to date, of all of these tests are discussed and summarized in this report.

(1) Joint Patent Pending, DeTech and Finnigan Corporation (2) Patent Pending, DeTech

THEORY

As discussed in a previous paper (1), the gain degradation (lifetime) of CDEMs has been found to depend upon the integrated number of electrons and ion impacts per unit surface area. Therefore, by designing a multiplier with increased surface area or replaceable surface area, one will effectively increase the lifetime of the multiplier. Computer modeling of the multiplication process revealed that an unrestricted electron cloud will rapidly expand to dimensions much greater than those of standard CDEMs. The model results show that after a gain of only 4000 the electron cloud has spread to over a 10mm full width at half maximum. Thus a detector with extended active surface area (compared to present CDEMs) should have an increased life when operated with the same input and gain. Other work has shown that the area of greatest charge concentration i.e.; the output end of the electron multiplier, is the location of the most damage during normal use of the CDEM. As the secondary emission ratio of the multiplier surface slowly decreases from the output toward the input, an overall decrease in gain results. An increase in detector voltage is then required to adjust the gain back to acceptable levels. Thus, a detector with a replaceable output segment will virtually duplicate its original gain, and then repeat its lifetime gain curve as each new segment is added.

EXTENDED LIFETIME DETECTOR SERIES

DeTech's Extended Lifetime Detector Series incorporated these developments into the new EverLast and Segmented CDEMs. As will be detailed, both of these detectors exhibit dramatically extended lifetimes compared to other available electron multipliers. Other important characteristics of detectors, such as noise, gain vs. voltage, and detection efficiency equal or exceed those of CDEMs presently in common use. The geometric aspects of these detectors have been adapted to CDEM specified hardware configurations, thus, direct retrofits are available for most equipment.

THE EverLast™ CDEM

The EverLast CDEM incorporates the theory discussed above directly in its manufacture. The EverLast is produced with a rectangular cross section that dramatically increases the active inner surface area. As the space charge progresses down this tube it expands across the total ID surface available. All tests were run, maintaining a gain of 10^6 . The term relative life is used as opposed to absolute life which depends upon a great many factors, such as operating pressure and proper handling. Absolute life can also vary with application. These tests were performed in a controlled, clean environment under conditions which can be repeated precisely for comparison purposes. Under these conditions, the EverLast CDEM's lifetimes averaged eight times greater than that of standard CDEMs. Customers in the field can expect to achieve similar results. This type of multiplier has a great potential for reducing equipment maintenance time and costs, while also reducing equipment downtime. Additional assets of the EverLast may also be apparent. The increased surface area allows a factor of 10 increase in the detector strip current. This in turn results in an increased output current since in a continuous dynode electron multiplier, the linear output is limited to approximately 10% of the strip current. For applications which require higher dynamic range, the EverLast configuration will increase this capability by the same factor of 10, or up to 20uamp output current. The EverLast design will be an asset in higher temperature applications. Many systems are being designed with the detector location running at an elevated temperature to reduce the problem of condensibles. The increased surface of the EverLast will allow for greater radiative cooling than standard designs of the past.

THE SEGMENTED CDEM

A second design for an extended lifetime CDEM has been jointly developed by Detector Technology, Inc., and Finnigan Corporation, San Jose, CA. In this device, the multiplier is constructed in two sections (segments). The rear segment of the CDEM is designed for easy and inexpensive replacement. Since the number of electrons rapidly increases as the electron cloud proceeds through the channel, the damage to the surface is greater further down the channel. Thus, the major factor limiting multiplier life is surface degradation occurring in the rear of the device. Simple replacement of the rear segment extends the lifetime of the detector. Tests performed at DeTech have revealed that the initial rear section of a properly designed device can be replaced three times before surface damage to the input section becomes the limiting factor and the entire unit must be replaced. When the exhausted rear segment is replaced the voltage required to produce a given gain drops dramatically back to almost the original voltage required. A gain of 10^5 was used throughout the tests. In-house testing has shown an increase in lifetime of a factor of 3 to 4. As with the EverLast, the actual increase observed in the field will vary somewhat with the application.

FIELD TESTING

DeTech has developed a dependable and reproducible, very high count rate, accelerated life testing procedure. This method was used for all the internal work. With the exception of work performed by Dr. Jon Amy at Finnigan, all field testing will be completed using normal rates. The results of field tests to date have been positive and follow:

FINNIGAN CORPORATION Dr. George Stafford of Finnigan is developing a segmented design for use in the Finnigan TSQ-70. Dr. Amy has evaluated a Segmented CDEM in an accelerated life test for this application using an engineering TSQ-70 and Finnigan 4500 electronics. Gain throughout the tests was calculated using analog methods incorporating an electrometer, which was installed to measure input and output current for the multiplier. Results were recorded at both 10^4 gain and 10^5 gain. He has reported the following: "Replacement of the back segment restored initial gains for four segments with applied voltage differences of less than 200 volts per segment. The data leads to the conclusion that the assumption that most of the gain is in the back section is valid. Replacement of this section is a practical method of restoring multiplier gain, and can be done four times with usable results."

VESTEC CORPORATION A Model 1340 EverLast has been installed in a Vestec Model 210 Mass Spectrometer at the Vestec Applications Lab. Dr. Marvin Vestal reports that in start-up operation this detector is similar to previous standard DeTech multipliers. Lifetime evaluations will be carefully tracked in this instrument for comparison to earlier work.

COMPUCHEM LABORATORIES Jim Chambers has been installing DeTech CDEMs in CompuChem instruments for four years and their Labs keep a record book for each instrument. CompuChem has complete history records, and can apply information with a large statistical base. Jim has pointed out that CompuChem has many instruments running on a 24 hour per day shift, and they put a large number of samples through their mass spectrometers. CompuChem can be considered to be a very tough test case for lifetime of multipliers. Lee Gregory installed an EverLast multiplier Model 1306 into an HP 5970 MSD. "Initial turn on indicated a low level of noise at a voltage of 1600, which was the voltage required to meet tune parameters. Over a period of several months, this new multiplier has continued to operate the same as the original equipment multipliers with a noise level that is equal to or less than the original. An important factor that we have seen is that the voltage level applied has remained fairly constant over a time of three months as compared to other units where the voltage has increased by four to six hundred volts over the same time frame. These facts lead us to believe that the new DeTech multiplier can give us an extended lifetime with a low level of electronic background noise."

SUMMARY

At this writing, both the EverLast and Segmented designs are living up to the extended lifetime expectations of original theory. A growing sampling of these detectors are in use, and a number of applications are being closely monitored. The results continue to be tabulated and published. Results to date show that performance parameters have either equalled or exceeded past performance in both designs, and models are compatible for retrofit in present equipment. The evaluations point out that the use of these new detectors will not only be cost saving, but will allow the mass spectrometer scientist to focus on the chemistry of his work with a major reduction in equipment maintenance considerations and details.

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HIGH SENSITIVITY ION DETECTOR PARAMETER STUDY

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Positive ion detection in mass spectrometry primarily relies upon the production of secondary electrons released from ions impacting a surface. The initial impact surface is either a Daly dynode (Ref. 1) or the surface of an electron multiplier. Since the secondary electron coefficient (the number of secondary electrons per impacting ion) is dependent upon the ion velocity, detection has been extended to high mass by applying high voltage to the conversion dynode to increase the impact velocity. Until recently, little attention has been paid to the production of secondary ions or to the tailoring of the work function of the dynode surface to favor the production of secondary particles including electrons and negative ions (Ref. 2).

Daly reported (Ref. 1) that large molecular ions produce more secondary electrons than single atoms or diatomic molecules even when impacting at the same high kinetic energy where the higher mass ions have the lower velocity. More recently, by both lowering the surface work function and increasing the impact kinetic energy, the authors substantially increased the detection signal for biomolecular ions (Ref. 2). For detection systems already employing post acceleration dynodes, lowering the surface work function resulted in a multiplication of the detector gain by more than an order of magnitude above 2,000 amu. For detectors that solely rely upon the electron multiplier accelerating voltage to produce the impact velocity (such as quadrupoles), the addition of a conversion dynode resulted in a multiplication of the gain by an order of magnitude at low mass and two orders of magnitude above 600 amu (Ref. 3).

In the present work, detection systems were designed to further examine this latter approach using different dynode geometries and a range of operating voltages on HP5982 and HP5988 quadrupole mass spectrometers. The results indicate that a large increase in signal and in signal-to-noise ratio was possible using a conversion dynode for all of the ion-producing modes of operation such as EI, CI, FAB, Thermospray and Particle Beam.

To measure the increase in signal, PFTBA was tested in the EI mode to compare the regular mass spectrometer detector with the experimental dynode detector. The experimental detector was also operated at zero dynode voltage, which simulated the regular detector and produced similar spectra. Figure 1 shows spectra produced at zero and at 9kV dynode voltages. The overall gain in signal and high mass enhancement is clearly observable. The gain is also seen in Fig. 2, which shows two curves for the average ratios of abundance with and without high voltage on the dynode for selected PFTBA peaks. A very small PFTBA peak at 564 amu was selected to illustrate the increase in signal-to-noise ratio with and without high voltage at the dynode (see Fig. 3). The spectral peaks were adjusted to have comparable abundances in order to convey the difference in noise levels. Figure 4 shows FAB spectra with and without dynode voltage applied, displaying the significant improvement gained by using this detector. FAB noise ordinarily observed on quadrupole mass spectrometers is strongly attenuated at certain dynode voltages. This phenomena and other features of the conversion dynode, along with the effect of low work function surfaces, are under further investigation in our laboratory.

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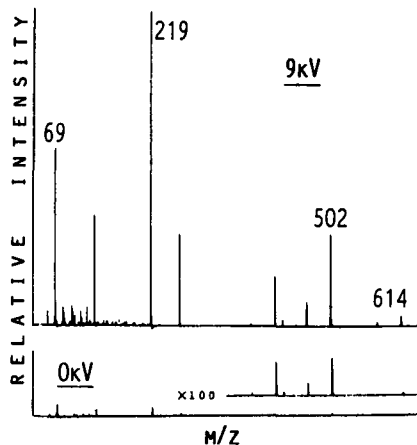


Fig. 1. PFTBA on an HP5982 with CDEM at 1.4kV for both spectra

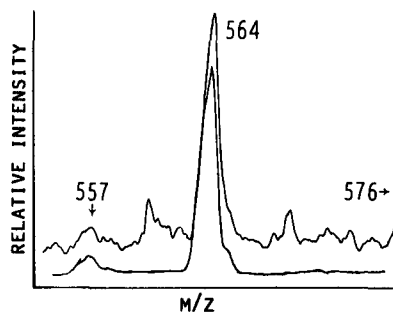


Fig. 2. Noise comparison using PFTBA on an HP5982

	CDEM	Dynode
Upper Spectrum	2.83kV	0kV
Lower Spectrum	1.70kV	9kV

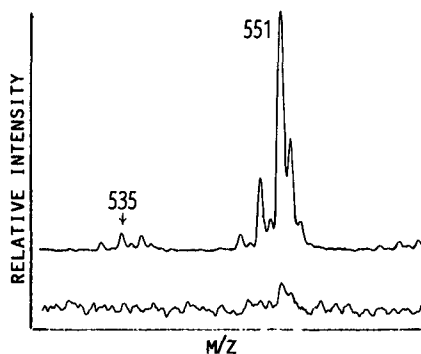


Fig. 3. FAB Spectrum of Arosurf on HP5982

	CDEM	Dynode
Upper Spectrum	1.55kV	8kV
Lower Spectrum	2.30kV	0kV

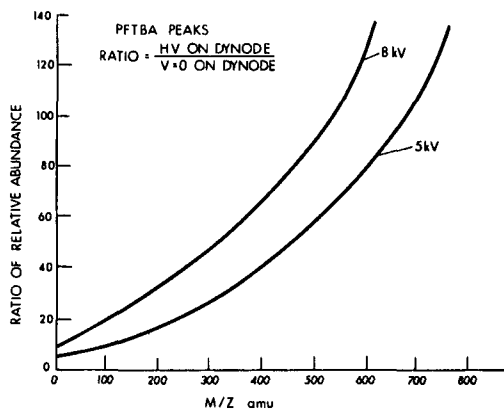


Fig. 4. Sensitivity increase (signal ratio) as a function of mass, with dynode voltage as a parameter

A FLEXIBLE ARRAY DETECTOR OF EXTENDED MASS RANGE FOR A HIGH PERFORMANCE TANDEM MASS SPECTROMETER J.A. Hill, J.E. Biller, S.A. Martin, K. Blemann, Dept. of Chemistry, MIT, Cambridge, MA, 02139
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INTRODUCTION Array detectors can be employed to utilize the more or less limited focal plane of Nier-Johnson type mass spectrometers. This results in an increase of sensitivity due to the simultaneous recording of a set of ion beams, and better S/N ratio due to the integrating properties of such detectors. Previously, we designed and constructed an array detector covering 6.6% of the mass range. That detector was placed into the focal plane of MS-2 of our JEOL HX110/HX110 mass spectrometer¹. Its major advantage is the 50-100 fold increase in sensitivity.

We now have redesigned this detector to record a variable and much wider mass range. It incorporates a novel ion-optical concept² which utilizes a pair of quadrupole lenses placed after the magnetic sector (Fig. 1) to vary the dispersion as well as the angle of the focal plane, which is then located at a fixed center point 12.7 cm in front of the normal collector slit. This makes it possible to move the detector into position with out interfering with the slit assembly. The design of the detector permits computer controlled setting of any desired angle via a stepper motor, while simultaneously applying the proper potentials to the quadrupole lenses. If the conventional slit/electron multiplier detector is to be used, the quadrupole lenses are grounded and the array detector moved out of the way of the principal beam using a linear motion feed through. The theoretical mass range accessible by this principle is 1:1.4 (i.e., 40% of the spectrum), limited by the width of the flight tube at the end of the magnet gap. We have presently achieved well focused ion beams covering the range of 1:1.055 at a detector angle of 17.7° to about 1:1.30 (i.e. 30%) at an angle of 40°.

RESULTS AND DISCUSSION Our main interest is in the recording of MS/MS (CID) spectra over a wide mass range, from m/z 60 to the $(M+H)^+$ precursor ion, while retaining unit mass resolution. The wide mass range at 40° detector angle is important at low mass to minimize the number of segments that need to be recorded, but a smaller range (therefore, angle) must be used at the higher mass range to retain resolution which is then limited by the 2048 elements of the 2" Reticon photodiode array. Two different angles generally permit minimizing the number of segments to be recorded for a single CID spectrum. As an example, that obtained with a peptide of $(M+H)^+ = m/z$ 2465.2 is shown in Fig. 2. For this spectrum the first 13 segments were recorded at a 40° angle ($R=1.29$) and the next five at 28° ($r=1.12$)

It has to be kept in mind that the kinetic energy of product ions in a CID spectrum varies and is proportional to the mass ratio of product/precursor ions. This energy spread has to be minimized to assure that all ions of the mass range to be recorded pass through E_2 and are properly refocussed on to the focal plane by the quadrupole lens system. This phenomenon becomes more severe with increasing mass range covered by the detector. For this reason we use 10 kV accelerating potential in MS-1 and operate the collision cell at 8 kV above ground.

This variable range array detector can also be used advantageously to record conventional FAB mass spectra. At wide angle (large range) a survey spectrum of low resolution ($R=500$) is obtained by stepping through a few magnetic field settings (in this situation the resolution limit of the photodiode array is irrelevant). When a peak is observed, it is roughly centered by changing the magnetic field (Fig. 3a). Stepwise decrease of the angle (and appropriate change of the lens potentials) permits "zooming-in" on the unresolved isotope cluster (Fig. 3b), which then can be resolved by changing the fixed ion source slit from 500 to 10,000 resolution (Fig. 3c,d). Simply increasing the integration time regains most or all of the signal intensity lost by narrowing the slit. To aid in integrating for longer times, the photodiode array has been cooled to about 0°C. This allows for integration up to 15x longer than with the detector at room temperature (Fig. 4).

In all cases the JEOL data system steps either B_1 (for conventional spectra) or $E_2/B_2 = \text{const.}$ (for CID spectra) to the appropriate center mass for

each segment. A separate dedicated computer controls all functions of the detector and the data are instantly transferred via DECnet to a VAX 750 for processing to the complete mass spectrum.

SUMMARY The array detector described offers an increase in speed and sensitivity with which conventional and tandem mass spectra of large molecules can be recorded. The most important features are the following:

(1) The complete system can be installed between the exit of the magnet and the collector slit of the JEOL HX110 mass spectrometer without any further modification of the instrument.

(2) The portion of the mass spectrum covered can be varied from a ratio of 1:1.055 to 1:1.3 (possibly to 1:1.4) by adjusting the angle of the detector assembly and the potentials on the quadrupole lenses.

(3) Complete spectra can be recorded either at a fixed angle or the angle can be changed (generally once or twice during the spectrum) to make use of a high ratio for low mass and a lower ratio at high mass to retain unit resolution (and sufficient mass accuracy) at higher mass. The fast, precise and reproducible setting of angle and lenses potentials are made by the computer which controls all functions of the detector.

(4) Survey spectra of a wide mass range are obtained with a few segments at high angle (wide mass range). Once a signal is detected the system zooms in on it by decreasing the angle and, therefore, the mass range recorded. Mass assignments are made at the lowest angle (highest dispersion).

References. 1) J.A.Hill, S.A.Martin, J.E.Biller and K.Biemann, *BEMS* 17, 147-51 (1988)
2) M. Ishihara *et al.*, This conference (paper WPA 135).

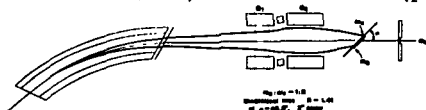


Figure 1 Ion Path with Quadrupole Lenses Placed After the Magnet of MS-2

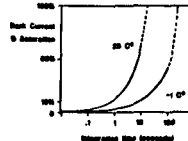


Figure 4 Dark Current vs. Integration Time for Cooled and Room Temp. Photodiode Array

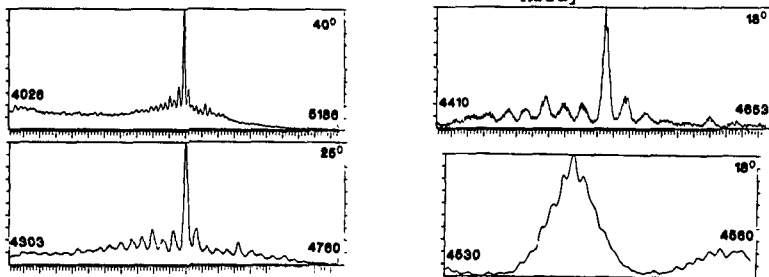


Figure 3 Zooming in on the Region of ACTH 1-39 (M+H)⁺ = m/z 4542.1 (avg.), 4539.3 (monoisotopic) a, b R=500, c R=10,000, d expanded plot of c.

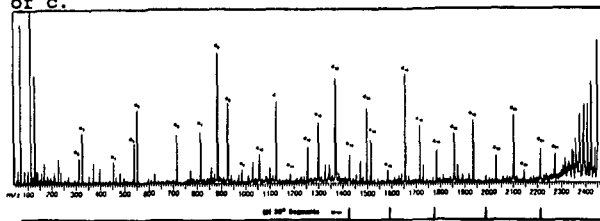


Figure 2 CID Spectrum of ACTH 18-39 (M+H)⁺ = m/z 2465.2 from m/z 60-2450 using 40° (R=1.29) below m/z 1450, then switching to 28° (r=1.12). Collision Gas: Xe. V_{acc}=10kv, E_{col}=2kv

A VERSATILE ARRAY DETECTION SYSTEM

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One of the most effective ways to increase the overall sensitivity of a magnetic sector mass spectrometer (MS) is the simultaneous detection of ions of different mass. This can be accomplished with a position resolving detector placed in the focal plane of the MS.

A number of different technical approaches have been used to implement focal plane detectors (1). Up to now commercial devices have been built around photo diode arrays (2). These systems suffer, however, from an inherent noise problem and the inability to perform a continuous wide mass range scan. The latter problem can only be circumvented by a series of static measurements.

We have designed a new focal plane detector, based on the principle of position and time resolved ion counting (PATRIC) which is able to acquire data by continuously scanning over the complete mass range. The detector is part of a new high performance sector field mass spectrometer, the Finnigan MAT 900. In the environment of the MAT 900 the detector covers a mass range of 8 % of the center mass.

The new detector is characterized by single ion sensitivity, full scan capability and the potential for post-acceleration.

Position determination:

Ions are converted into electron pulses using two micro channel plates (MCP's). A typical gain factor is 10^6 electrons per ion.

The electrons are collected on a multi-anode collector plate (Fig. 1). The anodes (conductive strips) of the collector plate are connected to each other by identical capacitors (Fig. 2).

The position of each ion impinging on the first MCP is determined by calculating the ratio of electrons flowing to either end of the collector plate.

This calculation is done for every ion arriving at the detector (ion counting).

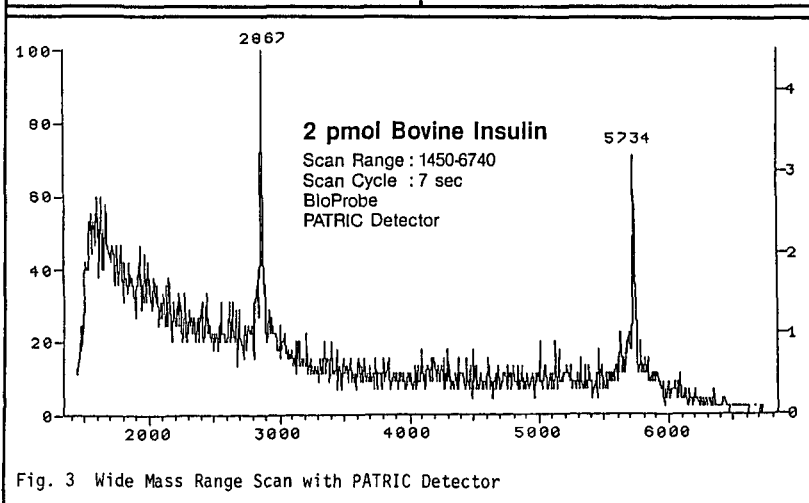
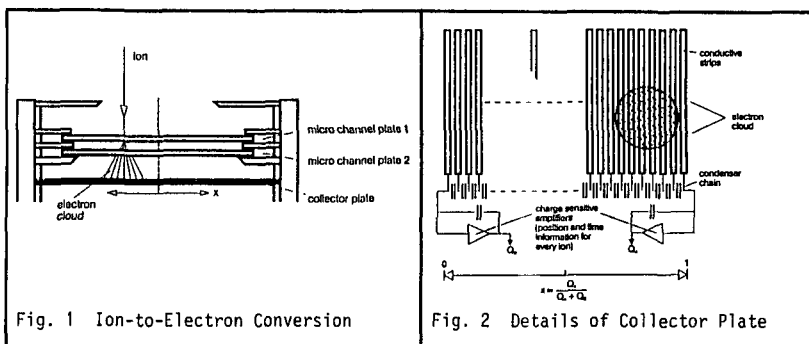
Scanning operation:

Since the response time of the system is extremely rapid (5 usec), it is known for each ion where and when the ion hits the detector. These two pieces of information enable the detector to function in a scanning mode.

-
- (1) Boerboom A.J.H.; Mass Spectrometry of Large Molecules;
Facetti S. Ed.; Elsevier, Amsterdam, 1985; pp 53-73
(2) Cottrell J.S., Evans S.; Anal Chem., 1987, 59, 1990-1995

In an exponential magnet scan all masses move over any given point of the detector with a constant speed. However, masses move over different points of the detector with different speed. The change in speed can be described with an exponential function.

In order to be able to scan and maintain resolution, it is necessary to exactly track the course of every mass over the detector. This is accomplished by a ring buffer mechanism. The buffer addresses are switched synchronously with scanning speed. The change in speed of every mass over the detector is compensated by an appropriate (exponential) detector internal mass calibration. Data from a wide mass range scan are shown in Fig. 3.



Detection of high molecular weight ions by channelplate detectors

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By the optimization of excitation parameters and sample preparation the mass range of the various desorption methods is extended year by year. The mass range of the time-of-flight analyzer is in principle unlimited, but the detection of high molecular weight ions becomes a serious problem.

The critical step in ion detection by electron multipliers is the ion-electron-conversion. The kinetic ion induced electron emission is a mass and velocity dependent process. Threshold velocities of some 10^6 cm/s have been reported in the literature [1,2]. Therefore high mass ions must be postaccelerated to high energies.

We have studied the mass discrimination of a channelplate detector in a high performance TOF-SIMS instrument. The design of the detector provides a postacceleration to energies up to 20 keV for positive and negative secondary ions. The velocity dependence of the detection efficiency was determined for polyatomic ions of different composition like polymers and clusters in the mass range up to 7000 u.

Measurements on Cr-clusters show that the electron yield of a molecule is the sum of the electron yields of the molecule's atoms: $\gamma_{mol}(v) = \sum_{atoms} \gamma_{at}(v)$.

Different polymers have been selected to study the mass discrimination of the detector. From the velocity dependence of the measured secondary ion intensities the detection efficiencies have been determined. These detection efficiencies are shown in fig. 1 for different oligomers of the polymer krytox. At $v = 1 \cdot 10^6$ cm/s the detection efficiencies are still above 0 so that a detection of high molecular weight ions is possible for velocities below $1 \cdot 10^6$ cm/s. In the mass range between 69 and 169 u threshold velocities of $2.5\text{--}4 \cdot 10^6$ cm/s have been obtained.

These measurements allow the determination of the detection efficiency as a function of mass for different postacceleration voltages (see fig. 2). No mass discrimination in the mass range up to 6500 u has been observed at the highest postacceleration voltage (20 kV).

Investigations of different polymer materials with, however, the same masses give raise to the assumption that the sample composition has a strong influence on the electron yields. From figs. 1 and 3 it can be derived that the electron yield and therefore the detection efficiency of fluorine containing polymers is higher than that of other materials. For substances which do not contain electronegative atoms (e.g. F) mass discrimination even below 6000 u has to be expected.

References

- [1] R.J. Beuhler, L. Friedmann; Int. J. Mass Spectrom. Ion Phys.; **23** (1977) 81
- [2] L.A. Dietz, J.L. Sheffield; J. Appl. Phys; **46** (1975) 4361

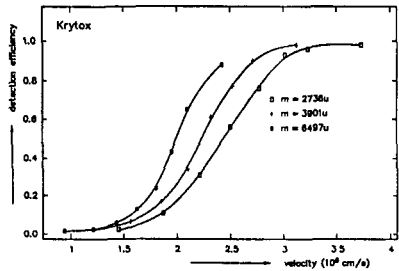


Figure 1: velocity dependence of the detection efficiency for 3 oligomers of the polymer krytox

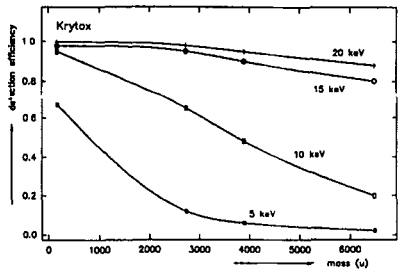


Figure 2: mass dependence of the detection efficiency for the polymer krytox

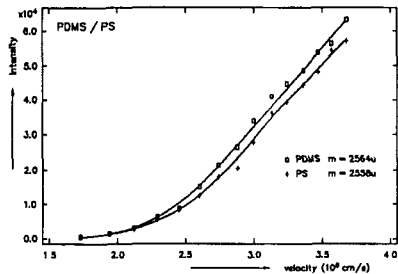


Figure 3: velocity dependence of the detection efficiency for the polymers PS and PDMS

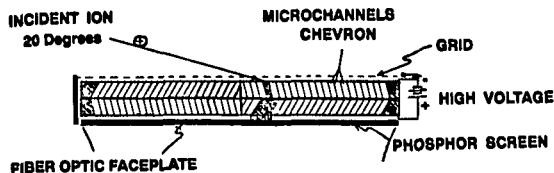
Development of an Array Detector for Wide Mass Range Detection, B. Musselman⁺, R. Eastman⁺, M. Ishihara^{*}, K. Sato^{*}, G. Ellis^o and M. Dixon^o, JEOL USA, Peabody, MA.⁺, JEOL Ltd., Tokyo, Japan,^{*} and Galileo Electro-Optics Corp. Sturbridge, MA.^o

The development of photodiode array-based (PDA) detectors for use on commercial mass spectrometers has been reported in the literature. Cottrell and Evans¹ reported on the design and fabrication of a detector for use in simultaneous detection of at least 4% of the mass range at a specific mass. Hill et al.² subsequently reported the development of a 2" MCP / 2" Photodiode array-based detector which permitted simultaneous detection of 6.6% of the mass range. This same detector was later utilized for the detection of greater than 20% of the available mass range when special ion optics were incorporated into the design of the tandem mass spectrometer in which it was installed.³

We have completed the fabrication of a PDA-based detector which has the capacity for simultaneous ion detection of 10% of the mass range of a conventional mass spectrometer, in this case a JEOL HX110. Two 37.5mm by 10mm active surface area microchannel plates (MCP) Chevron type, were placed end-to-end as shown in figure 1 to form a 75 mm MCP surface. The MCPs were mounted on a second set of identical MCPs to permit a gain of 106 in the detector. The microchannels are 1mm in depth with 12.5 micron diameter. The MCP sandwich was subsequently mounted on a continuous fiber optic element, the front surface of which has been coated with P-31 and aluminized to permit the conversion of secondary electrons to light which could be input to the PDA.

FIGURE 1.

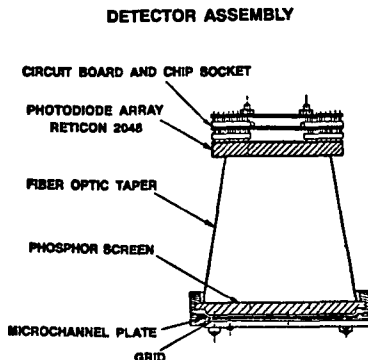
MICROCHANNEL PLATE ASSEMBLY



One end of a 3 inch fiber optic block was drawn down to 2 inches. The 3" end of the resulting fiber optic taper was mounted to the P-31 coated fiber optic faceplate. The 2" end of the taper was coated with a resistive finish to permit adequate grounding of the taper. A Reticon 2048 self scanning linear photodiode array was mounted to the fiber optic taper using a specially designed mounting bracket (figure 2). The incorporation of this 3:2 reducing taper results in the collection of a wider dispersion of ions than could be collected using a commercially available PDA and a forward geometry mass spectrometer of standard configuration. In

addition, the 3:2 taper also results in the collection of a greater percentage of available ion current in the horizontal z-axis. This greater collection efficiency may permit detection of up to 50% of the available ions.

Figure 2



The incorporation of a mechanism of rotation into the detector was completed so that acquisition of mass spectra under conditions of the "enhance resolution" experiment could be completed.⁴ This scanning mode is operational when a tandem mass spectrometer is operated as a single mass spectrometer ion optics system. The utility of this scan mode is that higher resolution measurements can be made without significant diminution of ion intensity. The angle to which the detector must be set for this experiment is 14 degrees off the incident ion beam.

Data acquisition and control of the PDA were completed with a Princeton Instruments ST-120 controller. (Princeton Inst., Princeton Junction, NJ.). This controller was interfaced to a Compaq 286 microcomputer. Software provided by PI and Galactic Enterprises, Salem, NH. was modified to complete data acquisition and reduction.

The effect of the fiber optic taper is to increase sensitivity by resulting in the collection of a greater percentage of the available ion current by the detector. The collection of up to 10% of the mass range of the HX110 mass spectrometer is also possible with this device, thus reducing the time required for collection of a complete mass spectrum.

1. J.S.Cottrell, and S.Evans, Anal. Chem., 59 (1987) 1990.
2. J.A. Hill, S.A. Martin, J.E. Biller, and K. Biemann, Biomed. Environ. Mass Spectrom. 17, (1988) 147.
3. K. Biemann, Hassler Award acceptance speech, Pittsburgh Conference, 1989.
4. T. Matsuo, M. Ishihara, S.Martin, and K. Biemann, Intl. J. Mass Spectrom. Ion Proc. 86 (1988) 23

PERFORMANCE RELATED CHARACTERISTICS OF
LINEAR ARRAY DETECTORS FOR MASS SPECTROMETRY

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Recent years have witnessed a rising interest in array detectors for applications in mass spectrometry. The major advantages of these detectors are increased sensitivity and spectrum acquisition speed. Although the concept has been around for some time, recent advances in component technologies has made the design and implementation of an Electro-Optical Ion Detector (EOID) System much more practical than was heretofore possible.

The performance of any EOID System depends upon such factors as application, instrument geometry, sample size, etc, however, the major components are similar in most cases. A typical detector consists of a combination of microchannel plate electron multipliers, phosphor screen, fused and flexible fiberoptic components and a photodiode or CCD array readout. The two primary design variations for current EOID systems place the solid-state readout either inside or outside the vacuum chamber. Each of these designs has certain advantages and disadvantages. Locating the readout inside the vacuum reduces the number of component and optical interfaces, however, most PDA or CCD device packages are not well suited to vacuum environments and cooling of the device in vacuum can be extremely complicated. On the other hand, placing the readout outside the vacuum eliminates the problems of vacuum incompatibility and excessively complicated cooling, but requires additional fiberoptic components with consequent loss of optical signal in transmission through these added elements.

Major system design considerations for an EOID concern the interfaces between the various electronic and electro-optical elements. In general there are six types of interfaces of concern in current EOIDs:

1. Ion/Microchannel Plate (MCP)
2. MCP/Phosphor Screen
3. Phosphor Screen to Fiberoptic
4. Fiberoptic to Fiberoptic (fused and/or flexible)
5. Fiberoptic to Solid-State Readout
6. Air/Vacuum (electrical or fiberoptic)

Factors affecting system performance to be considered during design of the EOID include the following:

1. Ion detection efficiency and channel size of the MCPs.
2. Charge spreading between MCPs or between MCP and phosphor screen.
3. Phosphor grain size and other phosphor screen characteristics.
4. Characteristics of the epoxy bonds between fiberoptic elements.
5. Fiber size and numerical aperture (NA) of the fiberoptic elements.

Typical interfaces and parameters of concern are illustrated in Figures 1 & 2.

Clearly, the optimization of these parameters is not a trivial task and is further complicated by the fact that most of the system components are non-standard in terms of performance or geometry. Progress toward optimization and standardization is being made, however, and the next generation of EOIDs promises improvements in overall system performance (such as detection efficiency and spatial resolution) as well as cost.

MCP/Phosphor/Fiber-Optic Interface

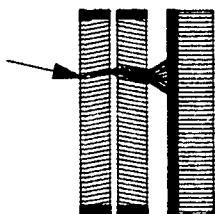


Figure 1

Fiber-Optic/Fiber-Optic Interface

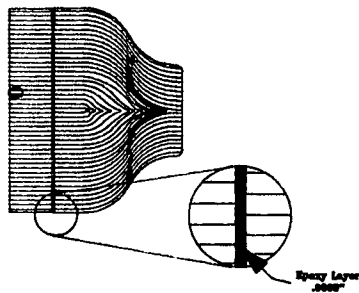


Figure 2

DESIGN OF AN ION OPTICS USING A QUADRUPOLE DOUBLET(QPD)
FOR ZOOMING MASS RANGE ON AN ARRAY DETECTOR

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The sensitivity of a conventional sector type mass spectrometer is increased by a factor of 10 to 100 by using an array detector placed in the focal plane (1). This method could provide new application capabilities. For example, MS/MS system equipped with an array detector (2) can be routinely used for the pico to femto mole level peptide sequence analysis.

For the conventional system, the mass range covered by the array detector is limited and fixed about 5%. Therefore, it requires many times of repetitive measurements to cover an entire mass range. To overcome this tedious time and sample consuming procedure, it is necessary to develop a mass spectrometer which can focus a wide mass range on the array detector. On the other hand, the mass resolution which is limited by the space resolution of the array detector is inversely proportional to the mass range.

Taking account of those factors, we have designed a novel ion optical system (Fig.1) which can easily vary the focusing mass range on the array detector. Basically the new system consists of a double focusing mass spectrometer and an electrostatic quadrupole doublet(QPD) placed in the third field free region. Thus existing mass spectrometer (i.e. JEOL HX110) can be modified to a new optical system. This modification does not affect any original performance of the instrument; when the quadrupoles are set to the ground potential, the new system will return exactly to the original ion optical system.

The mass range is continuously varied by the QPD without moving the detector position P1. Fig.2 shows the simulated ion trajectories under typical QPD conditions. The mass range covered by a 2"-detector under conditions of (a),(b) and (c) are 25%, 12% and 4%, respectively. The inclination angle (θ) of the focal plane depends on the mass range (R). We found that the relation between R and θ is given by

$$R = L/K \tan \theta \sin \theta$$

where L is a detector length and K is a constant depending on the individual instrument.

(Since the scale of the vertical direction is different from that of the horizontal, the angle θ shown in Fig.2 is not correct.)

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- 2) J.A.Hill, S.A.Martin, K.Biemann, Proc., 36th ASMS, 849 (1988).

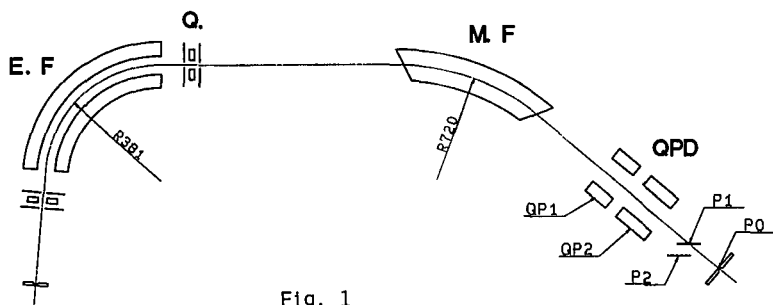


Fig. 1

New ion optics of the JMS-HX110 coupled together with a quadrupole doublet (QPD). P_0 is the point detector. An array detector can be moved away from the optical axis (P_1) to the off-axis position (P_2) when using a point detector.

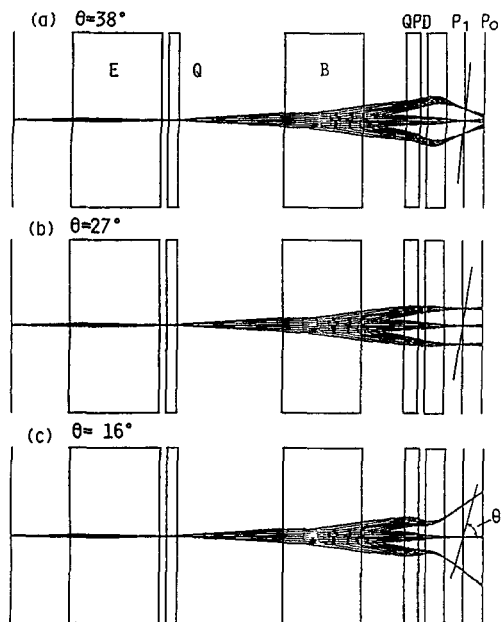


Fig. 2

Simulated ion trajectories show the unique focusing property of the QPD

DESIGN, CONSTRUCTION AND CHARACTERIZATION OF ION DECELERATION LENSES:
WHAT ARE THE SIMPLICITY/PERFORMANCE TRADEOFFS?

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Construction of a hybrid instrument of BEOQ geometry is currently underway in our laboratory for the study of the kinetic energy-dependence of gas phase ion/molecule reactions. A Varian MAT CH5-DF instrument (BE) provides a 3 kV ion beam, which must be decelerated to 1-100 eV and introduced into an rf-only octopole (O) collision cell. Reaction products will be analyzed by the quadrupole (Q) mass filter and Daly detector which complete the ion path through the instrument. For similar applications, a variety of deceleration lens designs have been successfully utilized. They are often fairly complex, however, requiring 30 or more lens elements and exponential fields to achieve deceleration without producing a severely divergent beam. When precise ion energies need not be known, or highly focused beams are not required, deceleration lens of simpler designs may be used. We have investigated the performance of both simple and "complex" deceleration lens systems using the ion optics modeling program SIMION (1), to determine what features of these systems are critical in achieving their performance. Our conclusion is that in some cases lenses with 30 or more elements perform well not because of the exponential fields, but because of the large aperture sizes relative to the dimensions of the ion beam traversing the assembly. If the ion beam samples the center of the field within the lens, where the lines of constant potential are flat and perpendicular to the direction of ion motion, then deceleration without divergence can be achieved. These insights lead to the construction of the deceleration system shown below. The design is shown in Figure 1 and the results of SIMION modeling are shown in 2. In Figure 2 an ion beam with an initial kinetic energy of 2800 eV enters the alignment stage, where X-Y deflection plates center the beam when necessary. Also shown in 2 are the lens components, ion trajectories, and selected lines of constant electric potential. It is clear that there is substantial curvature in the equipotential lines in the deceleration stage, and this curvature can yield a severely divergent beam. However, with the focusing stage before the deceleration stage, the beam dimensions can be decreased in this critical region, causing all of the ions to move up the center of the "potential hill" created by the lens. The resulting ion beam, having been reduced to an energy of 30 eV, exits with minimal divergence.

The device shown in Figure 1 has been constructed. Its performance was evaluated using a Channel Electron Multiplier Array (CEMA) with a phosphor screen, which allows for "beam visualization". The 3 KeV ion beam from the CHS mass spectrometer had dimensions of 4 x 0.75 mm, and a half-angle divergence of 0.25° . It was found that a static einzel lens potential of 5 kV (focusing stage) provided good focusing of the beam into the deceleration stage, and yielded a cylindrically-symmetric ion beam with final energies variable from 10-140 eV with a half-angle divergence of less than 5° in this energy range, which is suitable for injection into the octopole collision cell. Thus, we believe that deceleration lenses need not be complex for high performance operation, when the design/performance aspects are well-understood.

1. Developed by D.A. Dahl and J.E. Delmore at Idaho National Engineering Laboratories.

Figure 1. Cross-Sectional View of Deceleration Lens

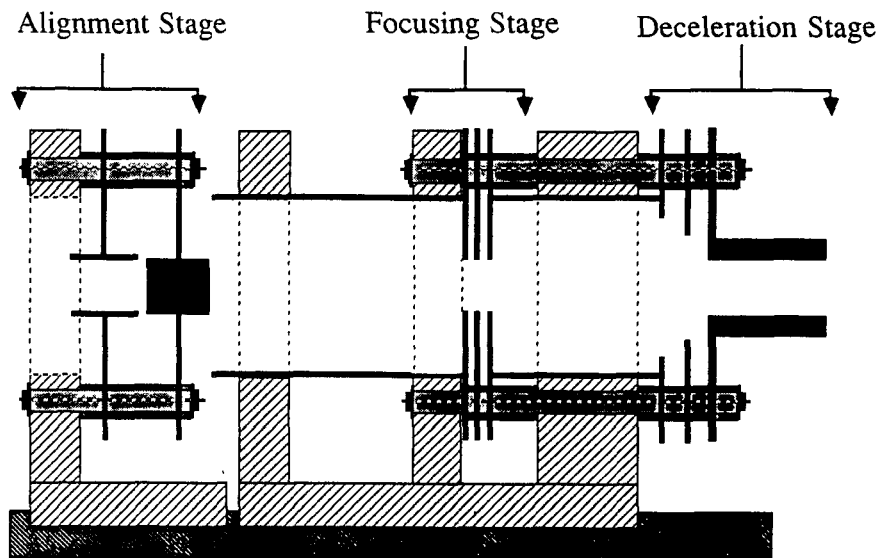
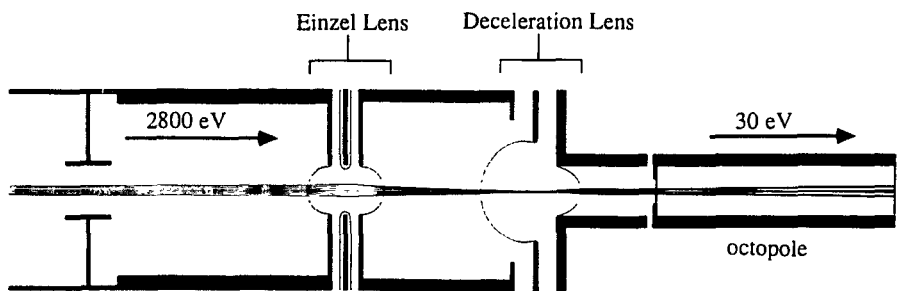


Figure 2. Simple Deceleration Lens with Einzel Focusing



IONIZATION TECHNIQUES BASED ON PROPERTIES OF SUPERCRITICAL FLUIDS:
II INSTRUMENTATION.

by
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The ideal "hyphenated" technique for the interface of chromatography to mass spectrometry for high molecular weight, highly polar compounds has the following properties. 1) The separation process is highly efficient. 2) The interface between the chromatography and the mass spectrometry is easily made. 3) The ionization process is capable of ultra-soft ionization, as well as, highly controlled energetic ionization. 4) The interface and the ionization processes are well suited for high performance mass spectrometers, such as FT-ICR or magnetic sector instruments.

We are developing new ionization techniques that are based on specific reactions that are invoked in gas phase ion clusters. The charge and the size of the clusters are controlled by varying the properties of the solvent before point of expansion into the mass spectrometer.

In this report, the instrument that is used for the development of these techniques was described in detail. The instrument consists of three chambers. In the first chamber the ions are initially formed. The second chamber provides significant ion focussing and deceleration. Finally, the third chamber provides mass spectral analysis by use of an Extrel high-mass quadrupole.

The design of the decelerator lens system was described in detail in this report. The initial stage of the design work was to start with an exponential decelerator originally designed by Vestal et al. (1). In our system a greater extent of deceleration was necessary. Also, the ion beam divergence was greater than that in Vestal's system. SIMION was used design a new exponential decelerator that provides high deceleration rates in less space than in the work of Vestal et al. (1).

- 1) M. L. Vestal, C. R. Blakely, P. W. Ryan and J. H. Futrell, Rev. Sci. Instrum. 47, 15 (1976).

HIGH ENERGY DYNODE (HED) ELECTRON MULTIPLIER DELIVERS INCREASED HIGH MASS SENSITIVITY FOR QUADRUPOLE MASS SPECTROMETERS.

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Previous work by Daley has shown substantial sensitivity increases for quadrupole mass spectrometers by the use of post mass filter acceleration devices. The increased sensitivity can be realized by adding a carefully shaped and positioned High Energy Dynode (HED) near a standard Continuous Dynode Electron Multiplier (CDEM).

The increase in sensitivity using the HED can be attributed to the fact that high mass ions having low initial velocities through the quadrupole analyzer, can be accelerated to a sufficiently high velocity to cause efficient conversion of ions to electrons at the CDEM.

Typical quadrupole mass analyzers yield best resolution at ion energies of five of ten volts. The detection systems used in most quadrupole mass spectrometers are the continuous dynode electron multiplier types which is operated between 1.5 and 3.0 kV. The CDEMs detection ability is highly dependent on the product of mass times velocity. Consequently, for higher mass ions with low velocities, the impact onto the entrance horn of the CDEM at two or three kilovolts of energy, is insufficient to yield a high conversion ratio of ions to electrons. Lower conversion rates of ions to electrons reduces the total current output from the CDEM and therefore the measured ion abundance. Higher ion velocities cause an increase in the conversion to ions to electrons resulting in higher measured ion abundance and higher signal-to-noise ratios.

By adding a HED near the horn of standard CDEM and setting the polarity of the HED to the opposite sign of the ion being analyzed, the ions are directed onto the dynode instead of the horn of the CDEM. The dynode has an operating potential of 2 to 10 kV to provide a high acceleration for increasing the ion velocity and conversion to electrons. The more efficient conversion of ions-to-electrons occurs at the dynode rather than the horn of the CDEM. The newly created electrons pass through the electrostatic field of the horn of the CDEM where further cascading the secondary electrons as the multiplication process occurs throughout the CDEM.

A diagram of the HED coupled with the common CDEM is shown in Figure 1. The dynode material was carefully chosen and positioned. The material and the surface of the dynode were shown to have a dramatic effects on the work function resulting in conversion rates from less than one to more than one. High fields which caused field emissions and high background noise levels were reduced by obtaining a 10 to 20 micro inch finish on the HED and all parts associated with HED system.

Figure 2 shows a plot of the signal abundance vs voltage applied to the HED for ion m/z 502 from a tuning compound. The plot shows the increased S/N which can be gained for the higher mass ions as the voltage on the dynode is increased. For this example, more than a factor of 25 increase can be observed.

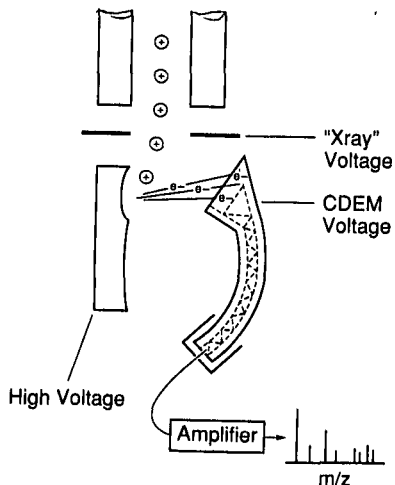


Figure 1. Diagram of High Energy Dynode combined with a standard Continuous Dynode Electron Multiplier for quadrupole mass spectrometers.

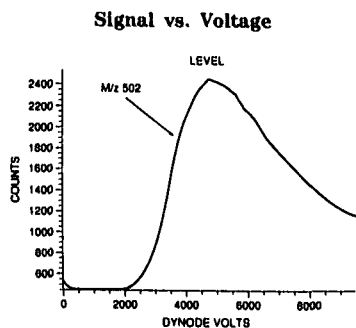


Figure 2. Plot of signal abundance for ion m/z 502, vs the High Energy Dynode accelerating voltage.

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ION TRAJECTORY MODELLING IN AN ION MOBILITY
SPECTROMETER EMPLOYING NONLINEAR ELECTRIC FIELDS

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Ion mobility spectrometry (IMS) can be thought of as time-of-flight mass spectrometry at atmospheric pressure. The techniques for using ion mobility at atmospheric pressure for spectrometry have been well established (1). IMS can be used for the detection of trace constituents of organic compounds in gaseous mixtures. Detection is achieved by ionizing trace molecules via ion/molecule reactions, the product ions of which subsequently drift through an electric field and are separated in time due to their velocity differences. The basic IMS instrument consists of a sample inlet, reaction region, shutter grid, drift region, and detector. In conventional IMS, a uniform or linear field, typically 200 V/cm, is applied across the drift region and the various ions from the sample separate according to their mobilities.

The performance of a conventional IMS system is limited in both resolving power and sensitivity. The shutter grid pulse width controls the sensitivity and resolution. A long shutter grid pulse is desired to allow more ions into the drift region and therefore, provides greater sensitivity, but a short pulse is required for greater resolution. Thus, IMS performance is a compromise between these two parameters.

An approach that uses linear and nonlinear electrical fields to control ion movement in the detection volume has been used to increase the performance of IMS systems (2). The ions of a particular mobility, K_0 , are located at a particular position in the drift region at each instant in time. Their motion can be changed and they can be moved to a different location in the drift region at a later time. This is achieved by providing the proper field between the present and desired location. Examples of drift field voltage control at various instances in time are shown in Figure 1. A linear and forward decreasing ramp can be used, followed by either a nonlinear or linear reversed field, which is then followed by a third ramp to move the selected ions toward the detector (see Figure 1A). By reversing the field at specific times, certain K_0 's can be isolated. If the field at the detector end is the reverse of the field at the shutter grid end (creating an ion well) ions will tend to drift toward the junction of the two fields. If the field strength is increased gradually, more ions will be accommodated and they will also have a reduced spatial distribution. From here they can be ramped toward the detector with a consequent increase in both sensitivity and resolution (see Figure 1B).

We have studied, via SIMION (3), ion trajectories under various dynamic electric field configurations in an IMS instrument. We have found that with proper timing of the field control (employing the user programming option available in SIMION), various K_0 's can be isolated. Modelling has enabled us to explore different electrode voltage magnitude and timing controls. This has allowed us to observe the resulting positional differences among ions of the same K_0 (with slightly different entrance angles and positions) for various electrode voltage changes. Table I gives the results of separating ions of the same K_0 with no angular divergence (ion number 1) from those that have a small divergence (ions numbered 2 or 3). The drift field begins with a linear decreasing ramp and is followed by a nonlinear field reversal that is active for 2 msec. This field sequence would be used to separate higher mobility ions from those of lower mobility. The ion separation can be optimized by adjusting the shape of the nonlinear field. Two conclusions that can be drawn from these data are: (1) with the same reversal function, it is advantageous to start the field at a later time, and (2) for a given

reversal duration, it is advantageous to use a higher order function for the adjustment of the electrode voltages. The duration of the field reversal affects the spatial dispersion of the ions although the flight time differences were small when compared to the nonlinear field results.

Table I

Field Reversal Start Time (msec)	$\Delta x_{1,2}$ (mm)	$\Delta x_{1,3}$ (mm)	Function
3.2	2.214	2.743	sqrt
5.2	4.143	4.626	sqrt
5.2	4.854	5.416	sqr

* $K_0=1.53$

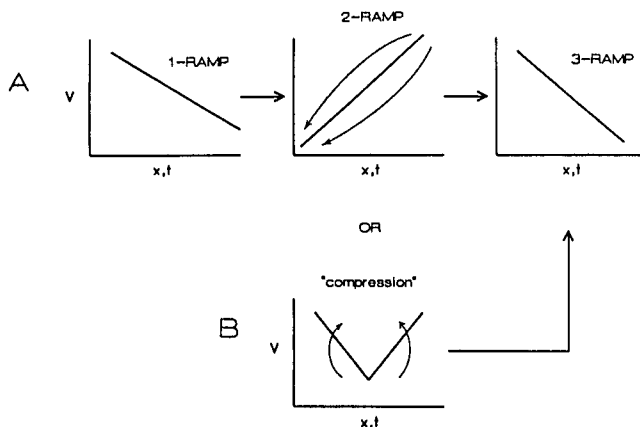


Figure 1

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CALCULATION OF ION TRAJECTORIES IN A QUADRUPOLE MASS FILTER WITH CIRCULAR CROSS SECTION ELECTRODES: A NUMERICAL STUDY

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POTENTIAL DISTRIBUTION

Exact analytical solutions to Laplace's equation are often difficult for geometries with irregularly shaped electrode boundaries even after applying techniques such as conformal transformation. In such cases it is possible to solve Laplace's equation numerically, by iterative evaluation of finite difference equations using relaxation methods. We have applied this approach to calculate the potential distribution within the real bounded quadrupole geometry. This configuration differs significantly from the ideal unbounded quadrupole case which consists of four hyperbolic cross section electrodes which extend asymptotically to infinity. Consequently differences in the potential distribution between the real and ideal cases are expected. We have applied the mathematically satisfying method described by Govorkov [1] for the solution to Laplace's equation in cylindrical polar coordinates.

The integrity of the relaxation method was tested by comparing results from a numerical solution with those calculated by analytical solution to the Laplace's equation for a mathematically well defined electrostatic analyzer (ESA). We found the relaxation had an accuracy of better than one part in 10^9 volt.

The potential ϕ , within four circular rod electrodes is given in polar co-ordinates by the multipole series expansion :

$$\phi = (U + V \cos \omega t) \sum_{n=0} C_n (r/r_0)^{2(2n+1)} \cos [2(2n+1)\theta]$$

The values of the coefficients C_n determine the magnitude of the higher order terms in the expansion and are therefore an indication of how the field differs from the hyperbolic potential distribution (A perfect hyperbolic field is defined when the value of the coefficient C_0 is unity and all the higher order coefficients are zero).

Comparison of the fitted potentials for the round rod electrode geometry with the ideal hyperbolic distribution reveals the regions where the potential distribution has the maximum deviation from the hyperbolic case. The best agreement, i.e. least difference is towards the center of the electrodes.

It is an experimental observation that reducing the entrance aperture into the quadrupole increases resolution, albeit with a penalty in sensitivity. This is consistent with restricting ions to the most ideal or hyperbolic central part of the field. The value of ratio r/r_0 determines the optimum condition for the circular rod approximation to the ideal hyperbolic field and historically the literature contains confusing ratios. We determined multipole coefficients for two r/r_0 values and this allowed other ratios to be linearly interpolated and in particular the value for $C_0 = 1$ was found for $r/r_0 = 1.1483$ which differs from that given by Denison [2], $r/r_0 = 1.1468$. In his work Denison states that the best approximation to the hyperbolic field quadrupole occurs when the value of the C_1 coefficient is zero. Here we observed that when C_0 is unity as required for the ideal hyperbolic case, then C_1 is non-zero. Equally using Denison's criterion that $C_1=0$ we find that C_0 now deviates from unity indicating the optimizing of the ratio r/r_0 for minimum deviation from the ideal hyperbolic case is in reality a subtle operation which has not yet been achieved. As pointed out by Denison [2], the internal radius R , of the earthed vacuum housing should be large enough to minimize field perturbations at the center of the quadrupole field formed within the electrodes. The ratio R/r_0 should be greater than 3.5 and we have used values of $R \sim 10r_0$.

TRAJECTORY CALCULATION : (A Finite Element Method)

The relaxation method has allowed the potential distribution within the quadrupole to be found and subsequent determination of coefficients in the multipole expansion allows the potential to be calculated at any point within the inscribed radius. The electric field components E_x and E_y are obtained through partial differentiation of the expression for the potential.

Since ;

$$E_x = -\partial\phi/\partial x \quad \text{and} \quad E_y = -\partial\phi/\partial y$$

So,

$$\partial\phi/\partial x = (U + V \cos \omega t) [C_0 (2x \cos 2\theta + 2y \sin 2\theta)/r_0^2 + \dots +]$$

and

$$\partial\phi/\partial y = (U + V \cos \omega t) [C_0 (2y \cos 2\theta - 2x \sin 2\theta)/r_0^2 + \dots +]$$

Thus the components of the acceleration a_x and a_y can be found:

$$a_x = zE_x/m \text{ and } a_y = zE_y/m$$

Now if we consider the Newtonian equations of motion then we can determine the distance traveled by an ion from the following expressions ;

$$s_x = u_x \Delta t + 0.5 a_x \Delta t^2 \text{ and } s_y = u_y \Delta t + 0.5 a_y \Delta t^2$$

where s_x and s_y are the resolved components of distance traveled by a particle in a time interval Δt , and u_x u_y are the initial particle velocity components (at the beginning of Δt) and a_x , a_y the acceleration components. Hence the resultant positional increments of a particle or ion subjected to acceleration in the x and y directions for a time Δt is calculated assuming rectilinear motion. It is essential to make Δt sufficiently small that changes in the field are negligible during the time interval and the overall particle trajectory is found by tracing successive positional increments Δs , using Newtonian equations for motion.

To ensure the trajectory is determined accurately it was found necessary to divide the z axis of the quadrupole into 300,000 finite elements corresponding to time intervals, $\Delta t = 2.742 \times 10^{-10}$ s for $u_z = 4.0$ eV for a rod length of 22.6 cm.

The accuracy of the finite element method was evaluated by comparing the trajectory of an ion in a pure hyperbolic potential distribution by both the Newtonian treatment and the analytical solution of the Mathieu equations. The hyperbolic potential is generated by taking the only the first term in the multipole expansion with $C_0 = 1$. Under these conditions both methods give essentially identical particle trajectories as shown in Figure 1 for the Mathieu solution. This establishes the validity of the Newtonian method and allows the trajectories of ions in the non ideal quadrupole field to be calculated by substituting the fitted coefficients into the multipole expansion for the potential. The x and y electric field components acting on an ion in the quadrupole now include terms that are dependent on both the x and y position. The effect of the round rod electrodes on the ion motion is shown in Figure 2 and contrasts significantly with the trajectory in the hyperbolic field, Figure 1.

SUMMARY

1. The ray tracing method allows individual ion trajectories to be determined in the round rod quadrupole geometry, however it does not conveniently describe the general transmission characteristics of the mass filter in the manner given by solution to the Mathieu equation. In order to map the stability diagram we have systematically traced ion trajectories for a series of different initial positions, energies and entry angles.

2. Significantly the apical region of the circular cross section rod quadrupole was found to be rounded and without the apex point described by the Mathieu stability diagram. This is consistent with previous findings by Dawson [3]. Moreover the round rod stability diagram was found to lie within that for the Mathieu solution consistent with a reduced transmission for the round rod case compared with the ideal case.

3. The use of circular cross section electrodes in a quadrupole mass filter has been shown to give rise to distortions of the sixth, tenth, etc order. Studies have calculated the degree of sixth order distortions produced by round rods and indicate that at high resolution precursor peaks may arise. Only resonance lines that pass near or through the apex of the stability diagram are important because they are intersected by the mass scan line. These resonance lines mark positions where the ion trajectories are unstable and appear as dips in the peak shape.

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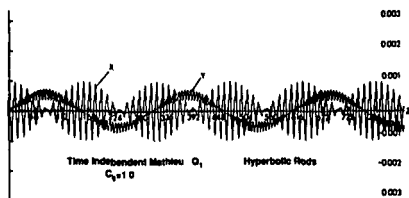


Figure 1 Ion trajectory for the unbounded ideal case with hyperbolic electrodes. (Mathieu Solution)

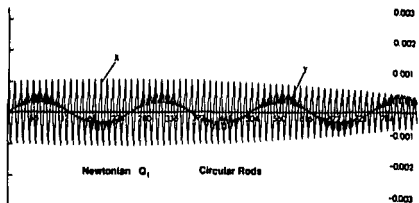


Figure 2 Ion trajectory for the real bounded case with round rod electrodes.

upon an Intel 8085 CPU controls DAC offsets, DAC enabling, scan, scan step time and settling time counters, and provides a sampling trigger for a PC which can be used to set all front panel controls. The data signal is fed to a Compaq 16 MHz AT-class 80386-based PC via a Data Translation DT2801-A board with a programmable gain buffer. Normal, daughter and parent ion scans were obtained and signal averaged using ASYST, a high level scientific software system.

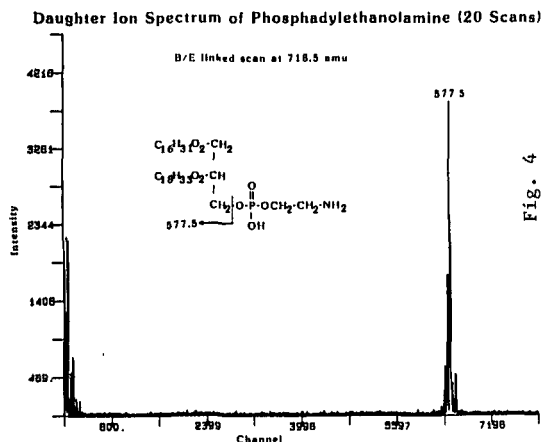
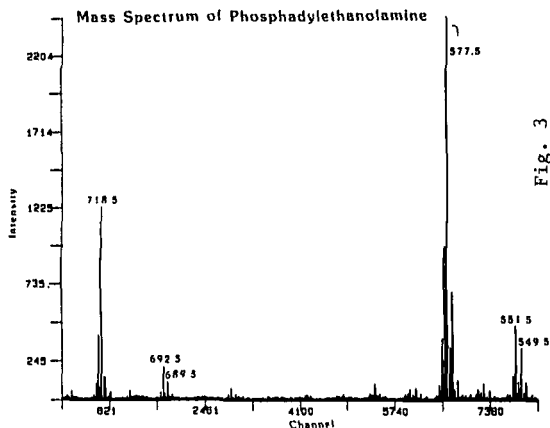


Figure 3 shows the signal averaged (7scans) normal mass spectrum of a phosphatidylethanolamine mixture. The peak at 718.5 amu corresponds to the MH^+ ion of PE with palmitic and oleic acid, while those at 692.5 and 689.5 represent minor components. The loss of the phosphoethanolamine head group from these three components results in peaks at 577.5, 551.5 and 549.5 amu. The B/E spectrum (sum of 20 scans) for the 718.5 amu peak shown in Figure 4 reveals only a single fragment peak at 577.5, and confirms the fact that the other peaks in the normal spectrum result from minor components, and not fragments.

An Improved Interface Design for Determining Radiochemical Purity by Radiogas Chromatography/Mass Spectrometry (RGC/MS). Patrick E. Kastl, Patrick W. Langvardt, Michael J. Bartels and Kathy A. Brzak. The Dow Chemical Company, Midland, MI

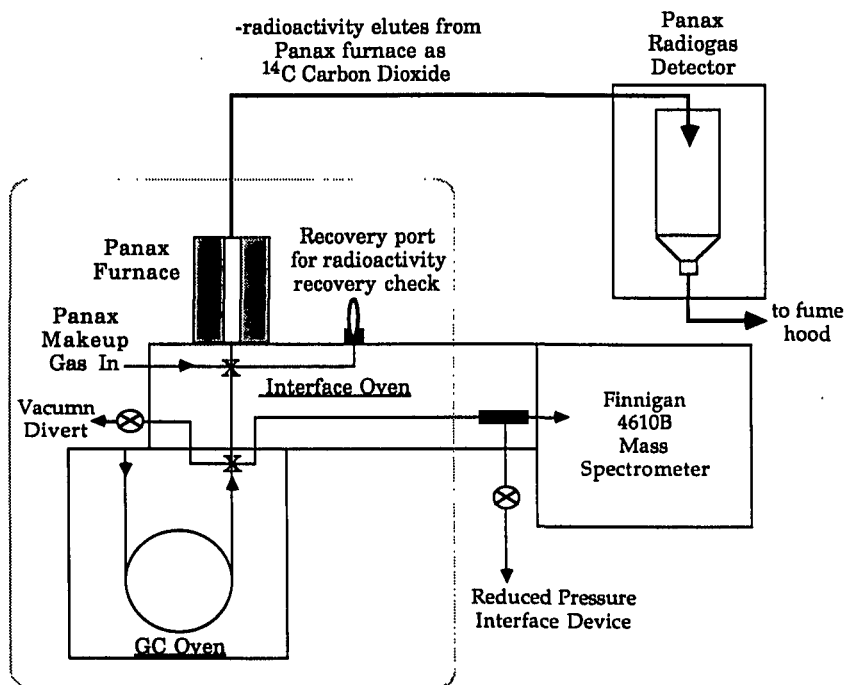
Radioisotopes are commonly used as a means for monitoring the fate of a chemical in a complex matrix. Therefore, determination of identity and the radiochemical purity of these materials is crucial when describing movement through a biological or environmental system. Radiochemical purity is often determined directly by a chromatographic method such as high performance liquid chromatography (HPLC) with an on-line flow through radioactivity detector or by thin layer chromatography (TLC) with a radioactivity scanner that allows for direct quantitation on a solid support. These methods are very versatile and sensitive in detecting small amounts of impurities, however, both HPLC and TLC are usually not useful for volatile compounds.

The objective of this work was to design an simple, maintenance-free interface between a Panax radiogas detector and a Finnigan 4600 GC/MS. The interface was designed to allow either simultaneous or independent operation of the radiogas detector and/or the mass spectrometer. This interface was also designed to allow for quantitative recovery of the injected radioactivity.

The interface was constructed with two (2) glass-lined stainless steel tees that are used to split the gas streams between the mass spectrometer, the radiogas detector and the recovery port (see Figure 1). During a RGC/MS analysis the first tee is used to split the GC effluent between both the radiogas detector and the mass spectrometer so as to simultaneously obtain a radio- and mass chromatogram. A vacuum divert line is also connected to this tee and is used to divert the solvent. The fused silica capillary line from this tee to the radiogas detector or the mass spectrometer may be disconnected and plugged thereby allowing independent operation of either. The second tee is used to route the radiogas detector gas stream (split at the first tee) to either the combustion furnace or to the recovery port. During a RGC/MS analysis the recovery port is plugged so that all the gas flow goes to the furnace and then the radiogas detector. During a radioactivity recovery check the fused silica capillary line to the mass spectrometer and the line after the furnace are disconnected and plugged thereby routing all the GC effluent through the recovery port. The recovery port was built with Cajon o-ring fittings that hold two 150 mg air sampling tubes which are used for collecting radioactivity. Following collection these tubes are solvent extracted and the radioactivity is counted by liquid scintillation counting. Both the Panax furnace and the recovery port are mounted on a separate interface oven cover to allow easy installation and removal. Fused silica capillary tubing was used for all the connections and the size used determined the split ratio.

Representative radiolabeled materials analyzed by RGC/MS include ^{14}C styrene oxide, ^{14}C 1,2-dichloromethane, ^{14}C 1,3-dichloropropene and ^{14}C 1,2-dichloropropane. Some of these materials were shown to contain radiolabeled impurities not originally reported by the manufacturer. Typical radioactivity recovery for compounds with boiling points below 200°C have ranged from 99 to 108% of the total radioactivity injected. To date, the RGC/MS interface has been installed and removed more than 30 times without requiring maintenance.

Figure 1. Overview of RGC/MS System



Legend:

— = 0.32 mm i.d. fused silica capillary tubing

— = 0.53 mm i.d. fused silica capillary tubing

X = location of split tee

⊗ = solenoid valve

CHARACTERISTICS OF A HIGH RESOLUTION ICP-MS INSTRUMENT

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Inductively coupled plasma source mass spectrometry has evolved over the past seven years into what is today possibly the most powerful elemental analytical technique available to the analyst.

All the current commercial instruments use the quadrupole mass analyser which allows the analyst to resolve mass peaks of approximately one mass unit with high ion transmission. This means that polyatomic species with the same nominal mass as the analyte isotope of interest will contribute to the measured signal assigned to that isotope.

Spectral interferences have been the subject of considerable investigation from the inception of the technique and an understanding of potential interferences is essential to the analyst. Whilst computational techniques can be applied to allow spectral correction these are not valid when the magnitude of the signal due to the interference is very much larger than the analyte signal. Such is the case when the interfering polyatomic results from matrix species for example. Other polyatomics can result from the introduction of solvent into the plasma and from the plasma gas itself.

We have developed an ICP-MS instrument utilising a high resolution double focussing mass analyser to resolve such interferences. The instrument consists of a standard VG argon inductively coupled plasma system utilising the Fassel type plasma torch. Sample introduction to date has been by nebulisation of solution in the usual manner. The plasma is coupled to the mass spectrometer by means of a Campargue type supersonic beam interface and a differential vacuum pumping system. The ion energy is infinitely variable up to 6keV and beam shaping optics are used to match the circular section ion beam from the source to the rectangular cross section required by the mass analyser. This ensures that ion transmission and hence sensitivity are maximised.

Resolution is easily adjustable by remotely controlled preset slit assemblies, between low resolution (a mode of operation analogous to that of the quadrupole mass analyser) and high resolution (up to 8000, 10% valley). As there is an inverse relationship between resolution and transmission the low resolution mode is more sensitive and high resolution operation is reserved for the difficult matrices or elements. For example, using a resolution of 4000, Si, S, V, Fe have all been completely resolved from the interferences that normally trouble them. In these cases limits of detection are now a few picograms/ml i.e. factors of 10 - 100 better than in conventional ICP-MS.

The use of the double focussing mass spectrometer has shown further advantages over the quadrupole. The background levels in quadrupole based ICP-MS systems have been reported as between 10 and 100 Hz. This background is universally distributed across the mass range and is believed to be mainly due to UV photons striking the continuous dynode detector and producing a cascade of electrons which register as an ion signal in the counting system. Although the pulse counting detector in our instrument is of a similar form, the long ion flight path, together with the ion optics configuration acting as optical baffles, means that no photons are incident on the detector. The measured background is 0.1 Hz and approaches the dark current limit for such detectors. The instrument also utilises an on-axis Faraday detector for ion beam measurement in order to extend the instrument dynamic range to 10 orders of magnitude.

The different mass response of the double focussing analyser leads to a mass response curve with a form different from that of the quadrupole ICP-MS systems. Greatest sensitivity is found for high mass isotopes. At present, limits of detection of 10 - 50 femtograms/ml have been achieved in low resolution mode.

The requirements with regard to the energy and trajectory of ions entering such an analyser are very different from the quadrupole analyser. The development of a source to give the high ion energies (typically greater than 4keV for high sensitivity) required has necessitated further investigation of the operation of the plasma sampling process and its interaction with the electrostatic focussing of the ion beam. These results show that the plasma sampling interface performs in a way similar to that in the PlasmaQuad instrument and that the acceleration of the ion beam does not appear to affect figures of merit such as the levels of doubly charged ions formed.

The analytical performance of the system has yielded information with regard to the source and nature of polyatomic species. This is of interest to users of the current range of commercial instrumentation in recognising potential problems in analyses and also demonstrates the benefits that high resolution ICP-MS offers the analyst.

ICP-MS MEASURABILITY LIMITS
IN CONCENTRATED SOLUTIONS

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The inductively coupled plasma source associated with the mass spectrometer technique (ICP-MS) is mainly known for the low detection limits (D.L.) it can achieve (sub ppt). D.L. may be defined as the calculated concentration of a pure element which produces a signal equal to twice the standard deviation of the background fluctuations.

Real life samples are trace elements contained in a heavy matrix. A more realistic criterion is then the measurability limit (M.L.). This is the concentration of an analyte that yields an intensity signal equal to the intensity of the background. This value is closely related to the degree of slope of the calibration curve.

With heavy matrix, sample dilution is required in order to obtain a concentration acceptable for the ICP nebulizer, torch and MS interface (possible clogging of orifices, plasma extinction or instability). The drawback is that ultimate dilution of the sample is dictated by the measurability limit of the apparatus used.

It is then important that the apparatus can work with the highest matrix concentration possible during all the time required for the measurements.

Our instrument, the PLASMASS use a standard C1 Meinhard nebulizer and a 1 mm diameter sampling hole but works at 40.68 MHz and use an analog mode of detection. We investigated the response of our instrument and present here three set of results .

The first one come from two different solutions : a matrix of Fe at 5 g/l and a matrix of NaCl at 5 g/l. Several elements (up to 11) have been observed during the same run. For quantitation purpose various low concentrations have been used. For each element the quantitation curve has been drawn. We used AUTOQUANT software for NaCl solution (area calculation), and SPECTRAL software for Fe solution (peak height calculation). For measurability limits calculations a statistical treatment has been used . Results are sum up in the following table.

The second set come from a solution with a Se matrix at 10 g/l. We looked for an identification of unknown elements by scanning a large mass range. We used the SPECTRAL software. On the one hand we recorded the matrix only spectrum (blank) on the other hand the unknown solution. The difference between the two

shows the unknown elements. A semi-quantitative estimation gives sub ppb level for P, Ag, Cd, Os and Pb.

The last set use the SCOPE software for instant display of analysis.

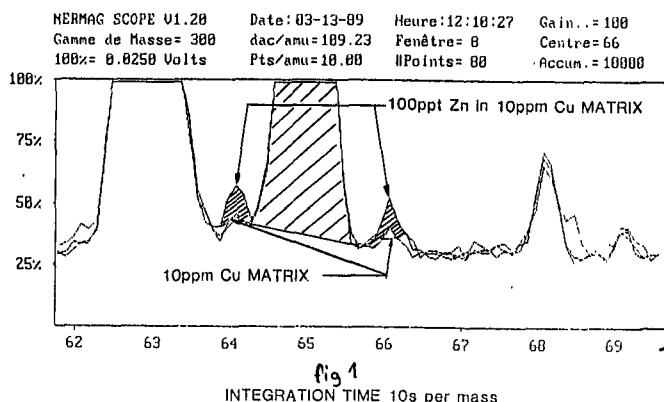
It shows various analysis of real sample as 0.1 µg/l of Zn in a Cu matrix of 0.01 g/l (see fig 1); or 10 µg/l of Mn in a 1 g/L Fe matrix ;or 10 µg/l of B in a red wine sample.

MEASURABILITY LIMIT

	5 g / l Fe Solution						
	11	27	34	44	52	114	120
	B	Al	S	Ca	Cr	Cd	Sn
M.L.	5.2	3.5	5.5	6.9	2.1	0.4	1.5
in solid							
ppb	26	18	28	35	11	2	8
in solution							

	5 g / l NaCl Solution					
	7	9	24	44	45	48
	Li	Be	Mg	Ca	Sc	Ti
M.L. ppb	1	0.4	2.9	3.5	0.4	0.5
ppm	0.2	0.08	0.6	0.7	0.09	0.1
in solid						

	75	88	98	138	139
	As	Sr	Mo	Ba	La
M.L. ppb	2.9	0.8	1	2	0.2
ppm	0.6	0.16	0.2	0.4	0.04



PRECISE MASS MEASUREMENT OF FAB IONS USING AN ALTERNATING SAMPLE PROBE AND PEAK MATCHING ON THE KRATOS MS50

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Objective

For several years FAB ionization has been used in this laboratory to obtain molecular weights for a range of compounds which cannot be handled by other techniques.

High resolution mass measurements using the peak matching technique have been made on a few unknowns in order to obtain elemental compositions but the reference ions used have been from the matrix or reference compounds mixed with sample. The results have been generally unsatisfactory because of difficulties in maintaining both sample and reference ions of suitable intensity during the measurement.

An alternating probe has therefore been built which allows the sample and reference to be deposited on separate surfaces and it has been used in conjunction with a new FAB source and gun mounted on a modified MS50 source housing. The details of construction and the results obtained are described.

The Alternating Probe

The probe consists of a 0.478" diameter shaft through which a 0.063" diameter inner rod passes. The end of the shaft carries an O-ring seal mounted slightly eccentrically relative to the axis of the shaft. The inner rod passes through this O-ring which is lightly lubricated with polyphenyl ether so that it provides a good vacuum seal but allows the rod to be rotated freely. At the vacuum end the rod carries a high voltage insulator and a clamp which allows sample/reference tips of various configurations to be mounted. For the initial experiments a simple cylindrical copper tip is used with two flat faces at 70° to the normal. The eccentricity of the shaft is such that the centre of either of the faces can be aligned with the centre line of the shaft by rotating the rod.

The other end of the rod is attached with a sleeve to a stub shaft carrying a gear wheel. This wheel meshes with a larger gear which can be rotated through a few degrees of arc by the action of a 24 vdc solenoid. A return spring reverses the motion when the solenoid is deactivated. The gear ratio and a mechanical stop are arranged so that the rod rotates exactly 180° when the solenoid is activated. A green LED lights when power is applied to the solenoid and a red LED is wired via a phototransistor sensor so that it will light only if power is applied to the solenoid but the gear mechanism does not complete its proper travel.

Power for the probe solenoid is derived from the MS50 via a relay which operates in parallel with the relay which selects the high mass/low mass position in the peak switching circuitry. A reversing switch allows the solenoid to be activated at either high or low mass.

The FAB Gun

A compact saddle-field FAB gun of a design similar to that already described [A.M.Hogg, Int. J. Mass Spec. Ion Phys., 49 (1983)25.] has been built. It has an integral flange with an O-ring groove and uses only two bolts to mount it to the source housing. It is therefore a very simple matter to clean or replace insulators or electrodes. A simple metal manifold supplies xenon to it via a Negretti needle valve. A standard Spellman power supply [RHR10P60/CR] provides the high voltage, normally 8kV at 1mA.

The Source Housing

The MS50 has been modified so that a source flange with an integral insertion lock can be mounted. This lock is used to admit the alternating probe and a very simple source assembly consisting basically of an extraction slit and two beam centering plates is mounted on the flange. A spring-loaded wiper applies the full accelerating voltage to both the probe tip and the extraction slit.

The FAB gun is mounted through a new port drilled between two of the existing source ports in the housing and a 3" diameter window has been added to one side of the housing so that the probe tip and source region can be clearly seen. The collimating magnets are normally removed to improve visibility and reduce the chance of high voltage breakdown.

Results and Discussion

Our earlier experience with exact mass measurements on FAB produced ions using a simple sample probe showed that devising a particular reference for each measurement attempted was too cumbersome a procedure for routine work. With the alternating probe, the ideal reference would be an alkali halide applied dry to the reference surface and left there for long periods while only the sample in liquid matrix on the other surface is replaced between experiments. CsI is the obvious choice for a reference but it suffers from the sole drawback that its series of cluster peaks are 260 daltons apart. This means that large ratios on the decade box may be required for ions which fall far from CsI clusters and measurement errors in peak matching generally become larger as the ratios used increase. The problem of course becomes less at higher masses and that is where most of the measurements will generally be required.

We have found that systematic errors in the MS50 peak matching system can be kept stable and linear over wide ratios provided both the Y and Z deflection plates are set to ground potential. The loss in sensitivity a 10,000 resolving power that this entails is negligible if all the other tuning parameters are carefully adjusted. It is then possible to use a CsI cluster as a low reference, measure the ratio to the next higher cluster before and after sample ratio measurements (normally just a rapid check), and use a simple linear correction for all the intermediate ratios.

Mixtures of alkali halides have been used as a reference but the advantage of closer spaced ions does not outweigh the simplicity of the single component except at very low mass. Pure NaI has also been used and it has the added novelty of producing a bright yellow glow in the fast atom beam.

Accuracy of measurement is partly dependant on the time spent making it. If the 0.1 ppm decade is not used, a quick measurement on the MH^+ ion of a peptide in the 1000 dalton range can be made with a single sample loading and is accurate to about 3ppm. If such a measurement is followed by withdrawing the probe, adding more matrix, and a very careful match made with a 3 sec sweep of the oscilloscope, 3Hz bandwidth and use of the 0.1 ppm decade, the measurement accuracy can generally be improved to 1 to 2 ppm.

The MS50 used for this work will soon be modified with a fully laminated magnet, shock mounts and a digital mass readout all of which should make this technique more routine and reliable.

FIELD DESORPTION OF ORGANIC POLYMERS

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Introduction

Field desorption (FD) is a useful ionisation method for many non-volatile compounds, e.g. peptides, porphyrins and carbohydrates. The method typically produces molecular ion species $[M]^+$ and/or pseudomolecular ion species such as $[M+H]^+$, $[M+Na]^+$, and can therefore provide molecular weight information. FD remains the ionisation method of choice in mass spectrometry of many synthetic organic polymers.

Experimental

A field desorption source is available on the Kratos Concept mass spectrometer. The FD source (Figure 1), as with the range of other ionisation sources offered on Concept, can simply be clipped into the source chamber of the mass spectrometer to allow quick and easy source switchover. The source is actually a dual source and can be operated in a FAB mode. This feature enables easy calibration at high mass. The FD emitter is introduced into the source on a probe via an insertion lock. The FAB source ion optics are located in front of the FD source optics, the FAB probe being introduced via a side insertion lock.

Results

Results for several organic polymers are shown in Figures 2-6. Figure 2 shows the FD mass spectrum of a sample of polypropylene glycol (av. mol. wt. 1000) from a single scan. Figure 3 shows the FD mass spectrum of a sample of poly (2,2,4-trimethyl-1,2-dihydroquinoline) from an integration of 15 scans. Figure 4 shows the FD mass spectrum of a sample of polystyrene (av. mol. wt. 2800) from an integration of 5 scans. Figure 5 shows the FD mass spectrum of a sample of polyoxyethylene terephthalate - "POET" polymer from an integration of 34 scans. Figure 6 shows the FD mass spectrum of a polysulfone polymer. As can be seen from the spectra the method is able to provide information on the actual molecular weight distribution obtained from the manufacturing process.

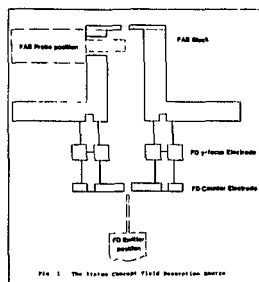
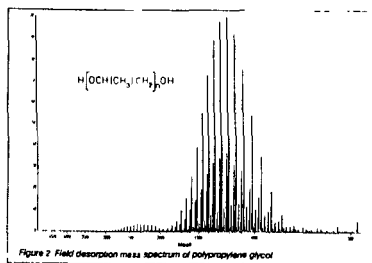
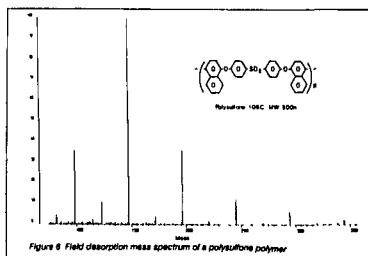
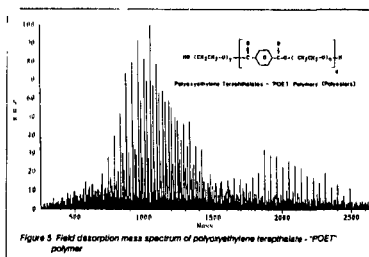
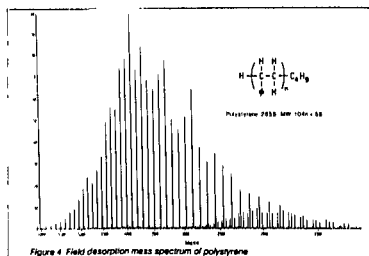
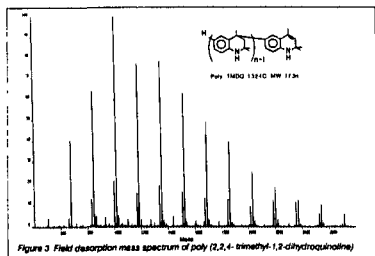


Fig. 1 The Kratos Concept Field Desorption Source





AN AUTOMATED SOLIDS INSERTION PROBE SYSTEM FOR AN ORGANIC MASS SPECTROMETER.

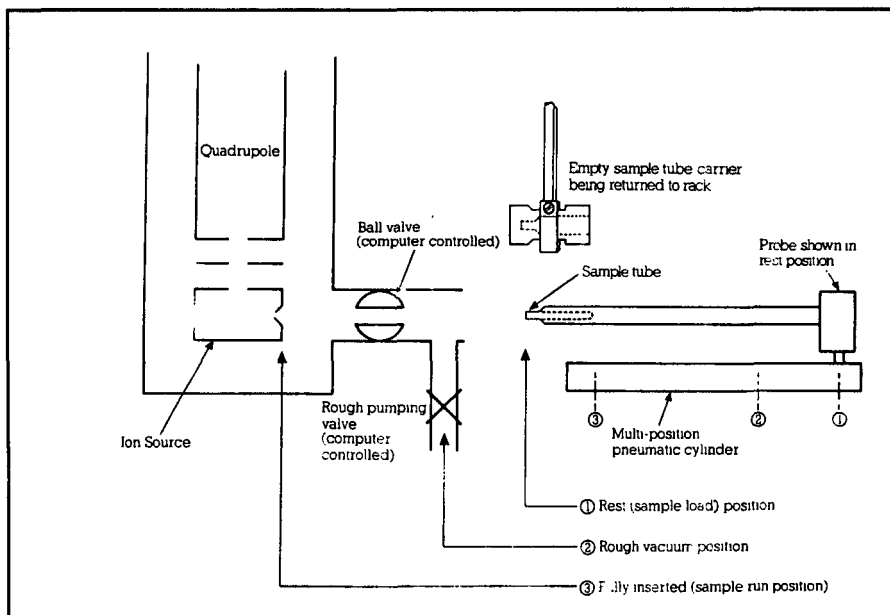
D.C. Smith, V. Gould and H. Mistry.
VG MassLab Ltd., Altrincham, Cheshire, UK.

A high throughput of routine probe samples is a common feature of many laboratories, particularly those in the pharmaceutical industry. This repetitive and tedious procedure involves little operator skill and is therefore ideally suited to some form of automation.

We have developed an automated solids probe system that automatically produces mass spectra and, if required, library search results for each of a batch of solid samples.

A laboratory robot is used to manipulate loaded sample tubes and to remove tubes after analysis. The solid sample insertion probe is automatically admitted to and withdrawn from the ion source using a pneumatically driven multi-position linear actuator. Water cooling and a powerful heater close to the probe tip provide precise control of sample temperature.

The introduction lock is of conventional design. The ball valve is pneumatically operated and the rough pumping is controlled by a solenoid valve. The complete system has been designed to be fitted to the mass spectrometer without interfering with other inlets.



The samples are first loaded into conventional sample tubes in the normal manner. Each tube is then loaded into a stainless steel sample tube carrier. (A separate carrier is used for each sample.) The carriers are placed, in order, on a rack ready for analysis.

The purpose of the sample carrier is two-fold:

The internal profile of the carrier ensures that the sample tube is accurately located to engage with the hole in the tip of the probe.

As each sample is placed within its own sample carrier, the possibility of cross-contamination between samples is eliminated.

The sequence of operation is as follows:

- 1] The laboratory robot picks up the first carrier from the rack and transports it to the tip of the probe.
- 2] The carrier is pushed over the tip of the probe, locating the sample tube in the tip.
- 3] The carrier is withdrawn leaving the tube in position. The tube is pushed fully home using the rim of the empty carrier before the carrier is returned to the rack.
- 4] The probe moves to the first stage of the vacuum lock and the roughing valve opens. When the roughing pressure is sufficiently low the ball valve opens and the probe moves into the ion source.
- 5] If necessary the probe tip is heated to evaporate the sample. Data is acquired in the normal manner.
- 6] The probe is withdrawn to the intermediate position and the vacuum valves close. The probe then moves to the rest position.
- 7] The sample tube is removed from the probe and returned to the rack. The next carrier is picked up and the cycle repeats.

A complementary system is available to allow liquid samples to be automatically injected onto the probe tip. Both systems allow repetitive, routine sampling to be carried out automatically, leaving the operator free to concentrate upon evaluating the results. In addition to relieving the tedium of manual probe operation, laboratory productivity can be significantly improved.

Prediction of Relative Sensitivities in Electron Ionization Mass Spectrometry. Charles Allgood and Burnaby Munson, Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716.

One problem in the quantitation of complex mixtures by any technique is the uncertain availability of the standards which are needed to establish the sensitivities or the relative sensitivities of the compounds in the mixture. Methods of predicting relative sensitivities or correlating sensitivities with molecular structures are of great importance in reducing the necessary number of standardization experiments. With the extensive use of gas chromatography electron ionization mass spectrometry (GC/EIMS) for the quantitative analysis of complex mixtures, the ability to predict the sensitivity of a compound based upon molecular properties would be extremely useful, particularly for environmental and fossil fuel samples for which individual components are not available.

If there are no significant mass discrimination effects in the mass spectrometer, then the total sample ionization as measured at the detector is proportional to the total sample ionization in the source of the mass spectrometer with very similar, if not identical proportionality constants for all compounds. In the present GC/EIMS experiments, performed on a Hewlett Packard Mass Selective Detector, the total ion current, TIC is obtained for each mass spectrum by summing the ion currents of all sample ions registered at the detector. These total ion currents are summed for each component over the time it elutes from the GC. From the ratios of total areas for mixtures of known composition, one can calculate the ratios of sensitivities and of ionization cross sections.

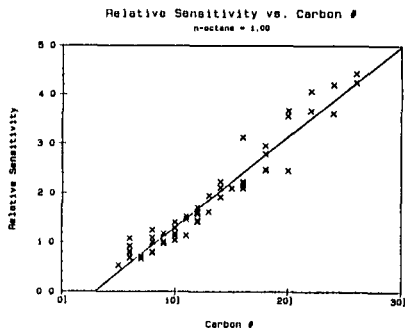
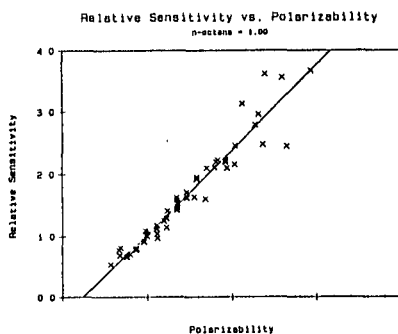
The tuning parameters for the HP MSD varied significantly over the period in which these measurements were made but the relative abundance of the fragment ions in PFTBA, remained fairly constant. For example, the abundance of the ions at m/z 219 (relative to the ions at m/z 69) ranged from .72 to .87 with an average value of $.76 \pm .07$. The relative abundance of ions at m/z 502 ranged from .05 to .14 and had an average value of $.10 \pm .03$. The variation in tuning parameters did not have a significant affect on either the short, medium, or long term reproducibility of the technique. The average standard deviation was about 3% for the short term and 15% for the long term experiments.

In order to assure no losses were occurring due to adsorption of high molecular weight compounds a set of experiments were done in which the temperature of GC/MS transfer line and the ion detector were varied $\pm 40^\circ\text{C}$ from their standard temperatures (280°C). The data showed no major variation in this range of temperatures. The value for the relative sensitivities of the higher molecular weight, less volatile esters were somewhat lower in the experiments using the lowest temperature (240°C) for the transfer line, then at 280°C or 320°C , however there was no indications of significant adsorption losses of these compounds at the temperatures routinely used in these experiments.

A comparison was made between the relative sensitivities for one set of compounds measured on two different mass spectrometers, the MSD and a Dupont magnetic mass spectrometer. The results are in very good agreement, the average ratio of the two results being nearly unity, 1.02 and the differences between the values being less than the uncertainties in the measurements. Other samples were introduced from a solids probe and also gave results in good agreement with the MSD indicating no significant discrimination effects of differential loss processes in the experiments.

The relative sensitivities obtained in these experiments can be compared to previously measured ionization cross sections (1) after appropriate scaling to a common reference compound. The average value for the ratios of these two sets of data was 0.98 with a standard deviation of ± 0.06 .

Linear correlations between the sensitivity or ionization cross section for a wide variety of compounds with a number of easily obtainable molecular parameters (molecular weight, carbon number, molecular polarizability) have been observed. The molecular polarizability, which can be calculated from the molecular formula, appears to give the most reliable prediction for the relative sensitivity of a compound in these GC/EIMS experiments.



1) Harrison, A., Jones, E., Gupta, S., and Nagy, G. Can. J. Chem., 1966, 44, 1967.

Internal Standard System for Use with APCI Mass Spectrometers

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At R.J. Reynolds, a SCIEX TAGA 6000 tandem mass spectrometer is used to sample environmental tobacco smoke (ETS) produced in a 16 m³ stainless steel test chamber. Real-time measurements of ETS constituents are typically obtained over a 0.5-4 hour sampling period. Our experience has shown that during this period, sensitivity of the spectrometer can slowly drift by as much as 10%. In addition, some analytes are plagued by interferences from other major constituents of the ETS matrix, and occasionally, abrupt changes in sensitivity occur.

A system to overcome changes in instrument sensitivity was developed. A steady concentration of an internal standard is blended with air sampled from the environmental chamber. Response of the spectrometer to the standard is used to correct data obtained for other analytes. Results from tests of this system with d5-pyridine as the internal standard are presented here. Pyridine vapor was generated in the chamber, and the internal standard was used to correct both natural and electronically induced changes in instrument sensitivity.

Figure 1 shows a block diagram of the internal standard system. The internal standard is generated in a Kin-Tek precision gas standards generator. A Kin-Tek d5-pyridine permeation tube (1021 ng/min @ 60°C) was placed in the generator. The temperature in the generator was set to 30° to reduce the permeation rate of the standard (est. ≈180 ng/min). In the work described here, flow through the oven was set at ≈250 ml/min, and dilution air was not used. The output stream from the generator was connected to the bottom of a "T" fitting. Diluted air from the ETS chamber was also drawn through the fitting. The combined flow passed through a 30 cm length of Teflon® tubing which was connected to the APCI source of the spectrometer.

Real time data was obtained with SCIEX's MIXTUR program. Internal standard data was collected in the first of eight reaction monitoring channels. Following a run, the raw data was converted to an ASCII file with MITEXT, a program specially written by SCIEX for that purpose. The data was then downloaded to a PC, and internal standard correction was performed using a program written in this lab.

Figure 2 shows an example of the application of the internal standard to the correction of both long term sensitivity drift and sudden changes in instrument sensitivity. The progressive separation of the two lines, particularly at 12-60 minutes, is due to drift in the spectrometer sensitivity. The sudden drop and rise in the uncorrected line at 60 and 180 minutes was induced by manually changing the potential on one of the lenses. The sudden changes shown here are of greater magnitude than those naturally observed in this lab. The internal standard was able to correctly for both changes in sensitivity induced in this manner.

Use of the internal standard to correct the concentration of pyridine measured in an ETS matrix is illustrated in Figure 3. The chamber was operated at one air change/hour during this test. Two Kentucky reference 1R4F cigarettes were smoked in the chamber between minutes 12-20. Pyridine is generated during the entire smoking interval. However, the concentration of nicotine present in the chamber during this test was sufficient to suppress the ionization of pyridine. Both pyridine and d5-pyridine have similar ionization characteristics, and it can be assumed that, in the presence of interferences, the signal from each of these compounds will be decreased by the same relative amount. Following correction of pyridine concentrations with the internal standard, the concentration profile obtained is consistent with the steady generation of pyridine over the smoking interval followed by decay due to dilution with incoming air.

The internal standard system described here provides a simple, reliable method for correcting sensitivity changes of an APCI mass spectrometer. Use of the system to overcome abrupt changes in sensitivity, sensitivity drift, and matrix interference have been demonstrated. The system consists of a stable source of internal standard (with sensitivity similar to the compound being analyzed) and necessary plumbing to introduce the standard into the gas sample being analyzed in the APCI ion source.

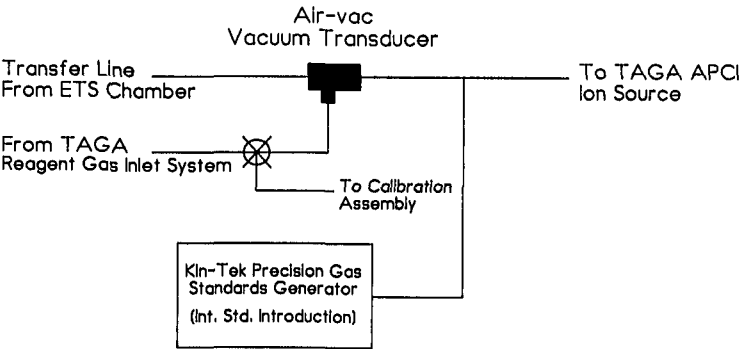


Figure 1

Smoothing of Electronically Induced Signal Variations with
Internal Standard

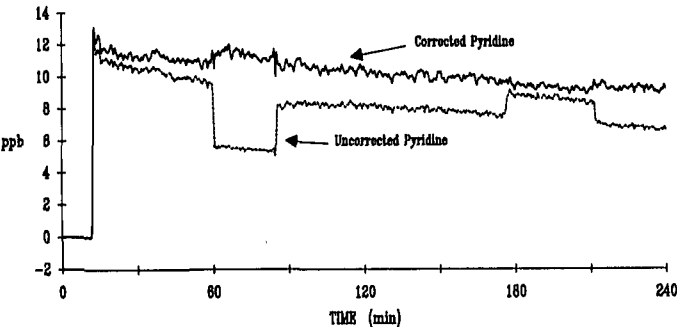


Figure 2

Correction of Pyridine in ETS Matrix with Internal Standard

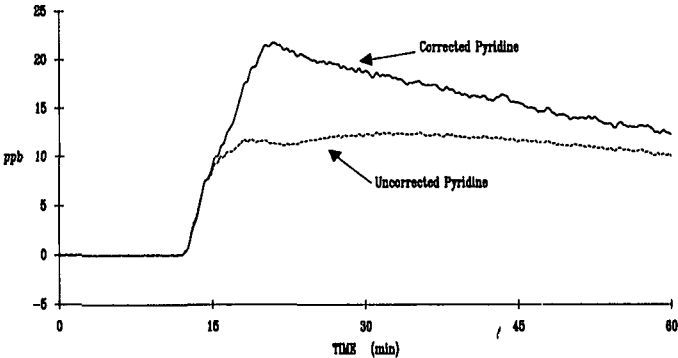


Figure 3

NON-CONTAMINATING CI MASS CALIBRATION
STANDARDS FOR QUADRUPOLE AND MAGNETIC INSTRUMENTS

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In an attempt to compare quadrupole and magnetic spectrometers for sensitivity in the mass range 1000 to 2000 daltons we found the widely-used calibrant tris(perfluorononyl) triazine to give much lower ion currents at m/z 1466 and m/z 1066 on a Hewlett Packard 5988A quadrupole instrument in positive ion electron ionization than was obtained on a VG ZAB SE magnetic instrument under the same conditions. Severe contamination occurred on both instruments, with visible residues accumulating on cool surfaces of the HP 5988A during use. The problem appeared to be due to low quadrupole sensitivity for this compound requiring large amounts of the calibrant to be introduced in order to obtain useful spectra. In an effort to obtain a less contaminating calibrant we investigated perfluoroacyl carbohydrates in this regard. Objectives in the design of a better calibrant for use in this mass range were volatility, usefulness via the direct insertion probe inlet and a spectrum with an adequate number and distribution of ions of significant intensity to be useful in calibrating magnetic instruments.

PROCEDURE: Cellobiose (4- $\{\beta$ -D-glucosido $\}$ -D-glucose) was converted to both homogeneous cellobiose octa(heptafluorobutyrate) and to the mixed ester heptafluorobutryl/pentafluoropropionyl (HFB/PFP) cellobiose as follows: 20 mg of the carbohydrate was heated for 48 hours at 60 degrees with 0.5 ml of acetonitrile and 0.5 ml of either heptafluorobutyric anhydride or a 3:1 (v:v) mixture of HFB anhydride and PFP anhydride. The derivatized substances have been found to be stable for several months at -20° C. if stored in the original reaction mixture.

RESULTS

NEGATIVE ION CI: The negative ion methane CI spectrum of octa (heptafluorobutryl) cellobiose on the ZAB had intense ions at M^{-} (m/z 1910) and $[M - HF]^{-}$ (m/z 1890). The same ions appeared in the spectrum from the 5988A with reversed intensities (m/z 1910 $>$ 1890). Other high-mass ions in the spectra from both instruments were m/z 1713 ($[M - C_3F_7CO]^{-}$), m/z 1697 ($[M - C_3F_7COO]^{-}$), m/z 1694 ($[M - F - C_3F_7CO]^{-}$) and m/z 1677 ($[M - HF - C_3F_7COO]^{-}$).

POSITIVE ION CI: In positive ion methane CI the ZAB SE spectrum of octa(heptafluorobutryl) cellobiose had ions of useful intensity at $[MH - HF]^{+}$ (m/z 1891) and ions resulting from the losses of heptafluorobutyric acid at m/z 1697, m/z 1483 and m/z 1269.

POSITIVE ION EI: These spectra had no high-mass ions of useful intensity on either instrument.

NEGATIVE AND POSITIVE ION CI OF THE MIXED DERIVATIVE OF CELLOBIOSE: In the negative ion methane CI spectra of heptafluorobutryl/pentafluoropropionyl cellobiose well-distributed ions of good intensity were obtained on the ZAB SE. The negative ion spectrum on the Hewlett Packard 5988A was similar in intensity to that of the ZAB but with differences in the fragmentation pattern as noted. The positive ion methane CI spectrum of the mixed derivative on the ZAB SE was similarly constituted with many ions of useful intensity. A characteristic of these spectra that makes them useful, aside from intensity of the ions, is that the mixed derivative spectra are made up of ions 50 u apart (i.e. the difference in mass between heptafluorobutyric and pentafluoropropionic acids) that result from molecules with different HFB/PFP ratios. Typically a 50 microgram sample introduced by an unheated probe and with a source temperature of 100° (HP) or 150° (ZAB) gave useful spectra for tens of minutes. Residual calibrant was not a problem, even the strongest ions were not evident once the probe was withdrawn.

Listed below are the calculated mass values and intensities of negative and positive ions in the spectra of the mixed heptafluorobutyl/pentafluoropropionyl derivative of cellobiose obtained by methane chemical ionization on a ZAB SE.

NEGATIVE ION CI (CH₄)

POSITIVE ION CI (CH₄)

Mass	Height%	Mass	Height%	Mass	Height%
R 2003.90990	4.46	R 1462.95409	9.60	R 1890.92429	0.25
R 1972.91150	8.46	R 1446.96059	12.50	R 1840.92749	0.20
R 1953.91309	7.08	R 1443.95709	15.60	R 1790.93069	0.30
R 1922.91420	8.46	R 1426.95439	10.50	R 1740.93390	0.40
R 1909.92319	3.08	R 1412.95729	6.90	R 1696.94409	0.30
R 1903.91629	7.69	R 1393.96030	6.80	R 1690.93709	0.30
R 1889.91699	7.40	R 1379.96109	11.20	R 1646.94730	1.30
R 1872.91789	2.90	R 1361.95049	7.20	R 1596.95049	2.00
R 1859.92639	9.10	R 1329.96429	10.10	R 1546.95369	2.10
R 1853.91949	5.38	R 1311.95369	6.20	R 1496.95689	1.50
R 1839.92020	28.00	R 1279.96750	4.50	R 1482.95769	0.50
R 1822.92110	3.85	R 1261.95689	3.20	R 1446.96010	0.30
R 1809.92960	18.30	R 1211.96010	1.00	R 1432.96089	1.70
R 1789.92339	60.30	R 1140.94440	1.20	R 1382.96410	2.50
R 1759.93279	20.50	R 1126.95000	7.60	R 1332.96729	2.10
R 1739.92659	74.50	R 1090.94759	1.30	R 1282.97049	1.50
R 1709.93599	19.20	R 1076.95000	8.80	R 1268.97130	1.40
R 1696.94460	4.90	R 1040.95079	2.50	R 1254.97209	0.50
R 1689.92979	60.50	R 1026.95000	6.40	R 1232.97369	0.40
R 1676.93839	3.90	R 990.95399	1.60	R 1218.97449	3.60
R 1662.94130	12.20	R 943.96069	0.60	R 1184.96909	0.30
R 1659.93919	11.30	R 912.96230	1.30	R 1168.97769	4.20
R 1646.94780	12.31	R 893.96390	1.30	R 1118.98090	3.00
R 1643.94429	22.10	R 890.96039	0.30	R 946.96390	11.20
R 1639.93300	34.90	R 862.96550	2.30	R 896.96709	22.70
R 1626.94159	16.50	R 843.96709	1.30	R 846.97029	21.10
R 1612.94449	21.00	R 812.96869	0.60	R 796.97350	10.50
R 1596.95099	32.40	R 793.97029	0.30	R 704.98259	7.90
R 1593.94749	36.00	R 743.97350	0.30	R 703.97479	9.90
R 1576.94479	30.10	R 426.96509	100.00	R 654.98579	13.50
R 1562.94769	25.60	R 407.96669	26.00	R 653.97800	17.70
R 1546.95419	34.60	R 388.96829	22.00	R 604.98900	9.40
R 1543.95070	42.20	R 376.96829	99.60	R 603.98119	12.40
R 1526.94800	30.70	R 357.96989	15.00	R 518.99109	88.40
R 1512.95090	17.00	R 338.97150	15.00	R 468.99429	100.00
R 1496.95740	23.60	R 326.97150	31.00	R 418.99750	48.90
R 1493.95389	28.30	R 307.97310	3.00	R 320.99959	21.30
R 1476.95119	21.20			R 307.02039	26.40

THE DYNAMICS OF IONS IN THE DRIFT REGION
OF A CORONA TYPE MS SOURCE:
SOME MATHEMATICAL MODELS
MARK BUSMAN AND JAN SUNNER,
DEPARTMENT OF CHEMISTRY
CURT VOGEL, DEPARTMENT OF MATHEMATICS
MONTANA STATE UNIVERSITY
BOZEMAN, MT 59717

Techniques using high pressure ion sources are becoming increasingly important in mass spectrometry. Examples are atmospheric pressure ionization mass spectrometry (API/MS) usually with a corona discharge and electrospray (ESP) mass spectrometry. In both of these techniques, because of high pressure, ions are transported through the source by ion drift in electric fields and by diffusion in concentration gradients,

$$J = \rho \kappa E - D \nabla \rho$$

where J is the ion current vector, ρ the number of ions per unit volume, κ the coefficient of ion mobility, E the electric field vector. Also, with corona discharges or electrospray, only ions of one polarity are present in the source. Typically, the associated space charge is the main factor determining the potential distribution and the electric fields in the source. Then the motion of the ions is determined more by the space charge distribution than by the potentials applied to the ion source walls, to extraction electrodes or to discharge needles or capillaries. The relative importance of the space charge effect depends on particular experimental parameters. Important factors are, for example, the discharge current, the applied electrode potentials, and the size and geometry of the ion source. In the extreme case where all electrodes and ion source walls are at the same potential (a field free source) ion transport will be determined solely by the space charge. There is presently very little information on space charge effects in mass spectrometry sources.

Here, we present a study of the effects of space charge on ion transport in high pressure sources.

A complete mathematical description of ion motion can be obtained by combining Maxwell's equations for electrodynamics with the Navier-Stokes equations for fluid dynamics. We are primarily interested in steady state behavior, and we assume that ion concentrations are sufficiently low that viscous effects are negligible. This results in considerable simplification of the model.

The relationship between the charge density ρ and the electrostatic potential, or voltage, u is given by Gauss' law

$$-\nabla \cdot \nabla u = 4\pi \rho / \epsilon$$

Here ϵ is the electric permittivity of the medium and is assumed to be constant. $E = \nabla u$ is the electric field.

The continuity equation

$$\nabla \cdot J = 0$$

essentially says the amount of current entering an infinitesimal volume element equals the amount of current leaving the element. The current density is given by

$$J = \rho \kappa \nabla u - D \nabla \rho$$

The first term represents ion drift, or transport, due to the electric field. The second term represents diffusion of ions resulting from the concentration gradient. κ is the coefficient of ion mobility, and D is the diffusion coefficient. Both κ and D are assumed to be constant. A further simplification in the model occurs if we ignore diffusion effects and set $D=0$. We refer to this as the diffusion free case.

For very special geometries and for the diffusion free case ($D=0$), we can obtain analytical solutions to this system. We present results for the following geometries in three dimensional space:

1. Two planes, both perpendicular to the x-axis.
2. Two concentric cylinders with circular cross sections centered at the z-axis.
3. Two concentric spheres centered at the origin $x=y=z=0$.

These geometries, while not exactly corresponding to any real physical system, help to give some indication of the effect that space charge can have in various types of sources. Furthermore, these analytical solutions set the groundwork for the extension of the method, both toward more realistic geometries and to a system incorporating diffusion effects.

This work offers some obvious areas for extension. First, the method needs to be made applicable to a wider variety of source geometries. However, this will require a great leap in mathematical complexity. Rather than utilizing the analytical methods, the solutions are going to need to be approximate. We have utilized Galerkin methods to discretize the system. We select basis functions satisfying homogeneous Dirichlet boundary conditions. The system can then be solved by iterative methods. There are several issues to address in the implementation of this method. One of the most important is the choice of appropriate basis functions and boundary functions. Taking into account the symmetry of system can reduce the dimension of the problem which consequently reduces the computational complexity.

Second, it would be desirable to extend the method to time dependent systems. Many applications would benefit from further understanding of non-steady state ion drift. Progress has been made in modelling such behavior in the most elementary geometries.

Third, for many situations, the neglect of diffusion is not realistic. The incorporation of diffusion represents another substantial increase in the mathematical complexity of the problem. Such complexity taxes the practicality of the problem from a computational standpoint.

INSTRUMENT-INDEPENDENT CAD DATABASE FOR IQQ INSTRUMENTS:



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Dynamically-correct (i.e., instrument-independent) branching ratios can be measured in XQQ (QQQ, BEQQ, etc.) tandem mass spectrometers (MS/MS) under single-collision conditions. That is, a kinetics-based measurement protocol can provide accuracy and precision for CAD measurements within XQQ instruments. This protocol can be used to develop an instrument-independent MS/MS database (library) for XQQ instruments. To do so, the key MS/MS parameters must be properly selected to compensate for (a) the reaction-induced mass discrimination within Q2 (the rf-only quadrupole mass filter which contains the collision region), for (b) the intrinsic mass discrimination within the Q3 mass analyzer, and (c) for the kinetic energy of the product ions entering Q3.

For the collisionally activated dissociation (CAD) of CH_3CO^+ at a center-of-mass interaction energy $E_{\text{cm}} = 2.4\text{--}38.6$ eV [the range of collision energies used for CAD], the sum of the branching ratios equals 1.00 ± 0.05 for all the fragment ions observed. That is, the rate of reactant ion decay equals the rate of product ion formation. For the source compounds studied [ethanol, oxirane, cis-2,3-epoxybutane, and $\text{CH}_3\text{CO-X}$ (where $\text{X} = \text{H}, \text{CH}_3, \text{CH}_2\text{CH}_3, \text{CH}_3\text{CO}, \text{CH}_3\text{COCH}_2$, and C_4H_9)], the reactant ion entering Q2 appears to be pure CH_3CO^+ . The collisional activation converts a small fraction of the CH_3CO^+ entering Q2 into the other $\text{C}_2\text{H}_3\text{O}^+$ isomers prior to their fragmentation. The product ion distribution for the CAD of $\text{C}_2\text{H}_3\text{O}^+$ at $E_{\text{cm}} = 2.4\text{--}38.6$ eV is quite different from that observed at 4–8 keV. The energy dependence of the branching ratios for $\text{C}_2\text{H}_3\text{O}^+$ from $\text{CH}_3\text{CO-X}$ source compounds is quite distinct from that observed for $\text{C}_2\text{H}_3\text{O}^+$ from ethanol or ethylene oxide (a carcinogen), or for the isobaric C_3H_7^+ from n-pentane. Hence, one can use the CAD of 43° to distinguish $\text{CH}_3\text{CO-X}$ compounds from other source compounds, including sources of the isobaric C_3H_7^+ . Therefore, the energy dependence of the branching ratios may provide a MS/MS "fingerprint" for ionic substructures. The "CBRIS" database format (Table 1) is proposed for the development of an instrument-independent CAD database of Characteristic Branching Ratios of Ionic Substructures.

Table 1. Proposed "CBRIS" Database Format ^{1,2,3}

Parent Ion: $\text{C}_2\text{H}_3\text{O}^+$ (m/z 43) ^{4,5} **Notes:** 9, 99, 999 ⁶
Source Compound: 2,3-butanedione (99.9%)
Ionization Mode: 70 eV electrons

E_{cm} (eV)	σ (Å ²)	α (13°)	β (14°)	γ (15°)	δ (26°)	ϵ (27°)	ζ (28°)	η (29°)
2.4	15 (20)	0	0	0.999 (2)	0	0	0	0.0010 (50)
19.3	22 (10)	0.0127 (15)	0.0493 (6)	0.915 (2)	0.0101 (7)	0.0013 (10)	0.0005 (35)	0.0114 (7)
38.6	21 (10)	0.0227 (10)	0.0750 (6)	0.839 (2)	0.0436 (6)	0.0055 (20)	0.0055 (20)	0.0086 (15)

- 1 CBRIS= Characteristic Branching Ratios of Ionic Substructures.
- 2 For this example we show the branching ratios (α - γ) vs. the center-of-mass collision energy (E_{cm}) for the CAD of ($C_2H_3O^+$) from the source compound biacetyl.
- 3 The numbers in the square brackets represent the maximum uncertainty in the cross section σ and in the branching ratios (α - γ), expressed as a percentage of each σ and of each branching ratio (e.g., for biacetyl at $E_{cm}=2.4$ eV, the maximum uncertainty in γ is $\pm((2\%)/100) \cdot \gamma$; i.e., $\gamma = 0.999 \pm 0.02$).
- 4 A reference citation would be provided for each CAD spectrum to identify the source of the data.
- 5 For the CAD of any given parent ion (e.g., $C_2H_3O^+$), appropriate corrections must be made for contributions from the concurrent CAD of isobaric ions (e.g., $C_2H_7^+$), regardless of their source. The isobaric ions may be co-produced in the ion source (i) from the source compound (e.g., ionization of $CH_3C(O)C_2H_7$ will produce both CH_3CO^+ and $C_2H_7^+$) and/or (ii) from a neutral impurity in a source compound (e.g., for a butanol impurity in a 2-butanone source compound, the butanol generates $C_2H_7^+$, while the 2-butanone generates CH_3CO^+). Fortunately, the CAD spectra of CH_3CO^+ and $C_2H_7^+$ are easily distinguishable. That is, for the CAD of $C_2H_7^+$, $C_2H_3^+$ (m/z 27) is the major CAD fragment for $E_{cm} = 2-80$ eV. By contrast, for the CAD of $C_2H_3O^+$, $C_2H_3^+$ is not produced at $E_{cm} = 2.4$ eV, and is only a very minor fragment for $E_{cm} > 2.4$ eV. Unfortunately, even minor impurities can contribute disproportionately to the CAD spectrum of a source compound because of differences in the CAD dynamics of isomeric and/or isobaric ions. Because of this problem, it is advisable that both the source compound and the target gas be of high purity ($> 99.95\%$). Otherwise, the impurities must be characterized so that appropriate corrections can be made for their contribution to the observed CAD spectrum.
- 6 The "Notes" field would be used to refer a user of the database to any information of special significance about the parent ion (e.g., structure of the ion, etc.). The notes enumerated in all the "Notes" fields of the CBRIS database would be collected together in a separate "NOTES APPENDIX". For the example given here, Notes 9, 99, and 999 would be found in the "NOTES APPENDIX", and might contain the following types of information.

Note 9: For a given E_{cm} , the branching ratio for each fragment ion is substantially the same for all CH_3CO-X source compounds (e.g., biacetyl, acetone, acetophenone, etc.). The reactant ion entering Q2 appears to be pure CH_3CO^+ in every case.

Note 99: The energy dependence (magnitude and direction) of the branching ratios is distinctly different for the isobars $C_2H_3O^+$ and $C_2H_7^+$. Hence, one can readily distinguish $C_2H_3O^+$ from $C_2H_7^+$.

Note 999: One can readily distinguish ethanol, oxirane, and cis-2,3-epoxybutane from each other, and from CH_3CO-X source compounds on the basis of the energy dependence of the branching ratios for the CAD of $C_2H_3O^+$.

STRUCTURE DETERMINATION OF PEPTIDES AND PROTEINS
BY HIGH PERFORMANCE TANDEM MASS SPECTROMETRY

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High performance tandem mass spectrometry has emerged as a powerful method for the determination of the structure of oligopeptides, namely, the sequence of the amino acids and modifications, if any, of the latter. The use of two double focusing mass spectrometers in tandem permits the selection of the ^{12}C specie of the isotopic $(\text{M}+\text{H})^+$ cluster as the precursor, which then results in a monoisotopic (devoid of isotope clusters) product ion (CID) spectrum. This approach results in a high reliability of the mass assignment for all peaks; the collisions occur at high energy (1-10 kV) which results in highly reproducible spectra, the features of which are very characteristic. From the study of the CID spectra of a few hundred peptides of known or originally unknown sequence and ranging from 5 to 25 amino acids in length, a set of general fragmentation processes and their dependence on peptide structure were deduced (1-3). This knowledge has been incorporated into a computer algorithm which allows the semi-automatic interpretation of the CID spectra of peptides in terms of their sequence (4).

A general strategy for the determination of the primary structure of proteins was developed based on this methodology for peptide sequencing (3). Briefly, the protein is digested first with trypsin, the complex mixture of tryptic peptides fractionated by HPLC into simpler mixtures, which are then subjected to tandem mass spectrometry for the determination of the sequence of each constituent peptide. This set of data is then augmented by similar experiments involving one or more other proteolytic enzymes of different specificity, which results in the formation of a set of peptides of very different sequences. The cumulative information derived from all these CID spectra generally permits the assembly of the complete sequence of the protein.

One example is the determination of the primary structure of the glutaredoxin isolated from rabbit bone marrow (5). This protein was not amenable to the conventional Edman degradation and therefore assumed to be blocked at the N-terminus. One of the CID spectra revealed an N_α -acetylated alanine (Fig. 1) which, therefore, must correspond to the N-terminal peptide. The complete sequence of 106 amino acids was derived from a total of 35 such spectra (Fig. 2).

One of the advantages of high energy collisions (>1 kV) is the formation of fragment ions (designated d_n and w_n), which are due to cleavage of the side chain of the amino acid at which the peptide backbone has been split (2,6). These processes permit the differentiation of the isomeric amino acids leucine and isoleucine. In the case of the rabbit bone marrow glutaredoxin, 15 of the 19 leucines and isoleucines could be differentiated in this manner but the peptides containing the remaining four did not give rise to either d_n or w_n ions. For these the formation of d_n ions was forced by formation of their N_α -(trimethylammonium)acetyl derivative, a reaction which places a fixed charge at the N-terminus of the peptide (7). The spectrum of the derivative of the octapeptide (positions 56-63) is shown in Fig. 3.

When this work was completed it revealed the close homology of the rabbit bone marrow glutaredoxin with that isolated from calf thymus (8), except that the latter had a rather different N-terminus (pyroGlu-Ala-Ala--) and was missing the four amino acids Thr-Val-Pro-Arg, which occupy positions 68-71 in the bone marrow protein. It was a simple matter to correct this discrepancy by tandem mass spectrometry of a chymotryptic digest of the calf thymus protein. The results revealed that the N-terminal sequence is actually Ac-Ala-Gln-Ala-- and that the missing four amino acids are present at the predicted positions (9).

Recent developments in detector technology have led to an increase in the sensitivity of our JEOL HX110/HX110 tandem mass spectrometer. An array detector covering 6.6% of the mass spectrum per step (10) made it possible to obtain excellent CID spectra with 10 pmol of peptide (11). An improvement which allows one to record up to 30% of the mass spectrum per

step (12) further improves the sensitivity and/or recording time.

This decrease in the amount of sample required makes it necessary to be able to manipulate protein digests at the picomole level and to introduce the resulting peptides into the mass spectrometer without losses or contamination. This has been accomplished by direct or indirect coupling of a packed capillary HPLC column to the ion source of MS-1 of the tandem system. For relatively simple mixtures, CID spectra can be acquired with the array detector while each peptide elutes from the column. In more complex cases, fractions are stored in sample loops attached to a multiposition trapping flow path valve, allowing the trapping of 16 or 23 fractions, respectively (13).

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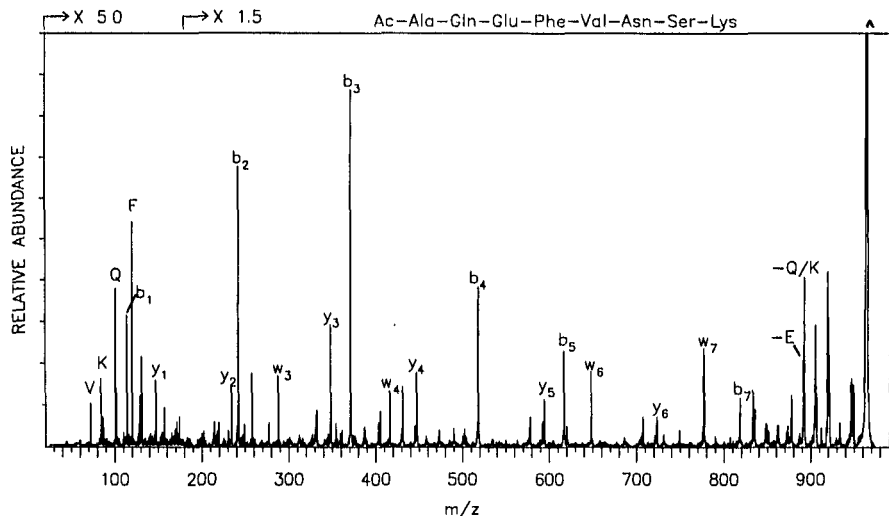


Figure 1

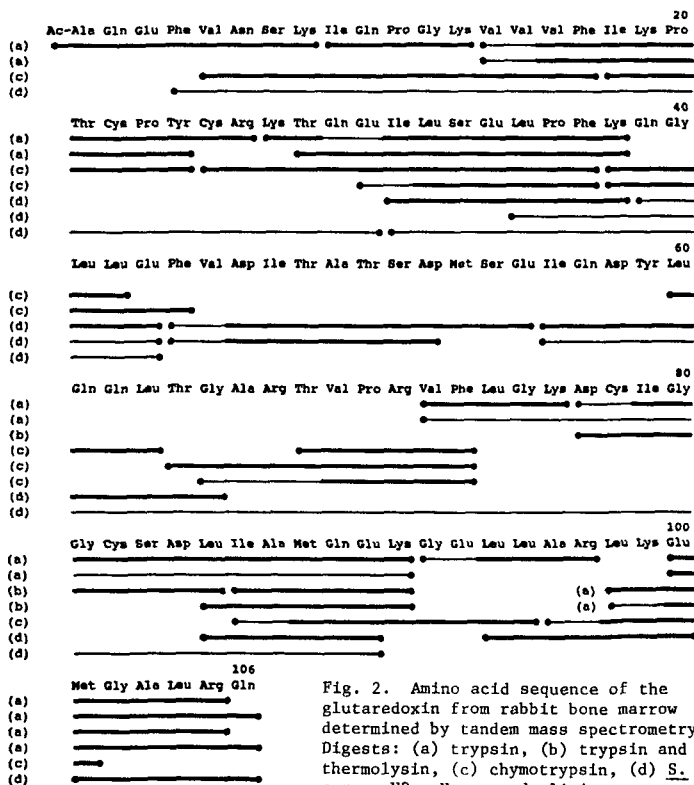
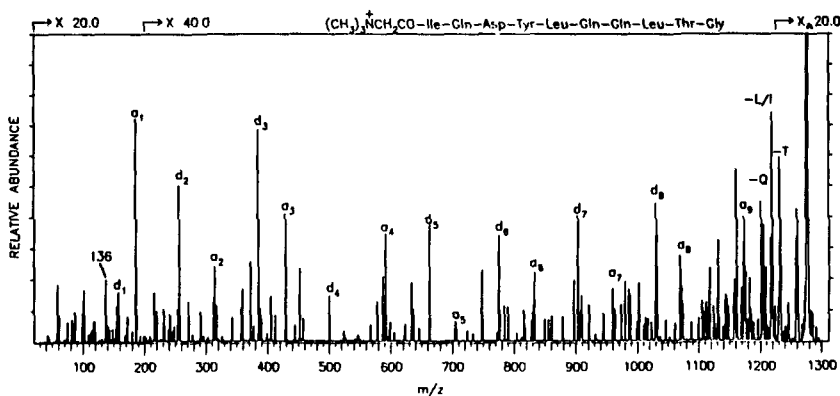


Fig. 2. Amino acid sequence of the glutaredoxin from rabbit bone marrow determined by tandem mass spectrometry. Digests: (a) trypsin, (b) trypsin and thermolysin, (c) chymotrypsin, (d) *S. aureus* V8. Heavy underlining: sequence derived from CID mass spectra.



PROTEIN STRUCTURAL PROBLEMS, THE ROLE OF TANDEM MASS SPECTROMETRY
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Development of new methods and instrumentation for the structural characterization of proteins continues to be a major goal of our research. Two instruments, a TSQ-70 triple quadrupole and a quadrupole Fourier transform mass spectrometer are employed in this effort. Ten proteins have been sequenced to date and more than forty additional proteins or protein systems are currently under study.

Typical steps employed in the sequence analysis of a protein include digestion of the protein with site-specific reagents such as cyanogen bromide, or proteolytic enzymes, partial fractionation of the resulting mixture of oligopeptides by microbore, high-performance, liquid chromatography (HPLC) and direct analysis of peptides in each HPLC fraction by the process of particle-bombardment, collision-activated-dissociation, on the TSQ-70 triple-quadrupole mass spectrometer and by particle bombardment, laser photodissociation on the quadrupole Fourier transform instrument.

Molecular weight measurements on the quadrupole Fourier transform instrument are routinely conducted on sample at the 0.1-20 pmol level in the mass range up to 6 kDa. Photodissociation of peptide (M+H)⁺ ions with an argon fluoride excimer laser is readily accomplished at the 10-30 pmol but generates complete sequence information for less than 30% of the samples examined. Most samples, including insulin, fragment to small ions in the mass range below 700 Da. Accordingly, the resulting spectra afford only partial structural information.

Sample quantities required for sequence analysis by collision activated dissociation on the TSQ-70 instrument are generally in the 1-10 pmol range for molecules of mol wt below 1,000 Da, 5-50 pmol for molecules below mass 2,000, and 30-300 pmol for molecules whose mol wts fall between 2,000 and 2,600 Da. At these levels the probability of generating a complete amino acid sequence for any peptide sample is generally greater than 90%.

Samples are desorbed from a matrix of thioglycerol (0.5 ul) on a gold plated probe with a Cs⁺ ion gun operating at 6 keV. Source and manifold are kept at 50° C to minimize vaporization of the matrix. Collision experiments employ a 2/1 mixture of argon and xenon at a total pressure of 3 mtorr as the target. Optimal peptide fragmentation is observed when the offset potential on quadrupole II (collision energy) is set between 15-35 eV. Quadrupoles I and III are operated at less than unit resolution to maximize ion transmission. Shown in Figures 1-2 are collision activated dissociation spectra recorded on the (M+H)⁺ ions of renin tetradecapeptide (5 pmol) and the methyl ester of ACTH CLIP (20 pmol).

Shown in Fig. 3 is the primary structure of the rabbit sex steroid binding protein (rSBP) obtained by a combination of tandem mass spectrometry and Edman degradation. Peptides are labeled according to the method used to generate them and the technique used to sequence them. Proteolytic and chemical cleavage methods are designated as follows: K = endo Lys-C, Tr = trypsin, C = chymotrypsin, E = endo Glu-C, El = elastase, D = endo Asp-N, M = cyanogen bromide, A = acid cleavage at Asp. Peptides sequenced by Edman degradation are labeled, -Ed-. Unidentified residues are designated by blank spaces. Peptides sequenced by collision activated dissociation on the triple quadrupole mass spectrometer are labeled by the nominal monoisotopic mass of the corresponding (M+H)⁺ ion. Primary structures deduced from laser photodissociation spectra recorded on the Fourier transform instrument are designated by the label, --FTPD--, followed by the average mass value observed for the isotopic cluster corresponding to the (M+H)⁺ ion. Sequences confirmed by enzyme specificity and molecular mass measurement on the Fourier transform instrument are designated by dashed lines plus the label, --FT--, followed by the average mass value recorded for the isotopic cluster corresponding to the (M+H)⁺ ion. Mass measurements on (M+H)⁺ ions for the corresponding peptide methyl esters were used to support assignments made by this latter method. Predicted incremental mass shifts of 14 Da/carboxylic acid group were observed in all cases.

Edman degradation proved to be the method of choice for generating sequence information at the N-terminus of the molecule, largely because the procedure could be carried out directly on the intact protein. Advantages of the tandem mass spectrometry method include the ability to sequence peptides present in mixtures and the speed with which one can obtain sequence information over the entire length of the protein. Only 3-5 hours of instrument time is required to obtain both molecular mass and sequence data on all peptides generated in any particular digest. As a result, ca. 75% of the rSBP primary structure was assembled into large non-overlapping segments with only several weeks of effort. Location of disulfide bonds is also greatly facilitated by mass spectrometry. Mass measurements performed on peptides from a particular enzyme digest, before and after reduction of the sample on the probe of the mass spectrometer, identifies those sequences attached through a disulfide linkage. This experiment requires only minutes of instrument time.

The major limitation of the above sequencing approach is that it is presently impossible to distinguish Leu and Ile, two amino acids of identical mass, under the low energy collision conditions employed on the triple quadrupole instrument. All 57 Leu and Ile residues in rSBP were assigned from either amino acid composition or Edman degradation data.

The region within the rSBP structure that proved most difficult to sequence by tandem mass spectrometry included residues 260-280. This region contains a paucity of acidic and basic amino acids and a large number of small hydrophobic residues. As a result, proteolytic enzymes produced either one or two peptides whose $(M+H)^+$ ions fell outside the mass range of the triple quadrupole instrument or a large number of small peptides, 2-4 residues in length, the sequences of which could not be overlapped to generate a unique primary structure for this part of the molecule. Automated Edman degradation of a 59 residue Asp-N peptide provided the necessary data to sequence through residues 260-280.

Figure 1.

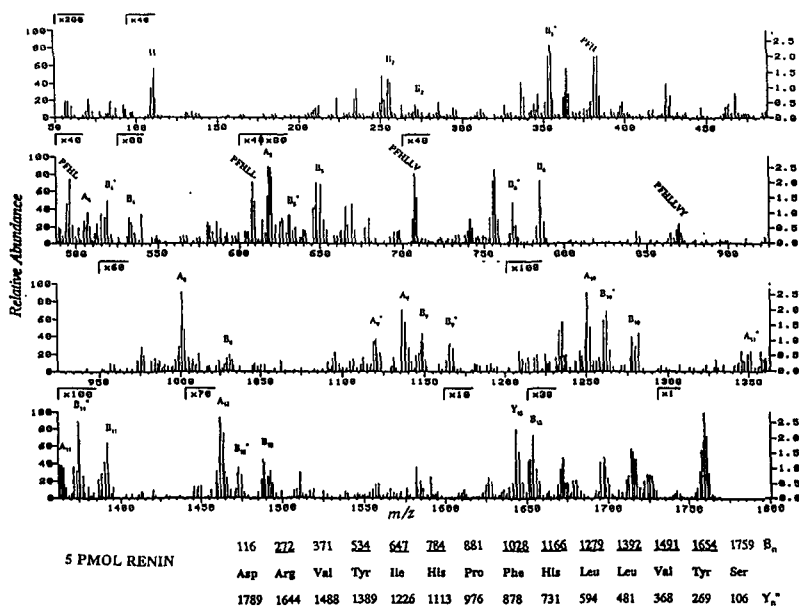


Figure 2.

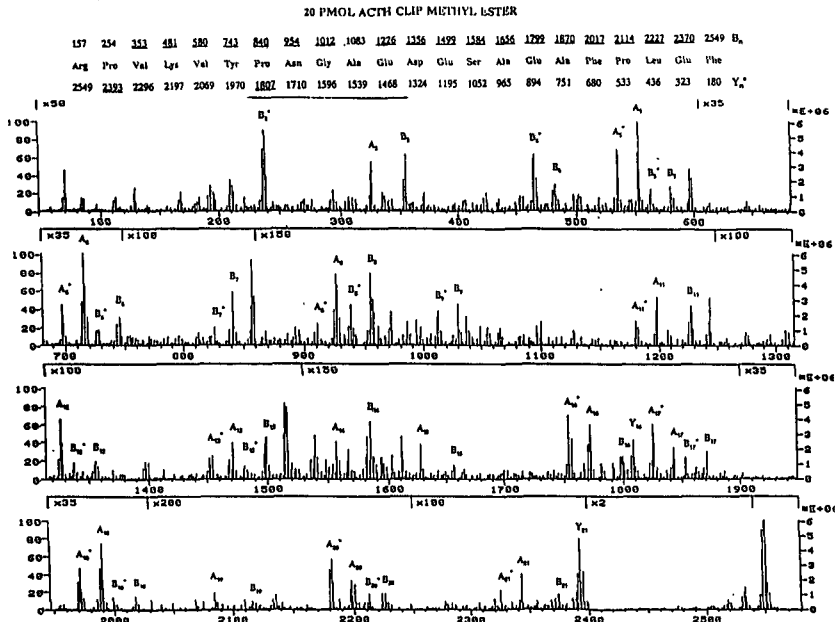
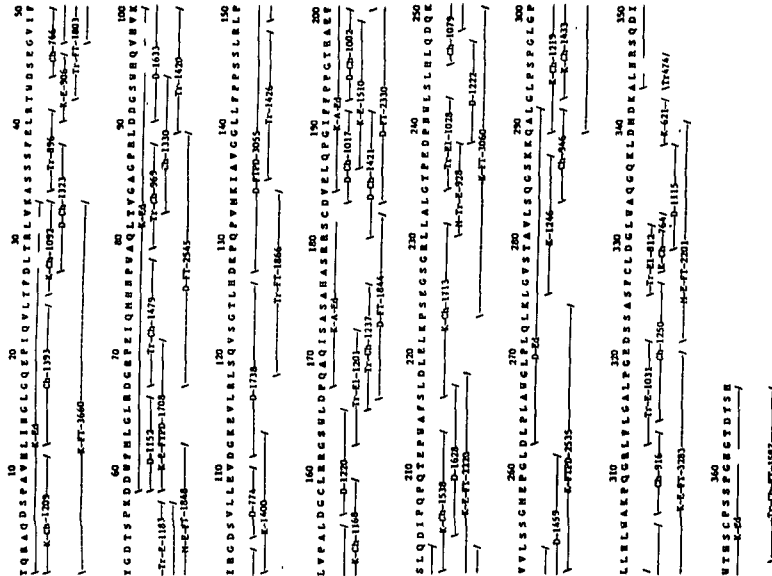
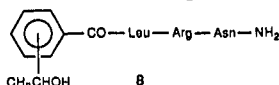


Figure 3.

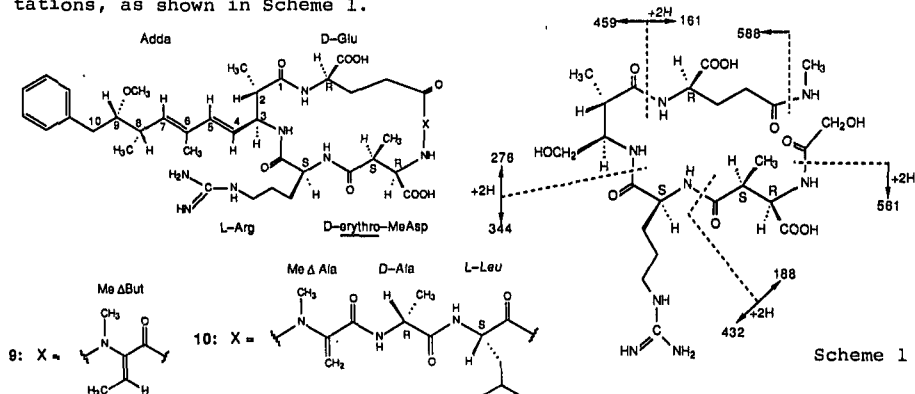


now at the University of Hamburg.³ All of the peptides to date contain C-terminal amide functions and most contain a pyroglutamyl N-terminal unit. However, two unusual peptides have provided intriguing challenges. One of these is Pol-RFamide II, derived from *Polyorchis penicillatus* (a hydromedusa). The amino acids identified by hydrolysis were lysine, leucine, arginine, glycine, phenylalanine, and glutamic acid, but these did not add up to the molecular weight. High resolution, however, established the presence of tryptophan, and the CID-induced MS/MS fragmentation indicated the sequence shown (7). Synthesis of 7 indicated the synthetic peptide had slightly different chromatographic and mass spectrometric properties from the natural product. We now tentatively (pending synthesis) assign an ϵ -lysine peptide link.

The peptide Antho-RNamide from the sea anemone *Anthopleura elegantissima* has provided a different surprise. Subtraction of the amino acid units involved from the high-resolution molecular ion of Antho-RNamide leaves a $C_9H_9O_2$ unit (149 amu). Mass spectrometry alone allowed too many options for the N-terminal unit, and we turned to nuclear magnetic resonance to assign that structural unit. 1H NMR showed the presence of a benzene ring and a CH_3CH group. Combining these data with the FABMS/MS spectrum of the acetyl derivative of Antho-RNamide allows assignment of the tentative structure 8, again subject to the results of synthetic studies underway.

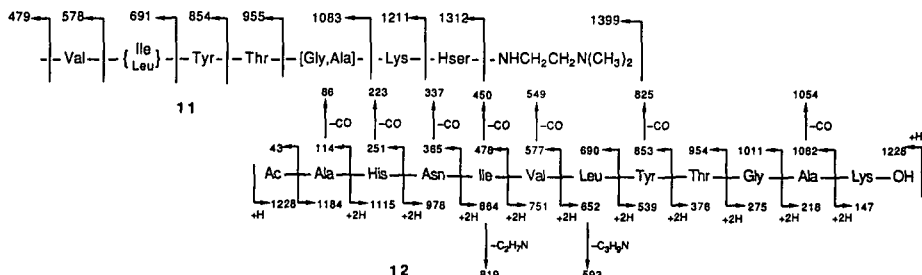


Cyanobacterial Hepatotoxins. Cyanobacteria produce a variety of toxins, often of significance to human health. The hepatotoxins which thus far have been found in *Microcystis* and *Nodularia* species are cyclic peptides containing the uniquely characteristic C_{20} amino acid Adda [(2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid]. We recently completed structural assignments of two of these compounds, microcystin-LR (9) and nodularin (10), including all stereochemical aspects of Adda.⁴ With these hepatotoxins, FAB fragmentation of the intact molecule and its hydrogenated counterpart did not occur, so that sequencing was not possible. Sequencing was, however, carried out on the partial hydrolysis product from dihydronodularin, and FABMS/MS analysis indicated it to be a mixture of at least three peptides. Successive application of FABMS/MS to successive fragment ions allowed partial assignment of the structures of the three peptides, which could then be combined to a partial structure for nodularin, the complete assignment resting at that stage on analogy to earlier assignments of microcystin structures. We have now developed what appears to be a useful general method for dealing with sequences of these cyclic peptides: this involves ozonolysis, followed by sodium borohydride treatment to give a linear protected peptide whose structure can be assigned by standard fragmentations, as shown in Scheme 1.



Scheme 1

Tryptic Digests of Aldose Reductases. In collaboration with Professor C. C. Doughty at the University of Illinois-Chicago and Dr. C. E. Grimshaw, Scripps Clinic and Research Foundation, La Jolla, we have recently been investigating the sequences of the aldose reductases from bovine lens and bovine kidney. Bovine lens aldose reductase was first cleaved with cyanogen bromide to give four large fragments. The C-terminal fragment was subsequently treated with trypsin, and the four isolated tryptic peptides were submitted to FABMS/MS sequencing. The three larger peptides indeed gave the fragmentations expected on the basis of earlier Edman degradation. More problematical was the N-terminal cyanogen bromide fragment, which was N-protected. While it was initially thought to contain 24 amino acids, this peptide gave mass spectral fragmentation consistent with half that molecular weight, that is, 12 amino acids, including the C-terminal homoserine lactone (protected as the dimethylaminoethylamine derivative). Partial sequencing was also successful (11), employing the B/E scan technique on our ZAB SE spectrometer. When a subsequent tryptic digest became available, FABMS/MS showed the N-terminal tryptic peptide to contain the expected 11 amino acids and confirmed the presence of lysine adjacent to the homoserine unit. Taking account of the amino acid composition of the undeca-peptide, we deduced the N-terminal group to be an acetyl and used the CID-induced fragmentation to assign the sequence shown (12). Initially the positions of leucine and isoleucine were regarded as interchangeable. Careful consideration of the FABMS/MS spectrum, however, and final comparison with two authentic synthetic samples containing the alternative locations of the leucine and isoleucine units established isoleucine at position 4 and leucine at position 6. With the N-terminal sequence in hand, the sequence of bovine lens aldose reductase was completed.



More recently, we turned our attention to the aldose reductase from bovine kidney. More specifically, we have compared the tryptic digests of bovine lens aldose reductase and bovine kidney aldose reductase with respect to the identifiable tryptic peptides present. No separation was attempted of the tryptic peptides, mass spectra instead being determined on the crude mixture. All peptides recognized were identified by their LRFAB mass spectra and most by their HRFAB spectra as well as FABMS/MS carried out on the 70 SE 4F spectrometer. These experiments are discussed in greater detail in another paper in this volume;⁵ it should be noted here, however, that approximately 27% of the sequence of bovine lens aldose reductase is found also in bovine kidney aldose reductase. More impressively, essentially all of the peptides found to date in the bovine kidney tryptic digest are also found in bovine lens digest. One major difference is the N-terminal peptide from bovine lens; it has not yet been found either in derivatized or underivatized form in bovine kidney.

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**AN INTEGRATED APPROACH TO PEPTIDE AND PROTEIN STRUCTURE
ANALYSIS UTILIZING TANDEM HIGH PERFORMANCE MASS SPECTROMETRY**

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The availability of large amounts of purified recombinant proteins together with the ability to specifically alter the structures of these compounds promises to revolutionize both the treatment of diseases and our understanding of their molecular bases. Many of these proteins are being developed as potential therapeutic agents, while others are being made for diagnostic purposes and for structural studies directed at elucidating their mechanism of action.

While cloning and sequencing of the gene coding for virtually any desired protein can now be accomplished with tremendous speed and efficiency, structural characterization of the recombinant protein product is often a relatively slow step. Amino-terminal and carboxy-terminal sequencing of the protein product are required to establish identity and internal sequences must be verified to check the fidelity of translation. Difficulties frequently arise with application of conventional sequencing methodologies such as Edman degradation since the N-termini of recombinant proteins are often blocked preventing confirmation of this region. Many other native or induced protein modifications cannot be identified by Edman. Clearly fast, sensitive, and reliable procedures are necessary to bridge the analytical gap between protein chemistry and molecular genetics. Unfortunately, no one technique is sufficiently accurate or rapid. Successful structural characterization of macromolecules like peptides and glycoproteins requires a coordinated, rational approach and close collaboration among spectroscopists, biochemists and chemists. Having the right instrumentation is only part of the solution.

In our laboratories at SKF we use MS and, since early 1986, tandem MS (VG ZAB-SE 4F) together with conventional chemical and enzymatic sequencing techniques to solve structural problems that are not readily soluble by any single technique. In our experience, peptide molecular weight determination by FAB/MS coupled with analysis of specific peptides by tandem MS is an ideal complement to Edman degradation for structural characterization of peptides and proteins. Tandem MS has been particularly useful in defining the location and structure of posttranslational modifications in peptides and proteins and for sequencing synthetic peptides incorporating unusual amino acids or in cases where the synthesis has gone awry (1). We have used this integrated approach to characterize androgen-dependent proteins from prostate and seminal vesicles, neuromodulatory enzymes and lymphokines. In these studies tandem MS has been used to determine the sequence of N-terminally blocked peptides (2,3), locate and correct errors in DNA and cDNA-deduced protein sequences (4), obtain internal and C-terminal sequence information for verifying the fidelity and the end of translation (3,4,7), define the site of attachment and sequence of peptides labeled with suicide substrate inhibitors (5) and naturally-derived peptides with unusual side-chain modifications (6), and pinpoint sites of deamidation in proteins (7).

The principles of this combined approach are illustrated by our structural studies of tumor necrosis factor (TNF), a protein which induces hemorrhagic necrosis of various tumors in animals. We were asked to check the overall sequence fidelity of a sample of TNF which had been found to have altered activity in a bioassay relative to the reference standard. Ninety-two percent of the protein sequence was corroborated in this single experiment. However, two signals, at m/z 1241.6 and 1397.7, could not be mapped to the deduced sequence. Both of these signals are 1 Da more than predicted for two expected tryptic peptides. FAB/MS of the tryptic peptide mixture after one and two cycles of manual Edman degradation established that the shifted signals were derived from peptides Arg32-Arg44 and Ala33-Arg44, respectively. Tandem MS of the peptides of $(M+H)^+ = 1397.7$ and 1241.6 (Fig. 1) demonstrated that the single mass unit shift was the result of deamidation of Asn39 to Asp. The tandem mass spectrum of the $(M+H)^+ = 1241.6$ peptide illustrates the typical appearance of the daughter ion spectra of tryptic peptides using high energy (keV) collision induced dissociation. The C-terminal fragment ions observed have the expected masses up to Gly8 based on the predicted sequence, but are shifted upward by one mass as fragmentation proceeds toward the N-terminus through residue seven, indicating that Asn (in-chain mass = 114) at this position has converted to Asp (in-chain mass = 115). Pinpointing the site of deamidation in TNF was completed in approximately one day by MS/MS, and did not require separation or purification of individual peptides.

Deamidation of Asn proceeds via a cyclic succinimide that can reopen to Asp or β -Asp in which the methylene group is now in the peptide chain (Fig. 2). Dodecapeptides Ala33-Arg44 containing Asp and β -Asp at position 7 in the peptides were synthesized and analyzed by FAB tandem MS with high energy collisions. Specific side-chain fragments indicative of Asp vs. β -Asp were obtained by conversion of the peptides to the corresponding trimethyl esters (Fig. 3, top and middle frames, respectively). Methylation of the tryptic digest of TNF and tandem MS of the peptide of m/z 1283.7 (trimethylated 1241.6) indicated that a mixture of Asp and β -Asp had been formed at this location in the protein. Tandem MS using high energy collisions will be useful in distinguishing these residues since no other routine methods presently exist.

- 1) See WPA-30, this volume; 2) *Biochemistry*, **25**, 4988-4993, 1986; 3) *J. Biol. Chem.*, **263**, 18678-18687, 1988; 4) *Biochemistry*, **27**, 4214-4221, 1988; 5) *Biochemistry*, **27**, 9093-9101, 1988; 6) *J. Biol. Chem.*, **263**, 18236-18240, 1988; 7) *J. Biol. Chem.*, **264**, 4948-4952, 1989.

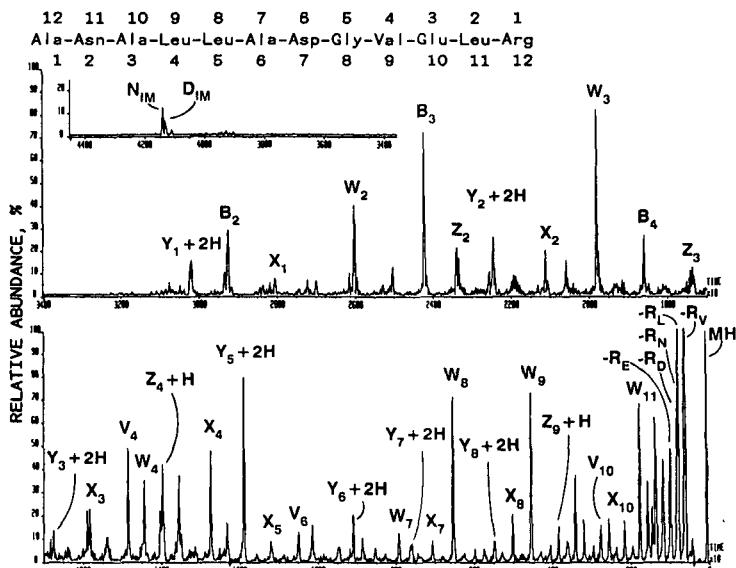


Fig. 1. Tandem mass spectrum of the peptide $(M+H)^+ = 1241.6$ present in a complex tryptic digest of TNF; ca 1 nmol of digest in thioglycerol. Resolution of MS-1 and MS-2 ca 1000; 10 keV collisions with He.

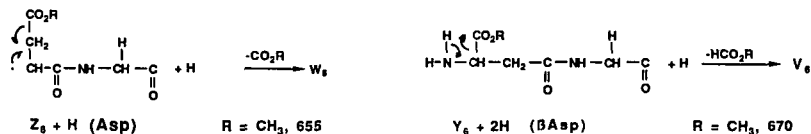


Fig. 2. Proposed origin of m/z 655 and 670 observed in the high energy tandem mass spectra of methylated analogs of the dodecapeptide in Fig. 1 with Asp vs. β -Asp at position 7, (see Fig. 3).

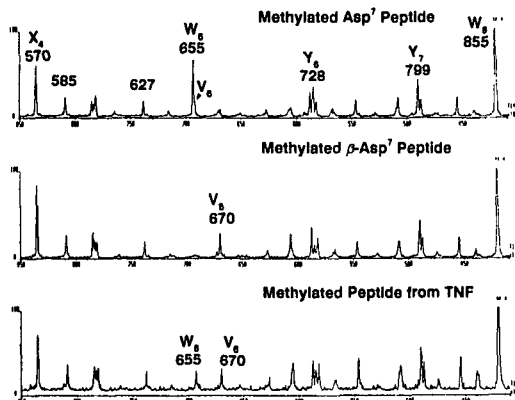


Fig. 3. Comparison of the relevant regions of the tandem mass spectra of synthetic, methyl esterified Asp7 and β -Asp7 peptides with the methylated peptide from TNF. The spectra were otherwise identical.

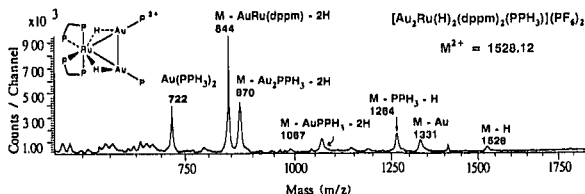
^{252}Cf -Plasma Desorption Mass Spectrometry of Metal Clusters

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We have examined several classes of metal clusters including mixed transition metal-gold complexes, small gold phosphine complexes and large gold phosphine clusters by ^{252}Cf -PDMS. The mixed metal clusters are of interest because of their potential use as bimetallic catalysts and catalyst models (1). The smaller gold clusters are the building blocks of larger clusters which are at the transition between particles with single atom character and larger aggregates that function as bulk materials. There is also potential biological applications of these clusters in the area of imaging and as chemotherapeutic agents when the gold is radioactive. The molecular structures of the small metallic complexes have been determined by a combination of NMR, x-ray crystallography and FABMS. However, the structural characterization of large gold clusters is problematic because they are often too large and heterogeneous to be characterized by x-ray crystallography. Conversely, they are too small to be examined by electron microscopy.

Fast atom bombardment MS has been used to structurally characterize the mixed-metal and small gold clusters (2). The results obtained by ^{252}Cf -PDMS are very similar to the FAB spectra. A typical positive ion spectrum is shown in Fig. 1 for a gold-ruthenium hydride compound. In this spectrum, the molecular ion has been identified as $(\text{M} - \text{H})^+$. However, higher resolution data is needed to confirm this identity. Fragment ions are formed by losses of the organic ligands and/or metal atoms. In the lower mass range, the $(\text{Au} + 2\text{H})^+$ ion is observed as well as small gold-phosphine and ruthenium-phosphine ions. In some cases, negative molecular ions are observed of the type $\text{M}^+ \text{X}_2^-$ or $\text{M}^{2+} \text{X}_3^-$ where X is the associated anion, but very few significant fragment ions are present.

Figure 1.

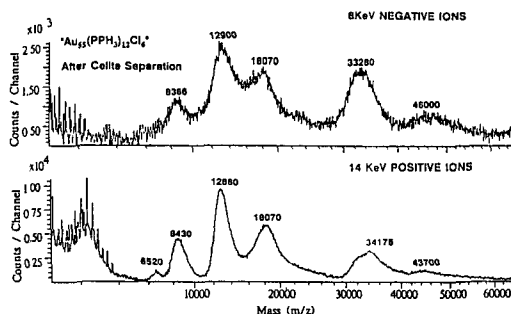


In these mixed metal clusters, the d-orbitals of the transition metal atom stabilize the complex. As the ratio of gold atoms to transition metal atoms increases, we observe that the molecular ions become less abundant and there is far more fragmentation. In the positive ion spectrum of $[\text{Au}_6\text{Pt}(\text{PPh}_3)_7](\text{NO}_3)_3$ there is no clearly identifiable molecular ion. A series of low intensity ions that are formed by sequential loss of gold and triphenyl phosphine ligands is observed. The $\text{Au}_5\text{Pt}(\text{PPh}_3)_3^+$ ion is the most prominent in this mass range. Below this mass, ions are observed that can be attributed to further sequential losses of metal atoms and ligands as well as ions formed by partial losses of the ligands which add an increased complexity to the fragmentation pattern. The positive ion spectrum of $[\text{Au}_9(\text{PPh}_3)_8](\text{NO}_3)_3$ resembles this latter region of the Au_6Pt complex. Again, it is difficult to unambiguously assign a peak to the molecular ion. Peak intensities are greatly reduced in this region compared to ions in the mass range of $\text{Au}_9(\text{PPh}_3)_3$ to $\text{Au}_3(\text{PPh}_3)_2$. The negative ion spectrum contains a series of Au_n^- ions extending beyond Au_{19}^- . Although there is a general decline in intensities, the gold clusters having an odd number of gold atoms are more intense than the adjacent even-numbered cluster. Each cluster is part of a pentuplet of ions that are formed by the addition of 24, 31, 55 and 79 mass units. The identities of the ions have not been completely resolved.

There has been some controversy over the structure of a gold complex believed to be $\text{Au}_{55}(\text{PPh}_3)_{12}\text{Cl}_6$. The structure determination has eluded chemists because of the problems previously stated. One proposed structure is a two-layer cuboctahedron in which a Au_{13} cuboctahedra is surrounded

by 42 additional gold atoms in a cubic close packed array (3). Teo has proposed an alternate structure of vertex-sharing icosahedra building upon each unit to form a highly symmetric supra-cluster (4). The positive ion ^{252}Cf -PD mass spectrum of one supra-cluster (5) contains three broad but distinct distributions centered at m/z 8200, 12800 and 16600. None of these correspond to the 14193 D mass of a Au_{55} cluster. These zones do fit fairly well for a series of two, four and six icosahedra having stoichiometries of $\text{Au}_{25}(\text{PPH}_3)_{12}\text{Cl}_6$ (mw. 8284), $\text{Au}_{46}(\text{PPH}_3)_{12}\text{Cl}_6$ (mw. 12421) and $\text{Au}_{67}(\text{PPH}_3)_{14}\text{Cl}_8$ (mw. 17153) (6). In the lower mass range of this spectrum a series of ions corresponding to almost all possible combinations of $\text{Au}_x(\text{PPH}_3)_y^+$ (where $y \leq 12$) is observed. Usually the $\text{Au}_x(\text{PPH}_3)_3^+$ ion is the most intense for a particular Au_x value; there is no clear evidence for chlorine incorporation nor, was chlorine incorporation observed in the mass spectrum of the smaller gold-phosphine compounds. The broadness of the distributions is undoubtedly due in part to these types of ions. If it is assumed that the centers of the distributions are gold clusters containing three ligands, with no chlorine attached, these distributions may correspond to $\text{Au}_{37}(\text{PPH}_3)_3$ (mw. 8075), $\text{Au}_{57}(\text{PPH}_3)_3$ (mw. 12014) and $\text{Au}_{76}(\text{PPH}_3)_3$ (mw. 15757) each having one more icosahedron than the preceding structures. In an effort to determine if part of the broadness of these bands was due to the presence of a mixture of various gold clusters, a sample from a different source (7) was passed through a column of diatomaceous earth. Mass spectra were recorded before and after purification. The positive ion spectra are shown in Fig. 2. The mass spectrum obtained after purification resembles much more closely that of the Pignolet gold cluster with the exceptions that there are some higher mass peaks and the m/z 16,600 peak is shifted to a higher mass. This experiment clearly demonstrated that the samples are heterogeneous in nature and that part of the broadness of the different distributions is the result of impurities.

Figure 2.



In summary, ^{252}Cf -PDMS has been demonstrated to be useful in characterizing the structures of various metal clusters. This is also the first application of mass spectrometry to the analysis of supra-clusters and opens up an exciting new application for mass spectrometry as well as providing important new insights into the structure of these large complexes.

- (1) Alexander, B.D.; Boyle, P.D.; Johnson, B.J.; Casalnuovo, J.A.; Johnson, S.M.; Mueting, A.M.; Pignolet, L.H. *J. Am. Chem. Soc.* **1987**, *26*, 2547.
- (2) Boyle, P.D.; Johnson, B.J.; Alexander, B.D.; Casalnuovo, J.A.; Gannon, P.R.; Johnson, S.M.; Larka, E.A.; Mueting, A.M.; Pignolet, L.H. *J. Am. Chem. Soc.* **1987**, *26*, 1346.
- (3) Schmid, G. *Structure and Bonding*, **1985**, *62*, 51.
- (4) Teo, B.K.; Hong, M.C.; Zhang, H.; Huang, D.B. *Angew. Chem., Int. Ed. Eng.* **1987**, *26*, 897.
- (5) This sample was a gift from L. Pignolet, University of Minnesota.
- (6) Fackler, J.P.; McNeal, C.J.; Pignolet, L.H.; Winpenny, R.E.P. *J. Am. Chem. Soc.*, accepted for publication
- (7) This sample was a gift from F. Furuya, Brookhaven National Laboratory.

**FABMS and FABMS/MS Studies on Inorganic
and Organometallic Compounds**

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We have recently employed FABMS to characterize a variety of inorganic and organometallic compounds of synthetic and biological origins. The main focus of our studies has been the two classes of synthetic materials listed in Tables 1 and 2, and the esters of a nickel-containing porphyrinoid isolated from *Trididemnum solidum*, a Caribbean tunicate (compounds 1-7).

The polyoxoanion complexes (Table 1) are important analogues to metal oxide surfaces used in industrial scale oxidations. These soluble complexes are potential homogeneous catalysts. The heterometallic natures and difficulties in obtaining uniform crystals of these compounds prevented complete characterization by elemental analysis, NMR and X-ray crystallography. Both positive and negative ion FABMS experiments have shown useful structural information at 1000 and 6000 resolution. Analysis of the isotope envelopes of negative ion FABMS obtained at 6000 resolution on a VG ZAB-SE confirm that the cyclopentadienyl ligands are strongly retained and that fragmentation of the core anions is dominated by multiple losses of WO_3 and O moieties.¹

We have also employed FABMS/MS on a VG 70-SE-4F instrument to study the fragmentation of the core anions of several rhenium carbidocarbonyl compounds (Table 2). The overall objectives of the mass spectral analysis of these compounds is to better understand the properties of metal clusters. Daughter ion scans have shown sequential losses of carbonyl groups from molecular ions before any breakdown of the rhenium core. Negative ion FABMS/MS studies of more complex ions containing one or more organometallic ligands are currently underway.

FABMS and FABMS/MS have also played a key role in the structure elucidation of compounds 1-7 and related compounds² since only a few micrograms of the tunichlorin esters have been isolated from kilograms of the organism. A structure of the ester side chain was proposed after an acid catalyzed transesterification and analysis of the products by GC/EIMS on an HP 5970 MSD. The data suggests that the resulting alcohols have rearranged to straight-chain methyl esters. Comparison studies with the appropriate model methyl esters showed identical fragmentation pattern and retention times under the same GC/MS conditions. Coinjection experiments also show coelution of the cleaved natural product and these model compounds.³ Experiments to conclusively determine the structures are in preparation.

TABLE 1. Trisubstituted Heteropolytungstate Complexes

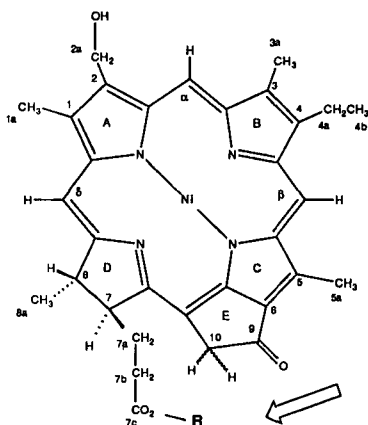
<u>Structural Composition</u>	<u>M.W.</u>	<u>Structural Composition</u>	<u>M.W.</u>
(Bu ₄ N) ₅ [CpRh-SiW ₉ Nb ₃ O ₄₀]	4052	(Bu ₄ N) ₄ [CpTi-P ₂ W ₉ V ₃ O ₆₂]	3558
(Bu ₄ N) ₃ Na ₂ [CpRh-SiW ₉ Nb ₃ O ₄₀]	3616	(Bu ₄ N) ₇ [CpRh-P ₂ W ₁₅ Nb ₃ O ₆₂]	6023
(Bu ₄ N) ₄ Na ₃ [CpRh-P ₂ W ₁₅ Nb ₃ O ₆₂]	5367	(Bu ₄ N) ₆ [CpTi-P ₂ W ₁₅ V ₃ O ₆₂]	5532
(Bu ₄ N) ₄ Na ₃ [CpRh-P ₂ W ₁₅ Nb ₃ O ₆₂]	5364	(Bu ₄ N) ₅ [CpRe-P ₂ W ₉ Nb ₃ O ₆₂]	5497
(Bu ₄ N) ₁₂ [H ₄ P ₄ W ₃₀ Nb ₆ O ₁₂₃]	11,176 **		

*Fragmentation observed in negative ion FABMS, $[M - (Bu_4N)_x + x H]^+$ and $[M - (Bu_4N)_x + x H + y Na]^+$
where $x = 2-7$ and $y = 0-5$, **only monomer units observed $[M/2 - (Bu_4N)_x + y H]$

STRUCTURES OF THE TUNICHLORIN ESTERS

TABLE 2.
RHENIUM CARBIDOCARBONYL
CLUSTERS INVESTIGATED

(Et₄N)₂ [Re₆C(CO)₁₉]
(PPN)₂ [Re₇C(CO)₂₁Cl]
(Et₄N)₂ [Re₆C(CO)₁₈(AuPPh₃)₂]
((Et₄N) [HRe₆C(CO)₁₈(AuPPh₃)])
(Et₄N)₂ [Re₆C(CO)₁₈(AuPPh₃)₂]
(Et₄N)₂ [Re₇C(CO)₂₁Hg(Cys)]
(PPN)₂ [Re₇C(CO)₂₁Ir(CO)₂]
(PPN)₂ [Re₇C(CO)₂₁Ir(CO)(C₂H₄)]
(Et₄N)₂ [Re₇C(CO)₂₁IrH(SiPh₃)(CO)]
(Et₄N)₂ [Re₇C(CO)₂₁Rh(CO)(PPh₃)]



<u>compound</u>	<u>-R</u>
1	- C ₁₄ H ₂₇ O
2	- C ₁₅ H ₂₉ O
3	- C ₁₆ H ₂₉ O
4	- C ₁₆ H ₃₁ O
5	- C ₁₇ H ₃₃ O
6	- C ₁₈ H ₃₃ O
7	- C ₁₈ H ₃₅ O

- 1 a. Finke, R.G.; Droege, M.W.; Cook, J.C.; Suslick, K.S.
J. Amer. Chem. Soc., **1984** *106* 5750-1.
b. Suslick, K.S.; Cook, J.C.; Rapko, B.; Droege, M.W.;
Finke, R.G. *Inorg. Chem.*, **1986** *25* 241-243.
- 2 Rinehart, K.L.; Kishore, V.; Bible, K.C.; Sakai, R.;
Sullins, D.W.; Li, K.-L. *J. Nat. Prod.*, **1988** *51*(1) 1-21.
- 3 Bible, K.C. Ph.D. Thesis, University of Illinois at
Urbana-Champaign, June 1989

FTMS STUDY OF THE ELECTRON AFFINITY OF TRANSITION METAL TRIS-(ACETYLACETONATE) COORDINATION COMPOUNDS

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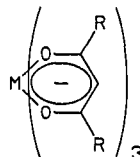
The thermodynamics of electron attachment to gas-phase ligated transition metal ions that have direct solution-phase counterparts have not been studied extensively. Such data are crucial to understanding the thermodynamic origins of electrode potentials in solution and the changes in bond energies that occur upon reduction of metal complexes in the absence of solvation. The compounds chosen for this study are the *tris*(acetylacetonate) and *tris*(hexafluoroacetylacetonate) complexes of the first transition metal series (Figure 1). The metal centers are formally in the +3 oxidation state and are coordinated by a pseudo-octahedral arrangement of 6 oxygen donor atoms. Each ligand has a negative charge, and the *tris* complexes are volatile neutral molecules.

At the experimental temperatures used in these studies, values of ΔG and ΔH for electron capture by gaseous molecules usually do not differ markedly and are close approximations to the true electron affinity. The data obtained in this study can therefore be used in thermochemical cycles that yield further important thermochemical information (Figure 2, where $D(M-L)_{avg}$ is the average heterolytic bond dissociation enthalpy).

Free energies of electron capture by the metal complexes were studied by using a Nicolet FT/MS 1000 ion cyclotron resonance mass spectrometer. Parent negative ions are made from a gas mixture containing a metal complex and an organic reference compound. The reference compound is taken from tables of organic acceptors for which the free energy of electron capture has been previously determined by pulsed high pressure mass spectrometry. Through a combination of free energy bracketing and observations of charge transfer equilibria, free energies

of electron attachment for each complex were determined. The results for the series of $M(acac)_3$ and $M(hfac)_3$ complexes are shown in Figure 3. In comparison to the values for electron capture by the free +3 metal ions, the values for the complexes are greatly lowered. This lowering of EA for the ligated metal ions reflects the higher average heterolytic M-L bond energies for the neutral complexes in comparison to the corresponding anions. The maximum occurring at manganese in both series results from the enhanced stability associated with the formation of the d^5 "half-filled shell" configuration, for which stabilization through electron exchange interactions is also a maximum. Other variations from a smooth increase in EA with increasing atomic number can be ascribed to splitting of the energy of the *d* orbitals in the complexes and can be more fully rationalized by a ligand field or molecular orbital analysis. The free energy of electron capture by the series of $M(hfac)_3$ complexes is approximately 50 kcal mol⁻¹ greater than for the $M(acac)_3$ analogs, but the relative order within the series is the same. The higher values for the $M(hfac)_3$ complexes are related to the lower polarizability of the fluorine containing ligand and the resulting lower value of $\Delta D(M-L)_{avg}$.

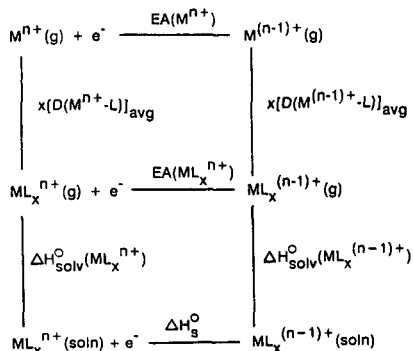
Acetylacetonate is close to H_2O in the spectrochemical series of *d*-orbital splitting. The ground state spin states for each member of the series of $M(H_2O)_6^{3+/2+}$ and $M(acac)_3^{3+/2+}$ couples are therefore the same. Comparison of the redox potentials for the $M(H_2O)_6^{3+/2+}$ couples to the free energy of electron attachment in the gas phase shows a similar trend across both series since electron attachment is to a metal center in a locally similar environment and differential solvation energies for the series of redox couples are roughly constant.



R	LIGAND
CH ₃	ACETYLACETONATO (ACAC)
CF ₃	HEXAFLUOROACETYLACETONATO (HFAC)

Figure 1

Included in this study was an estimation for the enthalpy of electron attachment to $\text{Ru}(\text{acac})_3$ at 298 K. This data as well as the reported reduction potential in water can be used to determine the differential enthalpy of hydration between $\text{Ru}(\text{acac})_3$ and its anion as shown in the lower part of the cycle in Figure 2. The absolute value for the hydration enthalpy of the neutral can be estimated from the reported values for the enthalpy of solution and the enthalpy of sublimation. This leads to a value for the enthalpy of solvation of the anion of $-60 \pm 5 \text{ kcal mol}^{-1}$. For anions of this type, the Born equation fails to predict accurate values from the crystal radii, and the large deviation can be partially accounted for by solvent penetration between the planar chelating ligands in solution. Similarly, in the upper part of the cycle in Figure 2, the difference between the electron affinities of a free metal ion and the corresponding metal complex yields the difference in the heterolytic bond enthalpies between the neutral complex and its anion. Absolute values for the neutral $\text{M}(\text{acac})_3$ complexes have been provided in the literature and so the values for the anions can be estimated. The trends in the bond energies are in accord with the crystal field model.

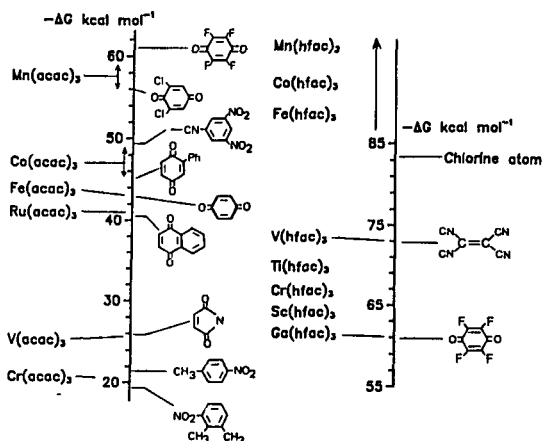


(Support by the NSF (CHE8700765) is gratefully acknowledged.)

Figure 2

Figure 3

ΔG FOR ELECTRON ATTACHMENT TO $\text{M}(\text{acac})_3$
AND $\text{M}(\text{hfac})_3$ COMPLEXES



SOLIDS ANALYSIS VIA GLOW DISCHARGE MASS SPECTROMETRY

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Glow discharge mass spectrometry (GDMS) represents the newest instrumental concept for the bulk analysis of solids. That GDMS is an important new technique is attested to by its recent and rapid commercialization. The use of glow discharge sources in mass spectrometry is described in an excellent review by Coburn and Harrison (1), two of the pioneers in this field. Some of the features that make GDMS an attractive analytical technique are: Direct analysis of metals for major, minor, trace and ultra-trace components with minimal sample preparation, ability to obtain isotopic information and a stable, reproducible ion beam that facilitates quantitative analysis.

The GDMS studies in our laboratory were done on a VG 9000 instrument. This instrument was equipped with a gas purification system to remove trace reactive impurities from the 99.999% Ar used to support the glow discharge. To evaluate GDMS as a quantitative technique we have used empirical sensitivity factors (S.F.). The sensitivity factor method compares the relative sensitivity of the analytical method for each sample constituent to some constituent that is designated a reference (usually a matrix species). The SF's are determined from standards and are then applied in the analysis of unknown samples of similar matrix. Sensitivity factors are defined as

$$SF_i = \frac{I_{ij} / (C_i f_{ij})}{I_R / (C_R f_R)}$$

where SF = the sensitivity factor of element i; I_{ij} = the measured signal of isotope j of element i; and f_{ij} = the isotope fraction of isotope j of element i. Subscript R refers to the relevant property of the reference element.

Table 1 lists sensitivity factors derived from averaged data from multiple analysis of the NIST 600 series low alloy iron standards. NIST does not provide certified values for N and O in these standards.

Table 1. Derived Sensitivity Factors

Standard	661	662	663	664		
Fe Wt. %	95.6	95.3	94.4	96.7		
Element	Avg.	%SD	Element	Avg.	%SD	
C	4.02	22	Co	1.13	12	
N	(12.8)	43	Ni	1.95	6	
O	(3.87)	72	Cu	6.84	14	
Al	1.47	18	As	5.03	11	
Si	2.16	19	Zr	0.64	3	
P	3.36	23	Nb	0.70	10	
S	3.07	29	Mo	1.15	16	
Ti	0.38	13	Sn	2.70	11	
V	0.53	8	Ta	1.55	14	
Cr	2.63	11	W	1.69	11	
Mn	1.65	15	Pb	3.23	10	
Fe	1.0					

(1) Coburn, J. W., Harrison, W. W., Appl. Spec. Rev., 17(1), 95 (1981).

Table 2 shows the results of analyzing NIST 665 as an unknown using the SF's reported in Table 1. In 665 the dopants are present at trace levels and below. GDMS analysis gives values for N, O, Zr, Nb, Sn, Ta, and W, elements that are not listed by NIST as being in the sample. With the exception of C, Al, Ti, and Mo, the observed ratios of GDMS/NIST values are significantly better than our lab does using spark source mass spectrometry on these same samples.

Table 2. NIST 665 Run as an Unknown

Element	Wt. ppm	<u>GDMS</u> NIST	Element	Wt. ppm	<u>GDMS</u> NIST
C	67	2.3	Co	70	1.1
N	*3.		Ni	410	1.1
O	*3.7		Cu	58	1.1
Al	(7)	2.1	As	(2)	1.1
Si	80	1.7	Zr	*7.	
P	25	0.7	Nb	*0.6	
S	55	1.2	Mo	50	2.4
Ti	6	0.3	Sn	*1.7	
V	6	1.8	Ta	*0.9	
Cr	70	1.2	W	*0.6	
Mn	57	1.4	Pb	0.15	0.7

*Values via GDMS analysis; these elements not certified by NIST.

As a test of the ability of GDMS to accurately measure major components in a sample, we derived a suite of SF's from the analysis of NIST stainless steel standards 447 and 449 and applied them to the analysis of NIST 446 and a vendor analyzed sample of 308 stainless steel. These results are presented in Table 3. The two sets of GDMS analytical values for NIST 446 were determined one month apart using the same suite of SF's. The results in Table 3 indicate that if suitable standards are available GDMS can return analytical values for major components that are comparable in accuracy to established methods, such as optical emission and X-ray fluorescence.

Table 3. Stainless Steels

Element	<u>Unknowns</u> <u>All Values are Weight Percent</u>				
	<u>NIST 446</u>			<u>308 S.S.</u>	
	<u>NIST</u>	<u>GDMS</u>	<u>GDMS</u>	<u>Anal Sheet</u>	<u>GDMS</u>
Si	1.19	1.22	1.37	0.35	0.26
V	(.03)	0.035	0.032	---	0.035
Cr	18.35	18.32	17.24	20.08	20.56
Mn	0.53	0.54	0.53	1.49	1.66
Fe	70.6	70.0	69.6	---	67.7
Ni	9.11	9.62	9.00	9.68	9.73
Cu	0.19	0.20	0.40	---	0.0047

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ANALYTICAL COMPARISONS OF DIRECT CURRENT AND
RADIO FREQUENCY GENERATED GLOW DISCHARGE ION
SOURCES FOR DIRECT SOLIDS ANALYSIS

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Recent advances in the field of glow discharge mass spectrometry (GDMS) have primarily employed the more common d.c. sustained discharge. In our laboratory we have employed a radio frequency (rf) powered glow discharge source for the analysis of both conducting and nonconducting sample types. When high voltage pulses, 13.56 MHz in this case, are applied to insulators a d.c. offset voltage exists which results pseudo-continuous discharge which serves as both the atomization and ionization source. Because the need for the sample to be conducting has been eliminated, a wide range of sample types are amenable to analysis employing this source. This range of applicability is demonstrated and other analytical characteristics of interest are compared in the analysis of brass, steel, and glass samples by both rf and d.c. plasmas (mixing of the glass with a conducting metal powder is necessary with the latter).

EXPERIMENTAL

Comparison of the rf and d.c. sources were made under the optimum operating conditions for each as determined by the signal intensity of the matrix material. Optimum source parameters for the d.c. source are typically 1-3 torr with 0.5-3.0 mA discharge current using a pin sample geometry. The rf source, operating at 0.33 torr and typical powers of 25-30W, utilized a 1/2" diameter sample. Comparisons are made relative to the power density (W/cm^2) of each.

Figure 1 is a diagrammatic representation of the double-quadrupole instrument employed in this laboratory. Ions exiting the source region pass through a differential pumping stage and into the analyzer region. These ions are focussed into a collision cell by a three-element lens system. For purposes of this study the collision cell was operated with no collision gas. The collision cell and mass analyzer are equipped with Extrel 3/8" quadrupole assemblies powered by 1.2 MHz generators. Ions traversing the rf-only quadrupole are directed into the analyzer by a single-element lens. All of the spectrometer scan functions, ion lens/pole offsets potentials, and ion detection are controlled by an Extrel C-50 mainframe.

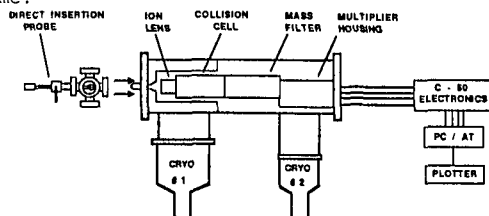
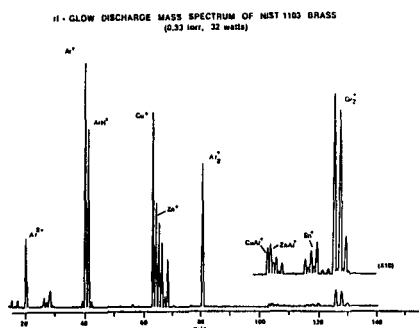
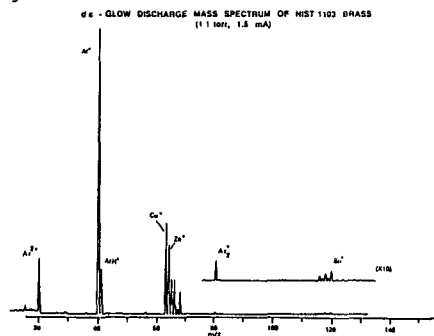


Figure 1. Double quadrupole glow discharge mass spectrometer system.

RESULTS AND DISCUSSION

Shown below are the d.c. and rf glow discharge mass spectra of NIST 1103 brass. These spectra illustrate the typical differences and similarities observed. The molecular interferences such as metal argides and dimers are common to each; however, the metal argide and dimer species are more prominent in the rf spectra. The absolute ion current observed from the ^{63}Cu ion in the rf source is sixteen times greater than that in the d.c.



Sputter rates were also determined for both sources and compared on the basis of power densities. Based on these power density considerations, the rf source appears to be a better atomization source with a sputtering rate twenty-one times that of the d.c. However, the ion currents per unit power density were only four times that of the d.c. indicating that the d.c. source is a more efficient ionizer.

Rf and d.c. powered glow discharges are comparable, with the rf having a distinct advantage in the ability to analyze both conductors and nonconductors. Future studies will involve the characterization and implementation of a dual rf/d.c. probe with subsequent evaluations of the parametric dependencies of the analyte signal intensity and spectral features. Following this, rigorous comparisons of the analytical figures of merit and fundamental studies of the atomization and ionization rates of the respective sources will be made.

PRECISE ATOMIC MASS MEASUREMENTS AND NEUTRINO PHYSICS: RECENT RESULTS AT THE UNIVERSITY OF MANITOBA*

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1. INTRODUCTION Recently, we have used the high resolution double focusing mass spectrometer at the University of Manitoba (Manitoba II) to precisely determine the energy available for the β and double β decay of certain nuclides. The results of these measurements provide data for experiments aimed at determining some of the properties of the electron ν .

2. EXPERIMENTAL DETAILS The Manitoba II spectrometer has been described in detail elsewhere [1]. The instrument is based on a design suggested by Hintenberger and Konig : $R_m = 62.74$ cm, $\Phi_m = 90.00^\circ$, $R_s = 100.00$ cm and $\Phi_s = 94.65^\circ$. The ion optics are correct to second order in both energy and radial angle. Typically resolving powers of 200,000 are realized at 10% of peak height.

A mass peak is generated by sweeping the ion beam across the collector slit by means of a small, sawtoothed magnetic field located near the exit of the magnetic analyzer. The ions that pass through the slit are detected with a channel electron multiplier and a mass spectra is accumulated by a multi-channel scaler/signal averager whose sweep is synchronized to the sawtoothed magnetic field.

The mass difference between two ionic species is measured by some variation of the peak matching technique [2,3]. The technique is based on a theorem given by Bleakney [4] which states that two ions with masses M and M' will follow identical paths through the mass spectrometer if all magnetic fields are unchanged and all of the electrical potentials applied to the instrument are changed from V_i to V_i' such that

$$MV_i = M'V_i' \quad (1)$$

$$\text{or} \quad \Delta M/M = \Delta V/V_i \quad (2)$$

where $\Delta M = M - M'$ and $\Delta V_i = V_i' - V_i$

Thus, on alternate sweeps, the potentials applied to the instrument are changed so that both members of a doublet follow the same trajectory through the spectrometer in succession. The coincidence of these two ion paths may be detected either by the "visual null" [2] or by the "computer assisted peak matching" techniques [3]. Once the required changes for the applied potentials are established one can determine the mass doublet spacing by measuring the ratio $\Delta V_i/V_i$ and combining the result with the mass of one of the doublet members. In practice the potentials applied to the electrostatic analyzer are used to establish this ratio because the beam position is most sensitive to this parameter.

3. RESULTS AND DISCUSSION Two modes of $\beta\beta$ decay are possible. The first is similar to ordinary β decay in that 2 β particles and two anti- ν s are emitted. In this mode lepton number is conserved and the energy spectrum of the emitted β particles is very broad. In the second mode two β particles are emitted but the ν emitted with the first β is absorbed during the emission of the second. Therefore, two electrons are emitted with the simultaneous emission and re-absorption of a virtual ν . Lepton number is not conserved and the energy spectrum of the emitted electrons consists of a single narrow peak corresponding to the total energy available for the decay. This provides a clear, unambiguous signature for the detection of this decay mode. The detection of this process would imply that the ν and its corresponding anti-particle were the same and that a lower bound (greater than zero) exists for the mass of a ν . Accordingly, various groups around the world have been searching for evidence of neutrinoless

$\beta\beta$ decay [5,6].

The half-lives associated with $0\nu\beta\beta$ decay are usually on the order of 10^{22} years. This results in extremely low event rates (a few counts per year) in existing detectors. It is therefore important that the $\beta\beta$ Q-value be accurately and precisely known, enabling experimenters to distinguish between the numerous background peaks that appear in the spectra and the signature of the decay. In past work [7] we have measured a Q-value of 2040.71 ± 0.52 keV for the $\beta\beta$ decay of ^{76}Ge . This has served to identify as background some peaks occurring near the region of interest (approximately 1 keV away) [5,6].

We have also determined the spacing of 4 doublets among isotopes of Te and Xe [8]. These measurements when combined with other mass spectrometric measurements in this region over-determine the energies available for the $\beta\beta$ decay energies for ^{128}Te and ^{130}Te . The results of a least squares adjustment in this region yield $\beta\beta$ decay Q-values of 930.3 ± 1.3 keV for ^{128}Te and 2914.4 ± 1.6 keV for ^{130}Te . Earlier data underestimated the value for ^{130}Te by 6 keV, a serious discrepancy. In both of these cases our new data improve the precision of these Q-values opening the way for future searches for these rare decay modes.

The observed flux of neutrinos from the sun is approximately a factor of 4 lower than that predicted by theoretical models. It has been suggested that a detector with a lower energy threshold may be able to resolve this discrepancy. The inverse beta decay of ^{205}Tl to ^{205}Pb caused by ν capture may provide such a detector. Through this process ^{205}Tl in geological samples has been integrating the solar ν flux. Since the half life of ^{205}Pb (1.5×10^7 years) is much shorter than the age of the earth little primordial ^{205}Pb is expected to survive in geological samples. A determination of the abundance of ^{205}Pb in thallium ores would therefore reflect the effects of the solar ν flux averaged over approximately 10^7 years. The ν absorption cross section of ^{205}Tl is very sensitive to the mass difference $^{205}\text{Pb} - ^{205}\text{Tl}$ (this is essentially the Q-value for the electron capture decay of ^{205}Pb to ^{205}Tl , Q_{ec}). This mass difference has been deduced from the intensity ratios of the X-ray lines emitted following electron capture as 41.4 keV. This value disagrees with that calculated from other nuclear reaction data, 53.5 ± 1.6 keV. This 12 keV discrepancy is sufficient to change the ν absorption cross section by a factor of 2. To resolve this discrepancy we have determined 4 new doublet spacings in the mass spectra of lead and thallium chlorides. The average precision of these measurements was $2.5/10^4$. When combined with existing nuclear reaction data these new measurements provide up to 4 different ways of calculating Q_{ec} . A least squares adjustment of all available data (excluding the X-ray measurements) results in a value of 53.40 ± 0.4 keV for this energy[9].

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A MASS-SELECTED REAGENT ION CHEMICAL IONISATION SOURCE

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A mass-selected reagent ion chemical ionisation (CI) source has been developed for producing novel reagent ions, for reducing chemical noise in the CI mass spectra and for studying the mechanism of the CI process. Reagent ions from a Balzers quadrupole mass filter (mass range = 255) enter the high pressure reaction chamber of a Kratos MS9 forward geometry double focussing mass spectrometer. The high pressure reaction chamber contains the sample and helium buffer gas. Ionic products are analysed by scanning the magnet as in ordinary CI. A diagram of the mass-select reagent ion CI source is shown in Figure 1.

The quadrupole mass spectrometer and its power supplies are floated at the accelerating voltage of the MS9 (in this case 6kV) using a specially designed insulating flange and an isolation transformer. Both the reaction chamber and the quadrupole housing are mounted on a PEEK insulator which has been inserted into a standard MS9 source flange. The quadrupole and the high pressure reaction chamber are differentially pumped so that there is little mixing of the sample gas and the reagent gas. The pressure in the reaction chamber is measured by a Baratron capacitance manometer and is variable from 0.1 to 1.0 torr.

The mass-selected reagent ions pass through a short rf-only quadrupole after the mass filter to collimate the ion beam. In order to inject the reagent ions into the reaction chamber, they are accelerated to 2 keV of kinetic energy through a lens system (designed using the SIMION program) and focussed on to a 0.5 mm diameter aperture in the high pressure reaction chamber. This technique overcomes the scattering caused by the jet of gas exiting through the aperture. After entering the high pressure region the reagent ions are decelerated to less than 5 eV by a voltage applied to a grid located 1.5 mm behind the aperture. Any ions formed due to collision-induced dissociation with the buffer gas necessarily have less kinetic energy than the reagent ions and cannot pass the grid into the reaction chamber.

When the reagent ions have passed into the reaction chamber they are rapidly thermalised by multiple collisions with the He buffer gas. Since the partial pressure of buffer gas exceeds that of the sample by at least a factor of 100, most of the thermalising collisions are with the buffer gas, not the sample. Using He as the buffer gas also has the effect of eliminating glow discharge ionisation in the reaction chamber. The thermalised reagent ions react with sample molecules by means of ion/molecule reactions and yield product ion spectra of the type shown in Figures 2 and 3.

Fig. 1.

COMPONENTS OF THE SELECTED-REAGENT ION CI SOURCE

1. Quadrupole Ion Source
2. Quadrupole and Mounting Flange
3. PEEK Insulator
4. MS9 Source End-Flange
5. Lens 1 and Screen
6. Lens 2
7. Lens 3
8. Lens 4
- PTFE Spacer (between 5 and 9)
9. 80% Transmission Screen and MS9 CI Source
10. Brubaker Lens (rf-only quadrupole)

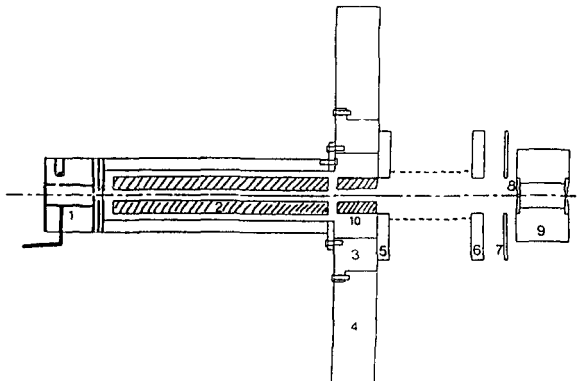


Fig.2.

AMMONIA IONIZED BY CH_4^+

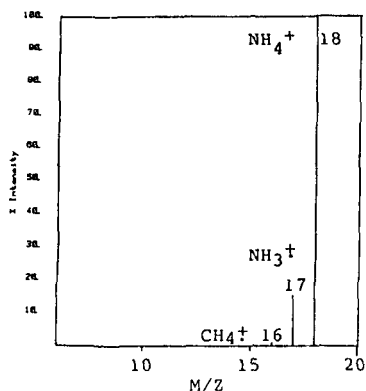
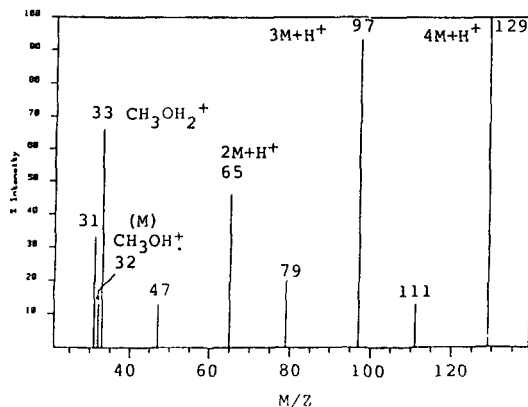


Fig.3.

METHANOL IONIZED BY CH_3OH^+



GC SAMPLE INTRODUCTION INTO THE COLLISION CELL OF A TRIPLE QUADRUPOLE MASS SPECTROMETER FOR MASS-SELECTED REACTANT ION CI

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A new gas chromatographic/tandem mass spectrometric technique has been investigated. This technique, gas chromatography/mass-selected reaction/mass spectrometry (GC/MSR/MS), involves mass-selection of reactant ions and reaction of these ions with the effluent from a GC column. This is accomplished by interfacing a gas chromatographic column directly to the collision cell of a triple quadrupole tandem mass spectrometer. Reactant ions are selected with the first quadrupole mass filter (Q1) and are allowed to react with neutral sample molecules which elute from a short (e.g., 2-3 m) open tubular GC column into the second quadrupole collision cell (Q2). The third quadrupole (Q3) is scanned for the products of the ion-molecule reactions.

Charge exchange (CE) and proton transfer chemical ionization (CI) reactions have been investigated. The selectivity of CE and proton transfer CI results from control of the energetics of the ion-molecule reactions between reactant ions and sample molecules. For example, a CE reaction may occur if the ionization energy (IE) of the sample molecule is less than the recombination energy (RE) of the reactant ion. Similarly, proton transfer CI reactions may occur if the proton affinity (PA) of the sample molecule is greater than the proton affinity of the conjugate base of the reactant ion. For both CE and proton transfer CI, the exothermicity of the ion-molecule reaction largely determines the extent of fragmentation of the molecular ion or pseudo-molecular ion. Since ion-molecule reactions can be performed with selection of a single m/z reactant ion, mixed-mode ionization, which is often observed when ion-molecule reactions are performed in the ion source, is avoided. Thus, mass-selection of reactant ions provides the highest possible selectivity for ion-molecule reactions.

One example of the intrinsic selectivity of ion-molecule reactions is demonstrated by the mass-selected CE chromatogram in Figure 1, where both argon and benzene were ionized in the source and the Ar^+ and C_6H_6^+ reactant ions were mass-selected with Q1 during alternating scans. As shown in the figure, the Ar^+ (RE = 15.8 eV) reactant ionizes all of the compounds in the mixture, while the C_6H_6^+ reactant (RE = 9.2 eV) ionizes only the aromatic components. Since the RE of the C_6H_6^+ reactant ion is less than the IE's of the aliphatic compounds, the aliphatic compounds are not ionized via benzene CE. The added selectivity of mass-selected reactions is particularly useful when combined with short-column GC to rapidly screen for specific classes of compounds. For example, C_6H_6^+ CE can be used to investigate the aromatic content of a jet fuel (a complex mixture containing aromatics and aliphatics) without interference from aliphatic constituents.

Another advantage of the GC/MSR/MS technique is that both structural information and molecular weight information can be obtained in the same chromatogram (i.e., a single GC injection) by alternating between reactants that have different recombination energies or proton affinities. Figure 2 shows a comparison of the spectra of 2,4 dimethylaniline obtained from Ar^+ and C_6H_6^+ CE from the chromatogram of Figure 1, as well as an EI spectrum acquired separately for comparison. Note that the Ar^+ CE spectrum resembles the EI spectrum (with more intense fragment ions), while the C_6H_6^+ CE spectrum exhibits an intense molecular ion with very little fragmentation. In addition, we have found that the mass-selected Ar^+ CE spectra provide reasonably consistent hits when searched against the NBS EI library. Thus, mass-selected CE spectra appear to be highly useful in providing both structural and molecular weight information for the classification of unknowns.

It was found that the detection limits obtainable with the GC/MSR/MS technique are comparable or better than those obtained with conventional GC/MS. Figure 3 shows mass-selected reaction chromatograms obtained with benzophenone as the analyte and C_6H_6^+ as the CE reactant ion. Figure 3a shows the mass chromatogram for the m/z 182 ion of 100 pg of benzophenone for a full scan of Q3 (80-240 amu in 0.5 seconds). Figure 3b shows the chromatogram obtained from 15 pg of benzophenone when Q3 was scanned from 181-183 amu in 0.5 seconds. The signal-to-noise ratios (S/Ns) for Figures 3a and 3b are approximately 9:1 and 30:1, respectively. For comparison, the specifications for the full scan sensitivity of the instrument used (Finnigan MAT TSQ70) with GC/MS are quoted as 200 pg for EI with a S/N of 10:1 and 100 pg with methane CI with a S/N of 25:1. The ability to obtain the molecular ion (or pseudo-molecular ion) with little or no fragmentation for an analyte provides the maximum sensitivity for trace analysis. Since such 'soft ionization' is easily performed with mass-selected reactions, the GC/MSR/MS approach provides the opportunity to control the ionization to obtain excellent limits of detection.

FIGURE 1.

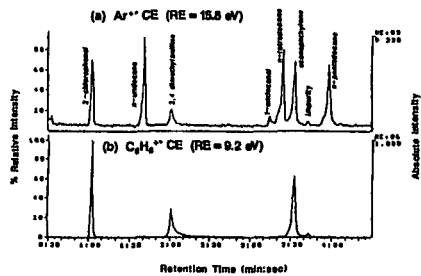


FIGURE 2.

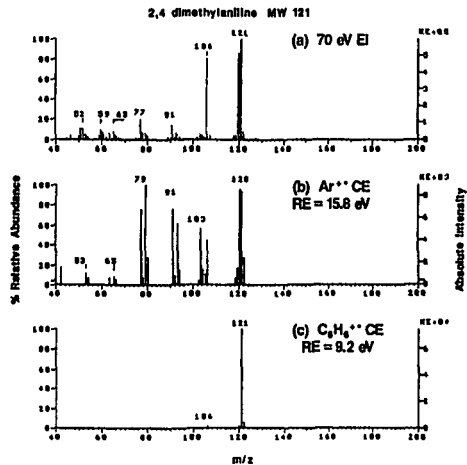
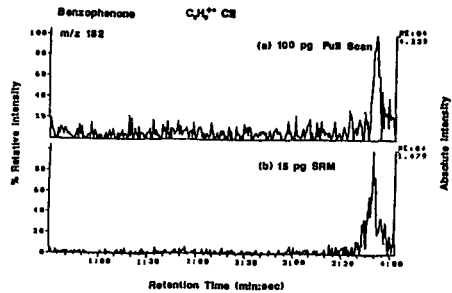


FIGURE 3.



ONE QUADRUPOLE ANALYSER, MS^{n+1} , ZERO-ANGLE (SID)ⁿ

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The surface induced dissociation process (SID) has been shown by P. G. A. Cooks to be a particularly efficient way of breaking ions difficult to fragment by other processes, such as Poly Aromatic Hydrocarbons (PAH) (1) or isomeric ions (2). Various instruments have been designed by this group and others for performing SID : B (SID) Q (3), Q (SID) Q (3) or Q (SID) Q (CAD) Q (4); recently a new instrument working under Ultra High Vacuum and in a wide range of angle has been built up (5); furthermore they have designed a "on line" SID device (6) that can be used in a conventional tandem MS/MS triple quadrupole instrument with a linear geometry. Current work from our laboratory has demonstrated that a quadrupole filter can be successively used as an analyzer and as an ion-trap in the same experiment (7). This led us to the idea of a new configuration for an MS/MS instrument using a zero angle SID:

source	quadrupole	surface	detector
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This configuration has potential advantages : (i) collision energy is maximum at zero angle, (ii) strong focussing properties of quadrupole field and good symmetry fit allow high collection efficiency. (iii) this configuration gives the virtual possibility of consecutive SID experiments allowing MS^n experiment with a single analyser.

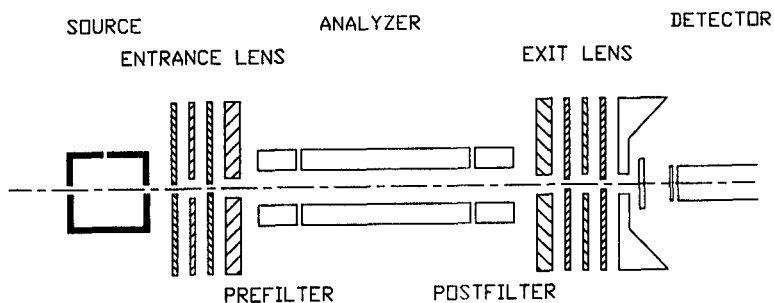
A typical SID experiment follows this scheme : (i) filling of the trap (RF-only), (ii) parent ion selection (resolving quadrupole), (iii) SID (RF-only, and opening of the exit lens), (iv) daughter ion selection (resolving quadrupole), (v) ion detection (switching on the detector).

Preliminary experiments show that using this SID configuration daughter ions can be obtained; improvement of the overall sensitivity is under way.

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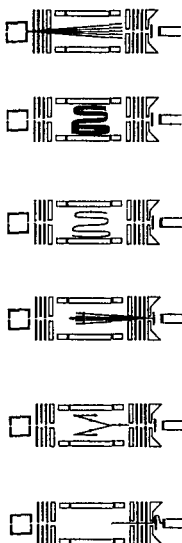
NEW INSTRUMENT



UNIVERSITY MADE CYLINDRICAL RODS
STAINLESS STEEL FILTER

-LENGTH 0.18 m (7")
-DIAMETER 0.015 m (9/16")

TIME AGAINST SPACE



USE OF A FOCUSED LIQUID METAL ION COLUMN
WITH A HIGH PERFORMANCE MAGNETIC SECTOR MASS SPECTROMETER

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The principal, distinguishing, optical characteristic of a focusing liquid metal ion (LMI) column is its very high brightness; in practical terms this means that a very large number of the emitted particles can be focused into a very small spot.¹ Because of this characteristic, we have suggested in the past² that an opportunity might exist for increasing the sensitivity of liquid matrix assisted organic SIMS by using a finely focusing LMI column to substantially reduce the absolute amount of analyte required for analysis, to increase the efficiency of ionizing the analyte molecules, and to increase the efficiency of collecting the analyte ions for transmission through the mass analyzer. Two years ago we attached a commercial, focusing LMI column (FEI Company) to a single focusing, magnetic sector mass spectrometer (Hitachi RMU-6E) and were able to obtain usable mass spectra from subnanogram amounts (~300 fmole) of several tripeptides.³

Although our earlier results demonstrated the potential for significantly greater sensitivity in liquid matrix SIMS, they were obtained with only small molecules on less than modern instrumentation. Consequently, we built a prototype LMI column for testing on a Kratos MS50TC-RF mass spectrometer. The LMI column delivers a positive ion beam of 1-5 nA into a spot on the target 3-10 μm diameter (1-70 mA cm^{-2}). The column is operable at accelerating voltages up to 25 kV. To prevent degradation of the LMI beam's optical properties as it approaches the sample target, the LMI column was built to float at the ion source potential of the mass spectrometer, and a small bore tube, also maintained at the ion source potential, was provided to guide the primary ions from the LMI column to the sample probe without interference from external electric fields. With the aid of a small electron multiplier located in the vicinity of the sample and a display monitor, the system could be operated in a scanning ion microscope or microprobe mode in which the sample could be alternately imaged and mass analyzed. This feature was used to tune the LMI column and to locate a sample and coarsely aim the beam onto it.

Samples, 10-100 nL, were introduced into the mass spectrometer on a small diameter wire either as a dried smear or as a small viscous droplet suspended on the wire like a bead. Secondary ion yield was maximized by accurately finding the optimum emission zone on the sample with the finely focused beam from the LMI column, and at the same time maximizing collection and transmission of the secondary ions by accurately positioning the sample within the acceptance zone of the mass analyzer. Tuning was monitored by observing the shapes and amplitudes of individual mass peaks on the instrument's oscillographic screen. Tuning proved to be difficult, but once achieved, was generally stable over an entire sample run requiring only minor adjustment to the LMI beam position to compensate for shrinkage of the sample ball. Even after a change of samples, little or no adjustment of the tuning was required. We were frequently able to exploit this feature when we attempted to analyze samples that had only short lifetimes in the mass spectrometer or that had such low concentrations that the analyte signal could not be observed in single scans.

Numerous mass spectra for each of the oligopeptides Met-Enkephalin (M,574), Bradykinin (M,1060), Bovine Insulin Chain B, Oxidized (M,3500), Glucagon (M,3550), Bovine Insulin (M,5735), Aprotinin (M,6510), and Ubiquitin (M,8560) and each of the oligonucleotides d(A)₄ (M,1190), dp(T)₄ (M,1234), dp(T)₆ (M,1842), dp(T)₈ (M,2449), d(GAT CGA TCG A) (M,3050), dp(T)₁₀ (M,3058), d(ATG CAT ATG CAT) (M,3642), d(GAT CCC TGC AGG) (M,3644), d(GTA AGG AGG TTT AC) (M,4340), d(TGA CCA TGA TTA CGA) (M,4573), d(CGG GAA AGG TTC CGT TC) (M,5223), d(GTA ACA ATT TTG TAA AAA) (M,5520) were recorded in both LMI and FAB modes for comparison. It was possible to obtain reasonably quantitative sensitivity data for most of the oligopeptides, but relatively little

quantitative data could be obtained for the oligonucleotides because of their low solubilities. A negative, integrated molecular ion profile, produced from 14 pmole (50 ng) of bovine insulin chain-B, oxidized, by bombardment with 25 keV Bi-ions is shown in Figure 1; this spectrum is typical of those produced from the other oligopeptides and the oligonucleotides. With respect to the relative sensitivities of the LMI and FAB modes on the MS50 for the two classes of biopolymers studied, it appears 1) that the two techniques have comparable sensitivities for either class of compounds up to 1,000 daltons, 2) that the LMI mode is roughly 5-10 times more sensitive than the FAB mode for oligopeptides over 1,000 daltons (bovine insulin being a notable exception that must be retested), and 3) that the FAB mode is slightly more sensitive than the LMI mode for oligonucleotides up to about 4,500 daltons and significantly more sensitive beyond that mass. The sensitivity using a focused LMI primary beam is not as great as anticipated due to what appears to be a radiation damage limit. Our results to date suggest that with technical improvements realistic liquid matrix, secondary ionization detection limits for biopolymers in the mass range of 1,000-10,000 daltons can be reduced to around 0.1 pmole for oligopeptides and 10 pmole for oligonucleotides. Of course, these are best case values and many compounds would be expected to have higher detection limits.

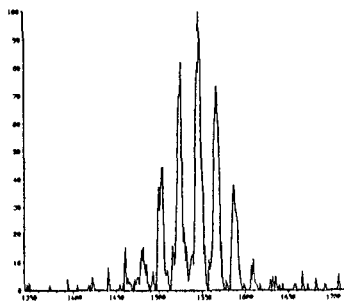


Figure 1

Acknowledgement

Funds for this work were provided by NIDDK DK36050, NIGMS/SBIR GM40250, and NIEHS ES00210.

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EXACT MASS DETERMINATION BY HIGH RESOLUTION TIME-OF-FLIGHT SIMS

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In many applications of static SIMS an exact mass determination is required to derive or confirm the composition of a molecular ion. This is true not only for the analysis of large biomolecules, but also for surface analysis of complex materials like polymers. In combination with a high mass resolution accurate mass determination eases the peak assignment and the interpretation of the spectra considerably.

First time-of-flight SIMS instruments (TOF-SIMS) had a rather poor mass resolution of a few hundred, but during the last years the resolution was increased considerably. With the high resolution TOF-SIMS, developed at the university of Münster, we achieved a resolution of 13000 /1/. The instrument is equipped with a mass separating pulsed primary ion gun (pulse width 800 ps), a reflectron-type TOF-analyzer, a postacceleration detector and a multistop time-to-digital converter (800 MHz resolution).

In this paper the precision of mass determination presently achievable by TOF-SIMS will be discussed.

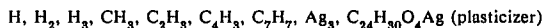
1. Mass calibration

For an electrostatic TOF-analyzer there is a strict and simple relation between mass and flight time:

$$m = c (t - t_0)^2$$

In TOF-SIMS deviations from the exact masses are mainly determined by the energy distribution of the secondary ions. The corresponding flight time deviations are quite large in a linear instrument, but they are reduced significantly by energy focusing. A precision in the ppm-range can be expected for a reflectron TOF-analyzer.

Due to the parallel detection of all masses well known peaks in the low mass range can be used for mass calibration. The two parameters c and t_0 were determined by a least square fit for up to 10 peaks in the mass range up to 500, which are present in almost any positive SIMS spectrum:



Therefore the calibration procedure is simple and no special calibration standards and peak matching methods are necessary.

Typical deviations of the calibration peaks from the fitted curve are less than 0.1 mmu for the masses 1 - 3 amu and less than 1 mmu for the higher masses.

2. Results

2.1. Low mass range (1 - 500 amu)

A large number of spectra were evaluated in the low mass range including fingerprint spectra of polymers like PMMA etc. /2/. Typical errors in this mass range are about 1 mmu or below (10 - 20 ppm).

2.2. High mass range (> 1000 amu)

The accuracy of mass determination in the high mass range was investigated with several spectra of the polymer Polydimethylsiloxane (PDMS) prepared on Ag. The spectra show high intensities of intact oligomers cationized by Ag in the mass range from 1157 up to several thousand amu. The calibration was performed by using the peaks given above. Then the masses of the oligomers and their deviations from the calculated masses were determined. Table 1 summarizes the relative mass deviations $m/\Delta m$ for four different spectra. A precision better than 10 ppm was achieved. From the measured masses of the oligomers the mass of the repeat unit of the polymer and the masses of the terminal groups could be determined with an accuracy of 1 - 2 mmu.

Table 1

sample	average deviation	standard deviation
1	0.5 ppm	2.9 ppm
2	5.1 ppm	3.9 ppm
3	-6.4 ppm	4.2 ppm
4	5.2 ppm	2.9 ppm

In addition the precision of mass determination was investigated for some peptides like Met-Enkephalin (MW 573.226), Bradykinin (MW 1059.561) and Substance P (MW 1346.727). The results are listed in table 2.

Table 2

sample	composition	average deviation
Met-Enkephalin	$C_{27}H_{35}N_5O_7S$	7.5 ppm
Bradykinin	$C_{50}H_{74}N_{15}O_{11}$	10.0 ppm
Substance P	$C_{63}H_{99}N_{18}O_{13}S$	19.5 ppm

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HIGH PRECISION CARBON $^{13}/^{12}$ RATIOS BY
GC / COMBUSTION / MASS SPECTROMETRY

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Although the method of combusting organic compounds eluting from a Gas Chromatograph and subsequently determine the $^{13}/^{12}$ Carbon isotope ratio with a dedicated multiple collector mass spectrometer has been reported as early as 1976 [1], considerable progress leading to commercially available products has arisen only recently [2,3].

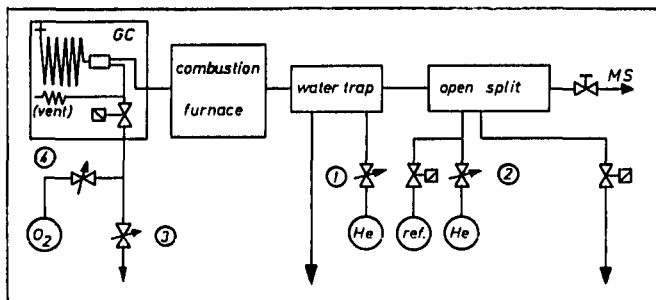


Figure 1: Combustion interface layout

In our system (fig.1) the GC eluate passes through a micro combustion furnace. Its quartz tube is 200 mm in length and has an i.d. of 0.6 mm. Filled with cupric oxide, organic compounds passing the furnace are oxidized quantitatively to CO_2 and water at 750 C. The water is removed continuously using a permeable membrane separator. A conventional open split assembly serves to introduce 0.6 ml/min gas into the ion source of the mass spectrometer. The combustion interface can be backflushed for peak cutting and for solvent peak suppression.

The mass spectrometer records the CO_2 masses 44, 45 and 46 (the latter for 17-O correction on 45) simultaneously in a triple Faraday cup arrangement. Fast data acquisition using a dedicated microprocessor board ensures continuous data integration without interruption.

Figure 2 shows the intensity of the 44 ion current as a function of time. Here, the separation of C-17 and C-18 n-alkanes from the saturated isoprenoids phytane and pristane in a crude oil extract is demonstrated. The upper trace monitors the evolution of the 45/44 isotope ratio over time. In all cases we have observed, the 45 ion current always precedes its 44 counterpart by several tens of milliseconds, resulting in an apparent "differential" peak shape. This is due to isotopic separation inside the capillary column. The exact amount of separation in time depends on a number of parameters like column type, flow rate, compound, temperature. However, in cases where the $^{13}/^{12}$ ratio is off the naturally occurring range, the trace will exhibit a "normal" peak shape (i.e. only positive or only negative), thus enabling a very sensitive detection of $^{13}\text{-C}$ labeled material down to the picogram range.

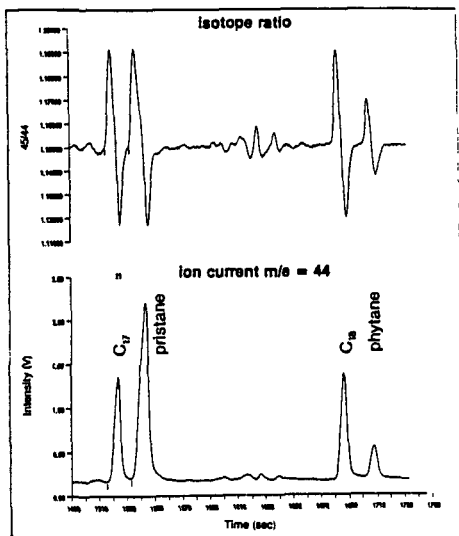


Figure 2: GC/C/MS traces

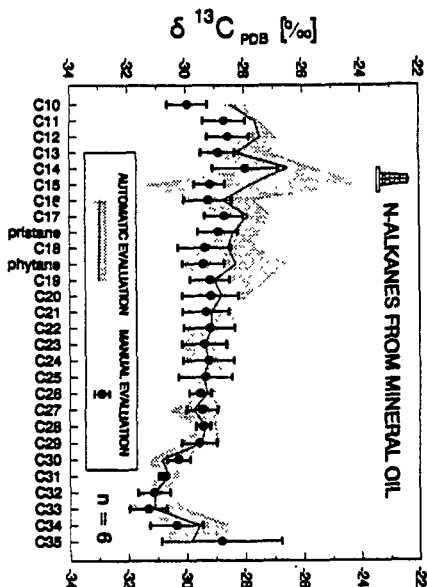


Figure 3: Crude oil ^{13}C record

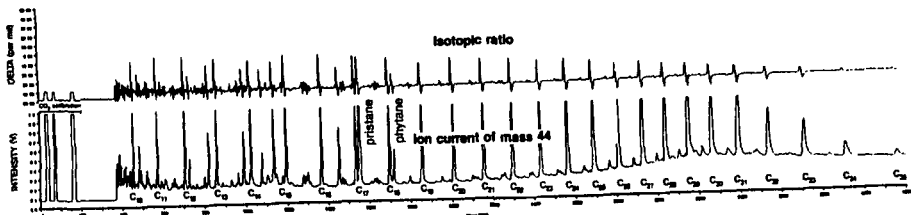


Figure 4: Crude oil extract

Finally, a complete carbon isotopic record has been obtained for a crude oil extract (Fig. 3 and 4) ranging from C-10 to C-35. The points were obtained by checking and correcting manually the automatic background and peak boundary assignments. In the lower carbon number region, corrections up to 2 permil were necessary due to peak overlap whereas for the higher carbon numbers corrections were negligible. From the isotopic record conclusions can be drawn to the history of the oil and the origin of the compounds. E.g. the C-30 to C-34 n-alkanes show an admixture from terrestrial higher plants which derive their carbon from atmospheric CO_2 by the Calvin photosynthesis cycle.

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AROMATIC SUBSTITUTION REACTIONS OF RADICAL CATIONS

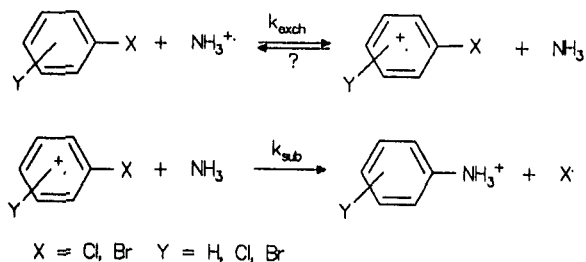
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The aromatic substitution is one of the most important organic reactions in solution, both by electrophiles and by nucleophiles. Usually these reactions proceed by a two electron transfer to and from the aromatic system to form a Meisenheimer und a Wheland intermediate, respectively. Besides these classical aromatic substitution reactions, other mechanisms involving a single electron transfer and hence radical cations and radical anions are discussed in the literature, in particular for photochemically and electrochemically induced reactions.

Aromatic substitution reactions in the gas phase were observed for certain benzene derivatives under $\text{Cl}(\text{NH}_3)$ conditions. Although radical cations participate very likely in this reaction, the mechanism is not very well known. The present paper gives the results of a detailed ICR study of the reaction between halogenated benzenes and NH_3 after electron impact ionisation, using an external ion source.

The isomeric dichlorobenzenes give rise to chloroanilinium ions in a $\text{Cl}(\text{CH}_3)$ plasma. The comparison of the CA spectra of these product ions with those of authentic reference ions reveals an ipso-substitution of the chloro substituent by NH_3 . The transfer of NH_3 radical cations into the ICR cell and reaction with mono- and dichlorobenzenes results only in an efficient charge transfer. However, the reaction of externally generated radical cations of halogenobenzenes with neutral NH_3 gives rise to nucleophilic substitution products.



The following reaction rates have been determined:

compound	$k_{\text{exp}} \times 10^{-10} [\text{cm}^3 \text{s}^{-1}]$	eff = $k_{\text{exp}}/k_{\text{AOC}} \times 100$
<chem>C6H5Cl</chem>	3.2	19 %
<chem>C6H5Br</chem>	3.2	20 %
<chem>o-C6H4Cl2</chem>	2.6	16 %
<chem>m-C6H4Cl2</chem>	1.4	8 %
<chem>p-C6H4Cl2</chem>	0.1	0.6 %
<chem>2-BrC6H4Cl2</chem>	4.0	25 %
<chem>3-BrC6H4Cl2</chem>	1.7	10 %
<chem>4-BrC6H4Cl2</chem>	0.4	2.4 %

The reactions proceed with an efficiency between 25% and 0.6%, and the rates decrease with the kinetic energy of the aromatic radical cations. The radical cations of chlorobenzene and bromobenzene exhibit identical reactivities, but for the dihalogenobenzene radical cations the rate depends strongly on the position of the second substituent and decreases in the series ortho > meta >> para.

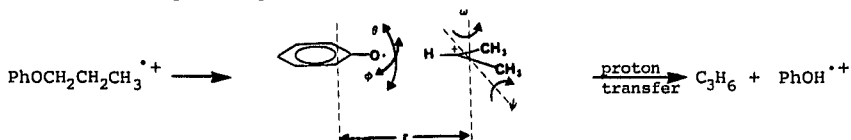
Furthermore, in the case of the bromo-chlorobenzenes a positional substituent effect on the reaction selectivity is observed. The 2-bromo-chlorobenzene radical cations substitute the Cl- and Br-substituent nearly at a ratio of 1:1 while substitution of Br is preferred in the radical cations of 4-bromo-chlorobenzene by a factor of 14.

These results indicate a multi-reaction step mechanism for the nucleophilic substitution of the radical cations of halogenated benzenes and a change of the rate determining step with the type of substitution of the benzene derivative.

SOLVOLYSIS AND PROTONATION: POSITIVE ION FORMATION IN THE GAS PHASE AND SOLUTION

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Bond heterolysis of a gaseous ion to form an ion-molecule complex can be described as a gas phase analogue of solvolysis, where a covalent bond (which is directional in nature) is converted to an ionic bond (where the ion is virtually free to rotate about its center of mass and to undergo structural rearrangements). In the gas phase the ionic bond consists of ion-dipole and ion-induced dipole components, rather than ion pairing. Otherwise the chemistry of ion-molecule complexes is very much similar to the chemistry of ion pairs in solution when they undergo E1 elimination reactions.



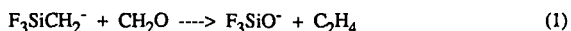
We have investigated a model for the formation of ion-molecule complexes from covalent precursors. Our model does not assume that there is any potential energy barrier separating the ion-molecule complex from a covalent isomer. (In practice, ion-molecule complexes can be reliably inferred only when the cationic fragment undergoes a rearrangement, such as the conversion of n-propyl phenyl ether molecular ion to the complex of isopropyl cation with phenyloxy radical, depicted above.) Although there is, in general, a barrier between the precursor and the complex, there is no potential energy barrier preventing the complex from returning to a rearranged covalent molecule (e.g. the collapse of the complex above to the molecular ion of phenyl isopropyl ether).

We consider the number of vibrational states that lead to simple bond fission, $G_{3N-7}(E^*-E_0)$, when the precursor molecule has internal energy E^* and a thermodynamic dissociation barrier E_0 . We compare $G_{3N-7}(E^*-E_0)$ for the simple fission transition state with the number of vibrational states corresponding to the ion-molecule complex. To get this latter number we compute the density of states of the ion-molecule complex, $\rho'_{3N-6}(E^*-E_1)$, where E_1 is the energy of the ion-molecule complex relative to its covalent precursor. The internal degrees of freedom for the ion-molecule complex are the 3 degrees of freedom of a point dipole in the central field (not necessarily symmetric) of the ion represented by r , ψ , and ω , the two librations of the dipole represented by θ and ϕ , the relative rotation about the axis connecting the centers of mass, and the internal degrees of freedom of the two fragments. When we compute $\Delta E \rho'$ (where ΔE corresponds to uncertainty broadening) and G_{3N-7} for phenoxy radical + isopropyl cation, we find that for ion-molecule complexes in the range $r = 7\text{\AA}$ to $15\text{\AA}$, complex formation is approximately as probable as complete fission ($r=\infty$) even when the total energy content corresponds to $E^*-E_0 = 0.5\text{ eV}$. This is taken to account for the predominance of ion-molecule complex products over simple fission in R2PI/Photodissociation expts.

SOLVENT AND STRUCTURAL EFFECTS ON GASEOUS ION ENERGETICS AND REACTIONS

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A number of gas phase ion molecule reactions have been investigated, using the techniques of Fourier transform ion cyclotron resonance spectrometry and high pressure mass spectrometry, which illustrate both the similarities and differences between reactions of ions in the gas phase and in solution. The considerable power of ion cyclotron resonance techniques to probe mechanistic details of ion molecule reactions has been exploited to examine an isoelectronic analogue of the Wittig reaction. Both fluorosilyl and methylsilyl substituted carbanions can be regarded as isoelectronic with a phosphorous ylid which serves as the key reagent in the Wittig reaction. For example, if $\text{F}_3\text{SiCH}_2^-$ is allowed to react with formaldehyde a Wittig style reaction occurs, presumably by a 4-centered intermediate, to yield F_3SiO^- and ethylene (eqn 1).



This reaction appears to be general for any combination of methyl and fluorine substitution on silicon in the silyl carbanion. However when exothermic proton transfer can occur it usually proceeds to the exclusion of the Wittig like reaction.

A second mechanistic investigation with a solution phase analogue involves the gas phase derivatization of carbonyl compounds. In solution carbonyl compounds are frequently characterized by the melting point of oximes which are generated in the reaction of the carbonyl with hydroxylamine in acidic solution. We have examined an analogous process in the reaction of protonated methoxyamine with carbonyl compounds. As in solution the reaction observed is elimination of water from an intermediate complex to yield a protonated oxime as, for example, in eqn (2).



High pressure mass spectrometry has proven itself to be an outstanding method for the determination of energetics of both exchange and clustering ion molecule equilibria. We have made use of this technique to examine the clustering of acetone and dimethyl ether onto methylated acetone. The adducts of both ether and acetone with methylated acetone are strongly covalently bound with binding enthalpies of 26 kcal/mol and 22 kcal/mol respectively. The principles of stereoelectronic control predict that the most strongly bound conformer will be that one in which a maximum amount of steric interference will arise. This prediction is borne out in the measured entropy changes for these two clustering reactions of -58 cal/mol-K and -42 cal/mol-K respectively (see Table 1). Construction of space filling models serves to verify that the ether adduct is indeed more sterically congested than the acetone adduct. In order to confirm that these anomalously large negative entropy changes are due to the restriction of rotations in the adducts we have also examined various deuterated analogues of these adducts. In each case the expected behaviour is observed with the magnitude of the negative entropy change increasing with increasing deuterium substitution. This study has thus permitted a quantification of the magnitude of steric effects in congested species. At higher temperatures it is observed that a marked change in the clustering energetics occurs with a shift to lower binding enthalpies but also to more normal less, unfavorable entropies. This change is associated with an isomerization to a more weakly bonded methyl cation bound dimer species with minimal restriction of internal rotations.

The advantages of FTICR and high pressure mass spectrometry have been combined in a new instrument which we have recently constructed at the University of Waterloo. An external high pressure ion source has been mated to our Spectrospin CMS-47 FTICR spectrometer to allow the production of cluster ions in the high pressure environment and to then transfer them to the ICR cell where they can be trapped for long periods of time and their subsequent ion molecule reactions with selected substrates examined. This facility allows us to probe the transition from gas phase to solution phase behaviour by generating ions with a specific extent of solvation and observing the change in reactivity as this extent of solvation is varied. For example we have recently examined the bimolecular reactions of protonated water clusters with a variety of substrates. If the $(\text{H}_2\text{O})_3\text{H}^+$ ion is allowed to react with acetone in the ICR cell the reactions observed are the addition of acetone followed by elimination of either one or two molecules of water. The new species formed then react in the same fashion until all of the water has been removed from the cluster ion. Interestingly the ion formed at m/z 117 is found by CID experiments to have a structure different than the proton bound dimer (I) which is produced in the direct association of protonated acetone with acetone. This ion most likely has the structure of a dimethyl-alkoxy carbocation (II). This is then a good example of how solvent can modify

ionic reactivity. Since the reactant ion is a protonated acetone coordinated to one or two water molecules the second acetone molecule cannot add at the proton since a water molecule already blocks this site. Instead, the acetone approaches the carbonyl carbon, the site thought of in solution as the more reactive centre. This reaction can be thought of as the reaction of acetone with a 12 M acid droplet in the gas phase.

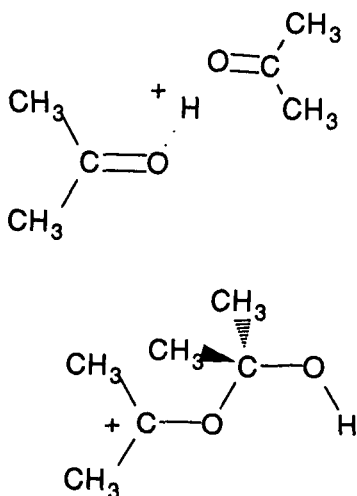


TABLE 1

Methyl cation transfer and clustering reactions and associated thermochemical data derived

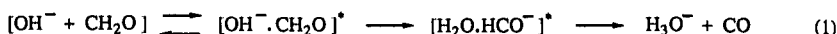
Reaction	ΔH° (kcal mol ⁻¹)	ΔS° (cal mol ⁻¹ K ⁻¹)
$(\text{CH}_3)_2\text{COCH}_3^+ + (\text{CH}_3)_2\text{O} \rightleftharpoons (\text{CH}_3)_3\text{O}^+ + (\text{CH}_3)_2\text{CO}$	-3.4	-12.0
$(\text{CH}_3)_2\text{COCH}_3^+ + (\text{CH}_3)_2\text{CO} \rightleftharpoons (\text{CH}_3)_2\text{COC}(\text{CH}_3)_2\text{OCH}_3^+$	-22.2	-42.0
$(\text{CH}_3)_2\text{COCH}_3^+ + (\text{CH}_3)_2\text{O} \rightleftharpoons (\text{CH}_3)_2\text{OC}(\text{CH}_3)_2\text{OCH}_3^+$	-26.4	-58.0
$(\text{CH}_3)_2\text{COC}(\text{CH}_3)_2\text{OCH}_3^+ \rightleftharpoons (\text{CH}_3)_2\text{OC}(\text{CH}_3)_2\text{OCH}_3^+ + (\text{CH}_3)_2\text{CO} + (\text{CH}_3)_2\text{O}$	-4.4	-16.0

BIMOLECULAR REACTIONS AND STRUCTURES OF IONS IN THE GAS PHASE

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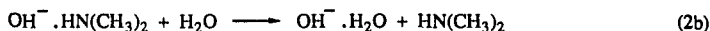
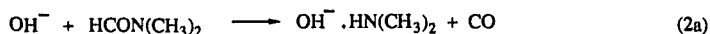
Bimolecular reactions and structures of ions in the gas phase constitute an important part of chemistry. Studies in that particular area can reveal similarities with and differences from corresponding reactions and species in solution, thereby providing a better insight into the role of solvent molecules and intrinsic properties of the species involved.

This will be exemplified by results to be presented below, which have been obtained via application of the elegant and powerful low pressure method of Fourier transform ion cyclotron resonance (FT-ICR).¹⁻⁴ A few years ago the reaction of naked hydroxide ions with formaldehyde has been found to lead to hydride solvated water molecules⁵ as shown in equation (1):

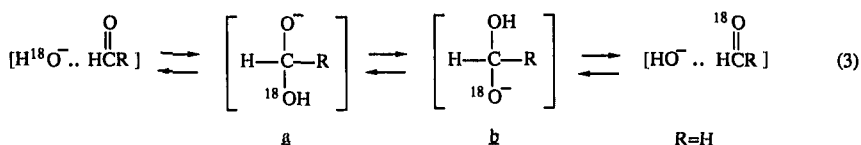


In this case the solvation energy gained upon formation of the collision complex, which is not carried off because of the absence of third-body collisions under the low pressure conditions of FT-ICR, fuels a proton transfer from formaldehyde being less acidic than water in the gas phase. A subsequent hydride transfer from the formyl anion to the water molecule in the correspondingly generated complex eventually leads to the products of H_3O^- and CO. These H_3O^- ions, which have also been prepared independently in an ion-beam experiment,⁶ contain exclusively the oxygen atom of the hydroxide ion as shown by ^{18}O -labelling experiments, while ^2H -labelling has indicated that the hydrogen atoms in H_3O^- are not equivalent.⁵

It is also possible to prepare under FT-ICR conditions hydroxide ions solvated by a single water molecule via the reaction sequence (2a) to (2b), where reaction (2a) resembles the well-known Riveros reaction.⁷



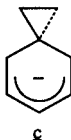
If the water solvated hydroxide ions are allowed to react with formaldehyde, long-lived complexes containing the elements of the hydroxide ion and water are generated. ^{18}O -labelling in combination with collision induced dissociation (CID) and chemical reactivity experiments on these long-lived complexes⁸ now show in contrast with the observations made via reaction (1) that the oxygen atoms have exchanged which can be explained on the basis of the reaction steps summarized in equation (3):



These observations demonstrate two things:

- (i) Upon reducing the internal energy of the collision complex between OH^- and formaldehyde, the chemistry shifts from a relatively simple initial proton abstraction by OH^- to an attack of OH^- upon the carbonyl carbon atom to form a tetrahedral species which is well known to play a role during the hydrolysis of esters in solution.⁹
- (ii) The proton shift required to interconvert the tetrahedral structures **a** and **b** in equation (3) means that they are intermediates and, therefore, correspond to potential energy minima.

Another result, which is thought to be relevant to solution chemistry, concerns the generation of the long-lived spiro [2,5] octadienyl anion **c** in the gas phase.¹⁰



This species has received considerable interest in the condensed phase, but its detection has been thwarted by counterion and solvation effects in addition to the possibility that it may be the radical rather than the ion.¹¹ Three routes have been explored to generate the desired $C_8H_9^-$ ion in the FT-ICR cell:¹⁰

- (i) Proton abstraction from spiro [2,5] octa-4,5-diene.
- (ii) Phenyl ring assisted expulsion of trimethylsilyl fluoride following F^- attack upon the Si-centre of 2-phenylethyltrimethylsilane, a route advanced by DePuy et al.¹² for the generation of a variety of stable carbanions.
- (iii) CO_2 loss by CID of the carboxylate anion of 3-phenylpropanoic acid, a reaction type which has been applied successfully by Squires et al.¹³ for the generation of carbanions whose corresponding radicals have a vanishingly small electron affinity.

All three routes have been found to be effective to generate $C_8H_9^-$ ions, whose structures have been probed and identified by specific ion/molecule reactions with hexafluorobenzene through comparison with reference $C_8H_9^-$ ions.

In this way it has been established that the $C_8H_9^-$ ions generated via routes (i) and (ii) are α -methyl benzyl and ortho-ethylphenyl anions, respectively, while those generated via route (iii) react similar to the structurally related 1,1-dimethylcyclohexadienyl anions.¹⁰ Moreover, the $C_8H_9^-$ ions generated via route (iii) react quite differently from those generated via routes (i) and (ii) with hexafluorobenzene in that the former react under expulsion of one molecule of HF, containing exclusively an original phenyl ring hydrogen as shown by 2H -labelling, whereas the latter two react under expulsion of two molecules of HF.¹⁰

In addition, the $C_8H_9^-$ ions generated via route (iii) have appeared not to be capable of transferring a hydride to suitable hydride acceptors.¹⁰

On the basis of all these observations it is therefore quite likely that the spiro [2,5] octadienyl anion 2 is formed by CID decarboxylation of the 3-phenylpropanoic acid carboxylate anion and that it can be a long-lived and stable species in the gas phase.

It is hoped that the few results presented are sufficiently convincing to state that gas phase ion chemistry studies can provide data which are of interest, if not essential, for the field itself and for the chemistry in the condensed phase as well.

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DRUG METABOLISM MADE EASY (SOMETIMES): THE USE OF TANDEM MS TECHNIQUES
FOR RAPID STRUCTURE ELUCIDATION
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Drugs and other xenobiotics typically undergo a series of biotransformation reactions that result in the formation of metabolites. These metabolites may have reduced, enhanced, or unexpected types of pharmacological activities, and the rate or extent of biotransformation can vary between individuals. A thorough understanding of metabolism is therefore essential to designing studies that can define the pharmacokinetics, pharmacodynamics, and toxicological profile of a given drug. It is for these reasons that the study of drug metabolism is an integral part of the process for drug discovery and development.

Traditionally, drug metabolism is considered a part of the development process rather than as a part of the drug discovery or design process. This is in part due to the comparatively long lead time required to fully characterize the metabolism of a drug. Conventional approaches to the study of drug metabolism typically involve the use of radiolabelled analogues to assist in distinguishing drug-related materials from endogenous compounds, and require the purification and isolation of individual metabolites prior to characterization by techniques such as NMR spectroscopy, mass spectrometry, and chemical synthesis.

The utility of MS/MS as a technique for rapidly determining the structures of drug metabolites was first proposed by Yost and co-workers in 1982 (1). In this paper, the authors pointed out that since metabolites usually retain some of the substructural features present in the parent drug, MS/MS scans that are specific for these substructures could be used to search for drug-related components present in crude sample extracts. The authors made use of a conventional direct insertion probe and CI conditions for sample introduction and ionization; conjugates were hydrolyzed enzymatically or chemically prior to MS/MS analysis. In subsequent papers by other investigators (2-4), it was demonstrated that when desorption ionization techniques such as FAB or thermospray were used, MS/MS scan sequences could be designed that were specific for the various types of conjugation products (eg., glucuronides, sulfate esters, glutathione conjugates, etc.). Thus, a combination of MS/MS scans specific for either the drug or for expected conjugation products could in principle be used to screen a sample extract for all of the possible metabolites of a given drug. Note that MS/MS is being used here as a method for enhancing the specificity of the mass spectrometer so that only drug-related components will be observed. This emphasis differs somewhat from the more ordinary use of MS/MS as a means for increasing the amount of structural information present in a mass spectrum. It is relatively straightforward to obtain structurally significant fragmentation on a given compound, but it can be very difficult or even impossible to devise a scan that has the requisite selectivity and sensitivity for screening purposes.

This approach, coupled with the fact that biotransformation is usually restricted to a limited number of metabolic pathways, was shown to be capable of providing useful data on metabolism in a few experiments, without the need for lengthy isolation procedures. The potential for a significant savings in time over more traditional approaches to metabolite identification attracted widespread attention, both by academic researchers and by the pharmaceutical industry.

The ideal case for the use of MS/MS techniques in drug metabolism occurs when the drug of interest is a low molecular weight (<500 d.) compound containing several distinct structural moieties, which will yield a CAD spectrum with fragment ions corresponding to each of the substructures. An example of such an ideal case is provided by the structure shown in figure 1. CAD of the protonated molecular ion ($M+H$)⁺ formed by FAB or TSP ionization yields fragment ions characteristic of the (N,N)-di-n-propylamino side chain (m/z 114) and of the indolone (m/z 160, 132). Precursor ion scans for these features (eg., parents of m/z 114 or 160) are an efficient means of detecting drug-related materials present in a urine or plasma extract. Conjugates such as O-glucuronides or sulfate esters can be detected by appropriate MS/MS scans (eg., neutral loss of 176 d. for glucuronides, neutral loss of 80 d. in negative ion mode for aromatic O-sulfates, etc.), and a fairly comprehensive understanding of the metabolism of this drug can indeed be obtained from a very limited number of experiments (5).

Over the past several years numerous examples of the successful use of MS/MS techniques for metabolite structure elucidation have appeared, but it has also become apparent that there are instances where the methodology provides ambiguous or even incorrect answers. For example, a structure that has a CAD spectrum that is dominated by loss of an acetate or other common substituent may not yield fragment ions that can be used to adequately distinguish drug metabolites from endogenous components (non-specific or trivial fragmentation). Another commonly encountered problem is that of overly complex CAD fragmentation, an example of which is shown in figure 2. Selection of a useful set of precursor ion scans for detection of metabolites of this structure would be difficult, although scans specific for conjugating moieties might still provide some useful information. Perhaps the most commonly encountered problem is that of a CAD fragmentation process that is not well understood. An example of the latter is shown in figure 3, where the apparently simple loss of MeOH from a methylbenzazepine N-oxide was shown by isotopic labelling to actually involve loss of benzylic carbons in a 1:1 ratio by a complex rearrangement reaction. This fragmentation appears to be unique to this particular structure, and MS/MS scans based on it (eg., neutral loss of 32 or parents of 180) are of no value for the detection of other metabolites of the parent drug.

While most of the early publications on the use of MS/MS for metabolite identification tended to stress the ability to examine crude mixtures directly (*ie.*, MS/MS as a substitute for a chromatographic separation), the tremendous complexity of biological samples dictates the use of some sample cleanup or extraction steps. Combined chromatography-MS techniques would seem to be an ideal method for dealing with this complexity, and most recent publications have taken this approach. HPLC-MS/MS systems using either thermospray, continuous flow FAB, or one of the variants of electrospray appear to offer the most flexibility for metabolism studies. These ionization methods can be successfully applied to most types of drug metabolites, especially polar, thermally labile conjugation products. A variety of additional techniques previously developed for MS studies of complex mixtures can be fruitfully applied to MS/MS studies on metabolism. One particularly useful technique is the use of "isotope twinning", where a 1:1 mixture of the drug and a stable isotope analogue is used. The unique isotope pattern that results can simplify the task of distinguishing drug-related components from endogenous compounds.

Techniques to improve the specificity of MS/MS scans for different classes of metabolites are also under active development. These include the use of reactive collision gases and the use of combinations of MS/MS scans. One interesting example is shown in figure 4. The bottom trace shows a total ionization chromatogram of a thermospray LCMS analysis, while the middle trace shows the TIC obtained for a neutral loss of 176 d. This MS/MS scan is highly specific for glucuronides (loss of $C_6H_8O_6$ via cleavage of the glycosidic bond), but is not absolutely specific. The minor peaks apparent at 25 and 29 min are in fact not glucuronides, but result from fragmentation of a heterocyclic compound (composition $C_{10}H_{16}N_2O_4$) that also happens to have a nominal mass of 176 d. Under the conditions of thermospray ionization with an ammonium acetate buffer, O-glucuronides form an NH_4^+ adduct with the carbohydrate moiety, yielding an ion of m/z 192. This species undergoes CAD to yield NH_4^+ at m/z 18. The top trace of figure 4 shows the enhanced specificity for glucuronides that is obtained by plotting both the RIC for m/z 18 in conjunction with the TIC for the neutral loss of 176 d.; the coincidence of both peaks provides additional evidence for the presence of a glucuronide. Improved specificity in this example could in theory be obtained by the use of high resolution MS/MS, but such experiments have not been reported as yet.

Extension to "Biotechnology" Drugs:

Drugs derived from biotechnology are just beginning to find their way into common use. These types of drugs are characterized by higher molecular weights (>1000 d.), low therapeutic doses, and complex structures encompassing many different types of functional groups. Examples include peptides, oligosaccharides, and lipopolysaccharides. Are the possible biotransformation products from these types of structures amenable to MS/MS screening techniques?

A good model for a complex polyfunctional compound is the immunosuppressant drug Cyclosporin-A. This is a cyclic undecapeptide incorporating a number of N-methylated amino acids plus a unique residue at position 1 ((4R)-4-((E)-2-butenyl)-4,N-dimethyl-L-threonine). This structure undergoes metabolism in a variety of species to give a series of mono- and dihydroxylated and/or N-demethylated derivatives (6).

Analysis by either FAB or thermospray ionization in the positive ion mode gives a prominent signal corresponding to $(M+H)^+$ at m/z 1202.8. MS/MS analysis of $(M+H)^+$ yields a variety of fragments which provide information about both the identity and sequence of the modified amino acids (figure 5). However, none of these ions appears to be specific enough for the Cs-A structure to serve as the basis for an MS/MS scan to selectively identify metabolites present in a crude bile or urine sample. The negative ion MS/MS spectrum of Cs-A derivatives, in contrast, is dominated by a single fragment corresponding to cleavage of the C7 side chain of residue 1. An example of this is shown in figure 6, for a typical (monohydroxylated) metabolite of Cs-A. A neutral loss scan for this fragment (112 d.) provides a highly selective MS/MS scan for metabolites that retain the butenyl-di-methyl threonine residue. In a similar fashion, metabolites formed by hydroxylation of this residue can be successfully detected by carrying out a neutral loss scan for 128d. in negative ion mode.

Conclusions:

It is perhaps most useful to consider MS/MS as a powerful adjunct to existing methods for characterizing the structures of metabolites, rather than as a replacement for those techniques. In favorable situations, MS/MS experiments can be used to selectively identify metabolites with minimal sample cleanup or isolation. However, there are numerous examples where significant or unexpected metabolites may be overlooked. Specificity is always a problem, and some form of sample cleanup will continue to be required in most cases. In addition, certain types of essential information are still difficult to obtain from MS/MS experiments. Quantitative data, for example, is usually only obtainable if isotopically labelled internal standards are used. Finally, the application of MS/MS screening techniques to the study of endogenous biologically active compounds such as steroids and eicosanoids is an area of emerging interest as MS/MS instruments become more generally available to researchers.

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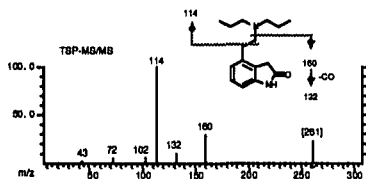


Figure 1

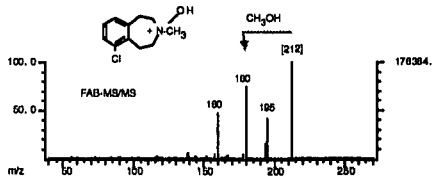


Figure 3

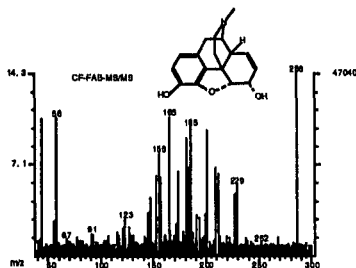


Figure 2

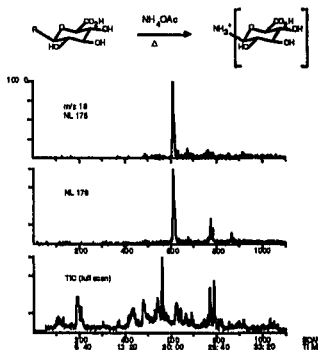


Figure 4

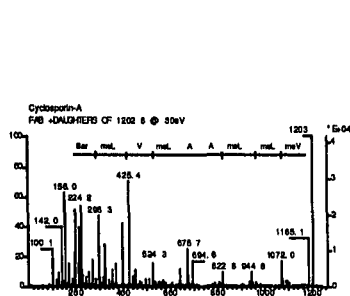


Figure 5

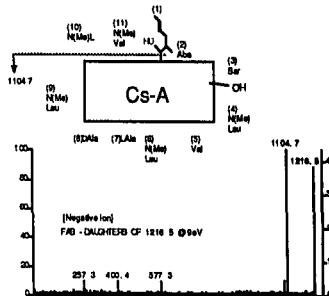


Figure 6

ULTRAVIOLET LASER DESORPTION/IONIZATION OF BIOMOLECULES
IN THE HIGH MASS RANGE

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INTRODUCTION

There is obviously a high interest in mass spectrometric techniques for the analysis of biomolecules up to masses of several hundredthousand Dalton. The problem for such applications lies with the generation of such large ions and several approaches have been taken over the last decade or so to develop suitable desorption techniques for ion generation. So far Fast Atomic Bombardment FAB with an upper mass limit of app. 10,000 Dalton and Plasma Desorption PD with an upper limit of app. 35,000 Dalton have been the most successful ones. Most recently Tanaka et al. and the authors group have extended the mass range to several 100,000 Dalton using matrix assisted UV-laser desorption with different matrices <1,2>. This report summarizes some of the most important features of laser desorption of proteins with a nicotinic acid matrix and nanosecond pulses of a quadrupled ND-YAG laser at a wavelength of 266 nm.

EXPERIMENTAL

The LAMMA 1000 instrument, used for these analyses has been described elsewhere <see e.g. 3>. In contrast to the intended operation of this instrument as a microprobe with a laser focus of app. 3 μm diameter, the laser was usually defocused to about 10 - 30 μm for best results in these experiments. Samples were mostly used as supplied by the manufacturer (Serva or Sigma) or by colleagues from different laboratories. Generally results are the better, both in ion yield and spectral resolution, the more salt free the samples are (compare e. g. the spectra of β -D-galactosidase in ref. <3> and <4>. 10^{-5} - 10^{-6} mol/l aqueous solutions were mixed with 10^{-2} mol/l aqueous solutions of nicotinic acid. Ca. 1 μl of this mixture was dripped onto a silver substrate followed by evaporation of the solvent either in air or in the vacuum to yield a reasonably homogeneous substance layer. Preparation of the samples and analysis in the mass spectrometer take typically 15 minutes.

RESULTS

Typically for molecules with masses below app. 100,000 Dalton, but in some cases even for larger molecules like Glucoseisomerase (MW 174,000 Dalton), molecular ions are so abundant that single shot spectra can be recorded with good signal to noise ratio. In other cases 10 to 100 spectra have been added. Spectra usually contain signals of the parent ion as well as those of oligomer - and multiply charged ions. Structural information on non-covalently bound subunits is frequently contained in the spectra; in some cases it appears, as if selected other structural information can also be attained (see e. g. Urease below). No unspecific fragmentation has ever been observed to any measurable degree; all signals below 500 Dalton can unambiguously be assigned to the matrix. The mass resolution is so far limited to typically 50; still the centroids of the peaks permit a determination of the parent molecular mass to typically 0.2%. The sensitivity of the technique was tested for a number of molecules in the range between 14,000 and 170,000 Dalton <5>. For lysozyme and trypsin the total amount of material used for the preparation could be minimized to 50 fmol without substantially sacrificing the quality of the spectra. For glucoseisomerase 200 fmol were needed. It was estimated that in all these cases the total amount of material actually used for the spectra was no more than about 10^{-17} mol.

The following three examples demonstrate the power of the technique. Fig. 1 shows the spectrum of a monoclonal antibody (IgG of the mouse against a specific human lymphokine). It is a typical example of spectra obtained from highly purified, carefully prepared samples. The spectrum contains exclusively signals of the singly and multiply charged parent molecule and its oligomers. From the spectrum the mass has been determined to 149,190 Dalton.

A spectrum of jack bean urease (Sigma) is shown in Fig. 2. This protein consists of 6

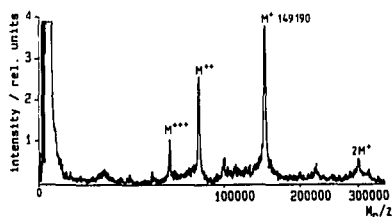


Figure 1. Laser desorption/ionization mass spectrum of a monoclonal antibody. Sum of 25 single spectra. For explanation see text.

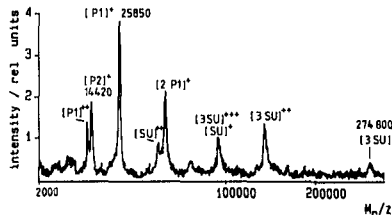


Figure 2. Laser desorption/ionization mass spectrum of jack bean urease. Sum of 100 single spectra. For explanation see text.

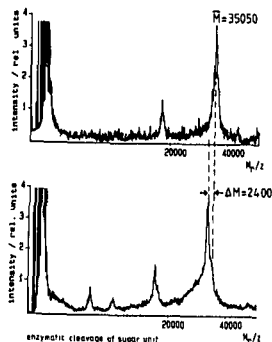


Figure 3. Laser desorption/ionization mass spectrum of violet phosphatase. Sum of 25 single spectra. For explanation see text.

equal subunit and supposedly has a total mass of ca. 545,000 Dalton <G>. The largest ion detected at mass 274,800 is the fragment containing 3 of the subunits; this fragment is known to be relatively stable. The decay into these fragments occurred possibly already during preparation rather having been induced by the laser during the desorption process. Several peaks in the low mass region cannot be assigned to multiply charged ions of the subunit or its oligomers. Though it cannot, at this time be excluded that these signals, tentatively assigned to proteins P1 and P2 represent contaminations of the samples, they reasonably match a pattern of active domains of the subunit chain as found by limited proteolytic cleavage. This would then represent the first demonstration of structural information in laser desorption/ionization spectra obtained through cleavage of presumably exposed covalent bonds.

Glycoproteins are accessible by the technique as well as is demonstrated in Fig. 3. Base peak in the top spectrum is the parent molecular ion of violet phosphatase at mass 35,050 Dalton. The bottom spectrum was obtained from the same sample after cleavage of the carbohydrate fraction of app. 2400 Dalton with endoglycosidase H.

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RECENT DEVELOPMENTS IN ISOTOPE ABUNDANCE MEASUREMENTS

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Sector field mass spectrometers have been revolutionised by improvements in ion optical design, vacuum technology, multicollector detector systems and microelectronics, whilst computers now play an essential role, not only in data collection and reduction, but also in controlling the spectrometer operation itself.

Ion optical design improvements include the use of non-normal beam incidence and curved pole pieces which enable the effective radius of curvature of the mass spectrometer to be extended whilst the physical size of the instrument remains unaltered. This increased dispersion improves the abundance sensitivity of the spectrometer and provides higher transmission efficiency without a deterioration in resolving power.

The ionisation efficiency of solid source mass spectrometers has been improved by the use of activators [1], resin bead techniques [2] and micro-electrodeposition [3]. These techniques have enabled sample sizes to be reduced without sacrificing accuracy and precision. In the nuclear industry, this reduction in sample size has been driven to some extent by safety requirements, but it has also provided the opportunity to tackle a range of previously inaccessible problems not only in nuclear science, but in fields as diverse as cosmochemistry and lunar science [4].

One of the most recent enhancements in mass spectrometer design has been the advent of multicollection. This enables simultaneous measurements to be made of a number of ion beams, thus removing errors caused by fluctuations of beam intensity in the peak jumping mode. Small samples can therefore be measured with greater precision than is the case for single collector instruments. Modern mass spectrometers can be equipped with a computer controlled, fully automated collector system which allows the analysis of a variety of elements with minimal operator intervention. The collector system can also be fitted with an electron multiplier or Daly Collector system for ion beams that are too small to be measured with Faraday Cup collectors.

Mass fractionation represents a fundamental limitation to the accuracy of isotopic ratio measurements in mass spectrometry. Mass fractionation can arise from a variety of sources - from evaporation processes in thermal ionisation sources, in sputtering processes in ion microprobe spectrometers and in some collector systems. Furthermore, the measured isotopic ratios change progressively with time during an analysis. It is also important to distinguish between instrumental-induced mass fractionation, and fractionation caused by natural processes [5]. The double spike technique can be used to eliminate machine-induced fractionation and enables physiochemical isotopic effects to be measured [6]. Light elements are particularly prone to mass fractionation. This can be overcome in large measure by measuring a heavy molecular species containing the light element.

The advent of modern computer-controlled mass spectrometers, which apparently provide excellent data with little operator involvement, require a comprehensive calibration regime to ensure that the output information is of optimum quality. Thus the linearity of the instrument should be periodically calibrated over a wide range of isotopic ratios. Synthetic mixtures of highly enriched uranium isotopes are now available to enable the mass spectrometry community to test the accuracy of their instruments [7]. Multi-collector detector systems also require the frequent calibration of the gain of each measuring channel to ensure that the isotopic ratios are measured accurately. The US National Institute of Standards and Technology

provide calibrated samples for a number of elements to enable the mass spectrometer performance to be checked against internationally approved standards.

The stable isotope dilution technique enables the abundance of over 70 elements to be determined in a wide range of samples. Its accuracy and sensitivity is approached only by neutron activation analysis. The abundance of an element is determined from the change produced in its isotopic composition by the addition of a known amount of a tracer of that element whose isotopic composition is different from that of the naturally occurring element. The technique has been widely used in nuclear science and in geochronology. It has also been used extensively in determining the amount of trace elements in geochemical reference samples. It is now being adopted in medical applications as an alternative to radioactive tracer techniques.

Negative thermal ionisation can be used as an alternative to the more commonly used positive ion technique. Negative thermal ionisation can produce high ionisation efficiencies for certain elements, and has the added advantage that organic impurities are reduced. The interaction of solar neutrinos with molybdenum produces extremely small quantities of technetium. This has prompted the development of a highly sensitive isotopic technique for measuring technetium using negative thermal ionisation [8]. Technetium samples of approximate size 1 pg can be analysed with high ionisation efficiency.

Concurrent with these developments in instrumentation has been the necessity to improve chemical processing procedures. This has been essential in order to reduce chemical blanks and to improve the purity of the extracted samples, so that the enhanced sensitivity in mass spectrometry can be fully utilized. Improved clean-room facilities and reagent purity are mandatory.

An alternative approach is to omit any form of chemical processing by analysing the sample in situ. The ion probe mass spectrometer has been successfully used to measure the isotopic composition of a number of elements in a variety of materials. A schematic representation of the Sensitive High Resolution Ion Microprobe (SHRIMP) at the Australian National University is shown in Figure 1. It has been successfully used to examine isotopic anomalies in titanium and magnesium from hibonite grains in carbonaceous chondrites [9], and has revolutionised zircon geochronology [10]. The technique offers great possibilities in deciphering the nucleosynthetic processes that are responsible for isotopic anomalies in meteorites. Apart from the minimisation of contamination, the ion microprobe technique also has the advantage that it does not extensively damage the samples and enables the isotopic composition of elements in mineral phases to be analysed.

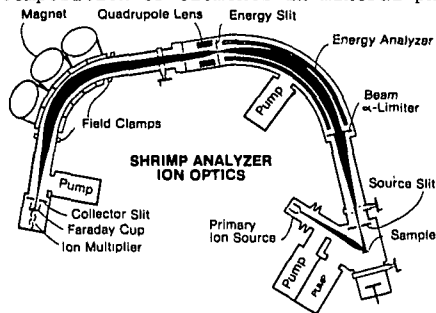


FIGURE 1

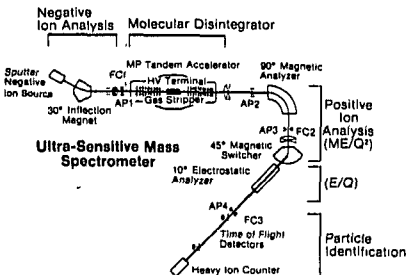


FIGURE 2

Mass spectrometry has progressively adopted technological advances developed in other scientific areas to improve the sensitivity and precision with

which isotopic ratios can be measured. The use of particle accelerators as ultrasensitive mass spectrometers has enabled isotope ratios below 10^{-12} to be measured. The basic concept in accelerator mass spectrometry is to discriminate between atomic species in the ion source by using negative ions to destroy interfering molecular species, and then to count identified individual accelerated ions. The ion beam transport system of the Rochester University tandem accelerator mass spectrometer is shown schematically in Figure 2 in which ion beam defining apertures are designated by AP, and Faraday cups for ion beam current measurements as FC.

One of the most important applications of accelerator mass spectrometry is carbon-14 dating, since it has sufficient sensitivity to make it superior to conventional counting techniques. Accelerator mass spectrometer systems can now measure the ^{14}C content of samples containing approximately a milligram of carbon in which the mass ratio $^{14}\text{C}/^{12}\text{C}$ is 10^{-13} . Satisfactory agreement between ages calculated from accelerator mass spectrometry and conventional ^{14}C dating techniques has now been demonstrated. The most celebrated use of accelerator mass spectrometry is the radiocarbon dating of the Shroud of Turin. Very small samples from the shroud have been dated in laboratories in Arizona, Oxford and Zurich. The results from the three laboratories are in good agreement, and showed that the age of the shroud is AD 1260-1390 at the 95% confidence level [12]. The results therefore conclusively prove that the linen in the Shroud of Turin is of mediaeval age.

A number of new mass spectrometers have been developed in recent years. Inductively coupled plasma source mass spectrometry (ICP-MS) offers a direct method of determining trace elemental and isotopic analyses on samples in solution. The technique has now been adapted to solid samples using a laser ablation system which carries the ablated particles into the ICP source. Resonance ionization mass spectrometry (RIMS) uses a pulsed laser to remove the electrons from a selected atomic species by tuning it to a discrete resonant electronic transition. The probability of ionisation increases enormously because of the step-wise absorption of photons. A laser microprobe mass spectrometer (LAMMA) utilises a laser pulse to vaporize and ionize trace amounts of an element which is then analysed in a time-of-flight spectrometer. The primary advantage of a laser microprobe is its ability to analyse organic and inorganic species with high precision.

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RECENT COMPUTER APPLICATIONS IN MASS SPECTROMETRY

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A rapidly-developing area of interest in analytical mass spectrometry involves the use of combined analysis systems (e.g. GC-FTIR-MS) to improve the reliability of mixture component identification. There are, at present, two such commercial instrument systems. One employs an infrared lightpipe GC interface and a high performance Fourier transform infrared spectrometer, designed for maximum sensitivity. The other is based on use of a matrix-isolation GC-FTIR interface, also for the purpose of enhancing infrared spectral sensitivity. Both systems utilize a simple quadrupole mass spectrometer (the Hewlett Packard mass selective detector) as the mass spectrometer component. An advantage conferred by use of two or more independent detectors for GC mixture analysis is that the performance constraints for the individual elements can be relaxed (i.e. lower spectral performance can be compensated by the increased information available). Thus, four wavenumber infrared spectral resolution and unit mass resolution are adequate for combined GC-FTIR-MS analysis. With the availability of such linked systems, capable of providing even more information than GC-MS alone produces, the challenge of developing computer interpretation algorithms is an even more important issue. In research to date, the primary approach to interpreting such data was to search independent computer-readable infrared and mass spectral libraries and to compare the results, looking for coincidences and (by inference) increased identification reliability when this occurred. Isenhour and coworkers have explored use of concatenated infrared and mass spectral libraries, analyzed by factor analysis and eigenvector projection (1) and, more recently, we have examined use of pre-search algorithms with a combined library (2). Applications of our new search strategies to complex mixtures, including environmental samples, have shown that such an approach can be highly reliable for both identifying mixture components or suggesting chemical class in cases where the unknowns are not represented in the spectral libraries. In the most recent studies, sample quantities between 10 and 80 nanograms in a thirty component model mixture were sufficient for a joint analysis which correctly identified 28 of the 30 components. An environmental soil steam distillate sample with unknown components present at similar levels also was successfully analyzed.

Efforts to combine computer-aided spectral analysis methods drawing on diverse spectral sources are not limited to directly-linked systems. An example of

another new approach to the interpretation of mass spectral data involves combining carbon-13 nuclear magnetic resonance data from an **unseparated mixture** with GC-MS data to develop algorithms which make topological inferences from the CNMR data and use those inferences to screen the results from GC-MS library searches(3). In this way, the rich information content available from NMR can be brought to bear on GC-MS interpretation. Due to the limited sensitivity of CNMR, this method is not suitable for trace analysis applications, but is a highly reliable adjunct to conventional library search techniques in applications which are not sample-limited. Thus, for a variety of test mixtures, it was found that false positive GC/MS library search identifications of mixture components were completely eliminated, when subjected to screening based upon quantitative carbon nuclear magnetic resonance spectra (3).

In summary, a promising new direction in computer-assisted mass spectral analysis is synergistic combination of additional types of information to improve reliability and efficiency.

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Acknowledgments

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**FABMS and FABMS/MS of Proteolytic Digests
of Bovine Aldose Reductase**

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The sequencing of peptides has become a routine analytical procedure in our laboratory employing a VG-70-SE-4F four-sector mass spectrometer. The complex mixture of a tryptic digest of an aldose reductase enzyme isolated from bovine kidney has been exhaustively analyzed by FABMS and FABMS/MS. Aldose reductases are members of the aldehyde reductase superfamily of enzymes which show non-linear kinetic behavior and a large degree of homology in their structures¹. These enzymes show a broad substrate specificity, and have been implicated as a central cause of kidney dysfunction and cataracts in diabetic and galactosemic animals². The main objective of this study is to show that mass spectrometry can be used as a primary sequencing method without chromatographic separations which often consume precious sample.

The determination of the complete sequence of bovine lens aldose reductase (BLAR)¹ was recently completed. The sequencing of a blocked N-terminal tryptic peptide of BLAR by FABMS/MS was conducted in our laboratory. Our studies have concentrated on showing homology in the sequences of BLAR and bovine kidney aldose reductase (BKAR).

Equal quantities of BLAR and BKAR were treated under the same trypsin digest conditions and the resulting mixtures were analyzed. Comparison of LRFABMS in the regions of 100 to 2500 daltons showed that 16 peaks corresponding to known tryptic peptides of BLAR were also present in BKAR. High resolution peak matching experiments were conducted on a VG ZAB-SE. The HRFABMS data showed excellent agreement with the predicted compositions, as shown in Table 1. Sequencing of the tryptic peptides was conducted using VG 70-SE-4F tandem mass spectrometer, with either argon or xenon used as CID reagents. A complete fragmentation analysis produced the unambiguous sequences shown in Figure 1.

With greater than 25 percent homology already demonstrated, an investigation of the tryptic peptide mixtures using off- and on-line LC/FABMS are in progress. Many of the higher molecular weight and the more hydrophobic tryptic peptides of BLAR were not observed in the LRFABMS of the mixtures. It is hoped that with LC/FABMS these peptides will be observed and additional homology will be demonstrated. Studies using other proteolytic digests will also be necessary to complete the sequencing of bovine kidney aldose reductase.

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Table 1. SUMMARY OF HIGH RESOLUTION FAB/MS DATA FOR BKAR

HRFAB/MS Data	Error*	Formula	HRFAB/MS Data	Error*	Formula
1556.8210	2.3	C ₆₉ H ₁₁₁ N ₂₀ O ₂₁	676.3867	- 2.3	C ₂₉ H ₅₃ O ₁₁ N ₇
1115.6304	- 1.7	C ₅₃ H ₈₇ N ₁₂ O ₁₂ S	660.3832	5.6	C ₃₁ H ₄₉ O ₇ N ₉
1106.6241	- 3.1	C ₅₀ H ₈₃ N ₁₃ O ₁₅	646.3745	- 4.7	C ₂₈ H ₅₁ O ₁₀ N ₇
906.5032	- 1.9	C ₄₁ H ₆₇ O ₂₇ N ₁₁	517.2978	- 1.5	C ₂₂ H ₄₀ O ₈ N ₆
901.5404	- 7.4	C ₃₉ H ₇₂ O ₁₂ N ₁₂	474.2916	- 2.5	C ₂₁ H ₃₉ O ₇ N ₅
882.5747	- 3.4	C ₄₁ H ₇₅ O ₁₀ N ₁₁	414.2749	- 3.3	C ₁₉ H ₃₅ N ₅ O ₅
796.5328	4.0	C ₃₈ H ₆₉ O ₉ N ₉	345.2490	- 3.7	C ₁₆ H ₃₂ N ₄ O ₄
688.3623	- 4.8	C ₂₈ H ₄₉ O ₁₁ N ₉	260.1990	- 1.6	C ₁₂ H ₂₆ O ₃ N ₃

* Errors are listed in millimass units.

COMPARISON OF PROPOSED BOVINE LENS ALDOSE REDUCTASE SEQUENCE AND IDENTIFIED TRYPTIC PEPTIDES OF BOVINE KIDNEY ALDOSE REDUCTASE ^{a,b}		
Bovine Lens Aldose Reductase ^{c, d}	Ac-AHNIVLYTGAK	MPILGLGTWK SPPGK
Bovine Kidney Aldose Reductase ^e		MPILGLGTWK
VTEAVK	VAIDLGYR	HIDCAHYVQNEVEGLALQAK
VTEAVK		LOEK VK-R-EDLEIVSK
		LOEK VK-R-EDLVSK
LWCTYHDK	DLVK GACQK	ILSDLK
DLVK		TLSDLK
		DLVYDLYLIHWPTGFK PGK
		DFVDTTWAMEELVDEGLVK
		ALGVSNENHLQVEK
		ILNK-PGLK
		YK PAVNQIECHPYLTQEK
		LIQYCNSK
		GIVTAYSPGLSPDR
		ILNK-PGLK
		PWAK PEDPSILEDPR IK
		AIADK -YNK
		ITAQVLIR
		FIQIR NLIVPK SVPER
		IK AIADK
		TTAQVLIR
		FIQIR NLIVPK SVPER
		IAENFQVDFELDK
		EMMTLLSYNR
		DWR
		ACALVSCASHR
		DYPFHEEF

NOTES:

- The amino acid sequence is denoted with the one letter abbreviations.
- The hyphens between the tryptic peptides represent incomplete digestions by trypsin.
- The proposed sequence of BLAR.
- The underlined portions of the BLAR sequence are observed in the LRFAB/MS.
- Peptides identified from tryptic digests of BKAR by LRFAB/MS, HRFAB/MS, and FAB/MS/MS.

**MOLECULAR WEIGHT DETERMINATION OF RECOMBINANT HUMAN GROWTH
HORMONE WITH AN ATMOSPHERIC PRESSURE ION SOURCE QUADRUPOLE
MASS SPECTROMETER**

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The use of living organisms for the manufacture of pharmaceutical products and the complexity of the resulting products require the development of specific analytical methods for quality control. In this paper we describe the use of ion evaporation ionization on an API quadrupole mass spectrometer to provide a rapid and accurate means of determining molecular weights of biologically active proteins produced in the biotechnology industry. In addition, further information may be obtained by molecular weight determination of proteolytic fragments of the desired protein.

For mass measurements of recombinant proteins, a Sciex Taga 6000E triple quadrupole mass spectrometer equipped with a standard atmospheric pressure ion source was used. An ion spray Liquid Chromatography/Mass Spectrometry interface was used to effect ion evaporation ionization from a continuous solution of analyte. Purified samples were typically dissolved in dilute aqueous solutions containing acetic, formic and/or trifluoroacetic acid from 0.1 to 1.0% concentrations. Typical sample concentrations were 1 to 100 picomoles/ μ L. Samples were infused with a medical syringe pump at 1-5 μ L/min with one minute full scan acquisition times. Molecular weight determinations over a limited scan range required only subpicomole quantities of sample.

Methionyl-human growth hormone (met-hGH, Protropin® and recombinant hGH (r-hGH)) can be distinguished by tryptic mapping techniques involving the use of enzymatic digestion and gradient HPLC methods. They can also be unequivocally distinguished via their ion spray mass spectrum. By infusing 2-4 μ L of aqueous buffered solutions (ca. 25 picomoles/ μ L) of these proteins to an ion spray LC/MS interface, intense ion evaporation spectra were obtained on 50-100 picomoles of material. Based on amino acid composition, met-hGH (Protropin) should have an average M.W. of 22,256.2. Molecular weight measurements on this instrument indicated a mass of $22,256.32 \pm 0.44$ d, derived from the multiply charged protonated molecular ions having 13-19 charges (Figure 1). Mass measurements of r-hGH showed a similar accuracy and precision. A synthetic mixture of met-hGH and r-hGH also yielded an intense spectrum and accurate molecular weights of both species could easily be determined (Figure 2).

A tryptic digest of met-hGH was run over a reverse phase HPLC column (5 μ L injected of 50 picomoles/ μ L, 1 mm x 10 cm C8 column, 40 μ L/min gradient of 5% to 70% CH₃CN:H₂O with 0.5% TFA in 60 min) resulting in the total ion chromatogram shown in Figure 3. The mass spectrum of the peak at scan 158 (Figure 4) is of the T11 fragment and is representative of the quality of data obtainable. As tryptic fragments generally possess two basic sites (the N-terminus and the C-terminal Lys or Arg residue with the noted additions of any His residues, Lys-Pro or Arg-Pro bonds or subtraction for the C-terminal fragment), the doubly charged ions are of greater intensity than the singly charged (M+H)⁺ species and should yield excellent MS/MS spectra.

Several observations may be made in conclusion. The generation of multiply charged ions at atmospheric pressure and their mass spectrometric detection is an extremely useful technique for the determination of protein molecular weights. The technique is rapid, requires minimal sample handling and is amenable to high sample throughput. Low picomole amounts of material are typically required for analysis. Components in a mixture may be distinguished. The ultimate mass range obtainable depends on the number of charges which a protein can accommodate (total number of basic sites) and on the resolution of the mass analyzer. With this particular instrument it should be possible to attain accurate molecular weight information on 200-300 Kd proteins.

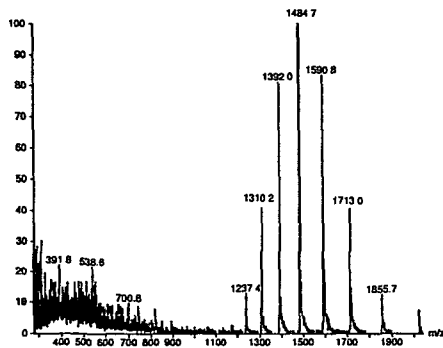


Figure 1. Ion Evaporation Mass Spectrum of met-hGH. 100 picomoles consumed. Average MW 22256.32 $\pm$ 0.44.

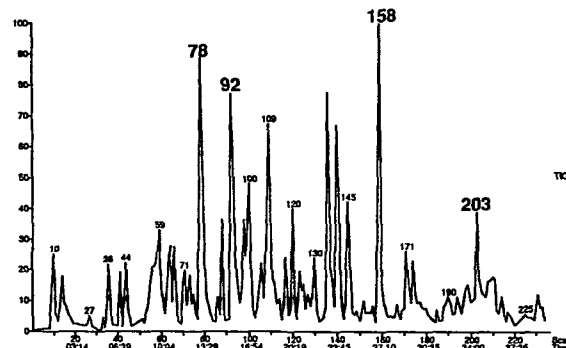


Figure 3. Ion Evaporation LC/MS TIC Chromatogram of a Tryptic Digest of met-hGH. 250 picomoles injected.

average MW of met-hGH = 22,256 expt^{TM} = 22,255.8 $\pm$ 1.6
 average MW of r-hGH = 22,125 expt^{TM} = 22,124 $\pm$ 1.6

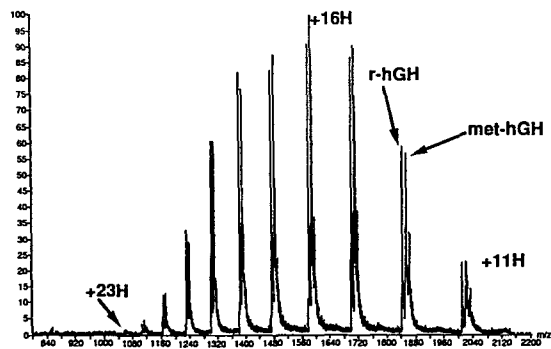


Figure 2. Ion Evaporation Mass Spectrum of a Mixture of met and r-hGH. 100 picomoles consumed, total basic sites=23.

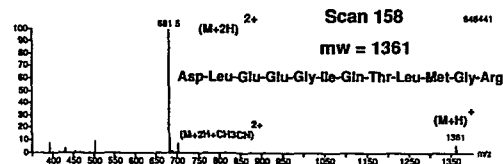


Figure 4. Ion Evaporation Mass Spectrum of the Peak at Scan 158.

QUANTIFICATION BY GC/MS OF SEPARATED ENANTIOMERS OF ARABINITOL TO AID THE DIFFERENTIAL DIAGNOSIS OF DISSEMINATED CANDIDIASIS.

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The use of serum total (D + L optical isomers) arabinitol concentrations to diagnose and monitor disseminated candidiasis in immunosuppressed patients has been limited because of the inability to differentiate between increased arabinitol of fungal and non-fungal (i.e., due to renal dysfunction) origin (1). There is evidence that fungal arabinitol is all D (2) and endogenous arabinitol is a mixture of D and L isomers (3). Our objective was to develop a GC-MS technique for the separation and quantification of the D and L optical isomers of arabinitol in serum and urine.

SAMPLE PREPARATION. Serum: 200 μ L serum + 100 μ L I.S. (5 ng/ μ L) + 500 μ L hexane, vortex, centrifuge 10 min, 3000 g; to aqueous layer add 1.8 mL acetone, vortex, centrifuge 10 min, 3000 g, dry supernatant with nitrogen, waterbath 35 C°. Urine: filter 500 μ L urine through 10,000 dalton cutoff Centricon 10 (Amicon Co.) filter; 50 μ L filtrate + 100 μ L I.S. (5 ng/ μ L), dry with nitrogen. Derivatization: dried samples + 200 μ L methylene chloride + 100 μ L TFA, heat 75 C° for 20 min, cool to 25 C°, dry with nitrogen, add 50 μ L toluene, vortex, inject 1 or 1.5 μ L.

GC-MS CONDITIONS. Perpentylated α -cyclodextrin glass capillary (4), 0.25 mm ID, isothermal 100 C°, 7 min (20 m column) or 18 min (40 m column), increase to 150 C° at 20 C°/min, hold 4 min. Carrier: helium. Injection: splitless, 200 C°; transfer line 220 C°. Mass spectrometer: Model Trio-3 (VG Masslab); CI: isobutane; multiplier 650-950 V. Full spectra: scan 100-650 amu in 1 s. Selected ion monitoring of base peaks: m/z $\approx$ 519 and m/z = 526 (int. std.) ions corresponding to the loss of an OCF₃CO group from the acetylated compounds (fully deuterated arabinitol, int. std.).

CHROMATOGRAPHIC SEPARATION. Fig. 1 illustrates the separation of 1 ng each of D- and L-arabinitol, adonitol and xylitol, using a 20 m long column. The retention times of D- and L-arabinitol were between 4 and 5 min and the separation was nearly at baseline. Using 50 m long columns, retention times increased to 16-18 min as illustrated in Fig. 2 (see below). The longer columns offered no advantages, thus most experiments were carried out using 20 m columns. As the columns aged, chromatographic resolution decreased, and often the separation was poorer than shown in Fig. 1 (e.g., compare Fig. 4A and 4B).

D-ARABINITOL IN CANDIDA. The D/L ratio in normal serum (Fig. 2A) changed drastically after inoculating and incubating with *Candida albicans* (Fig. 2B) showing that D-arabinitol is the major metabolic product; Fig. 2C shows the addition of L-arabinitol.

D/L IN NORMAL SERUM AND URINE. In normal serum (Fig. 3) from the blood bank (n=16), the mean $\pm$ s.d. of D/L ratio was (based on peak heights) 1.5 ± 0.4 (range: 0.9 - 2.2). The mean for cancer patients without candidiasis (n=10) was 1.6 ± 0.4 (range: 1.0 - 2.2). Defining the upper limit of normal as the mean + 2 s.d., D/L ratios > 2.3 are considered abnormal. In normal urine (n=14) the mean $\pm$ s.d. of D/L ratio was 1.6 ± 0.3 (range: 1.1 - 2.1). Defining the upper limit of normal as the mean + 2 s.d., D/L ratios > 2.2 are considered abnormal.

D/L PROFILES (Fig. 4). A = confirmed candidiasis, increased creatinine, B = confirmed candidiasis, normal creatinine, C = no candidiasis, high creatinine (dialysis), D = no candidiasis, high creatinine (renal dysfunction). In 12 confirmed candidiasis samples, D/L was > 2.3 in 10 cases (range 2.4-14.1), there was one false negative, and one was borderline.

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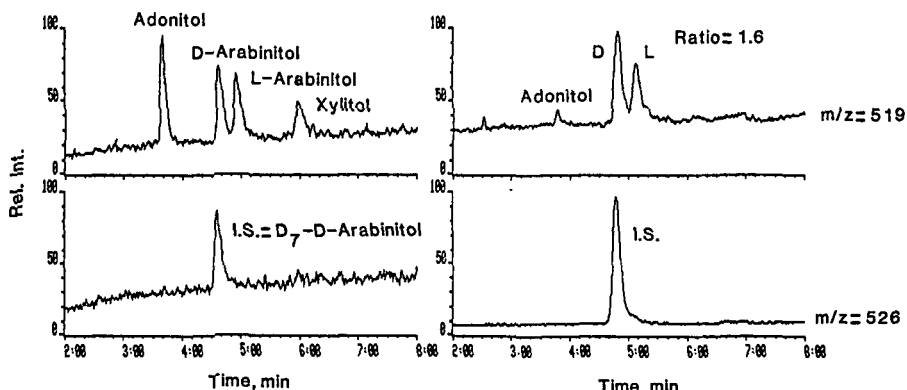


Fig. 1.

Fig. 3.

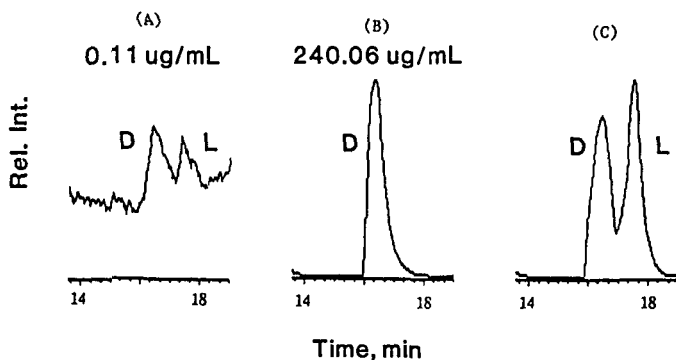


Fig. 2.

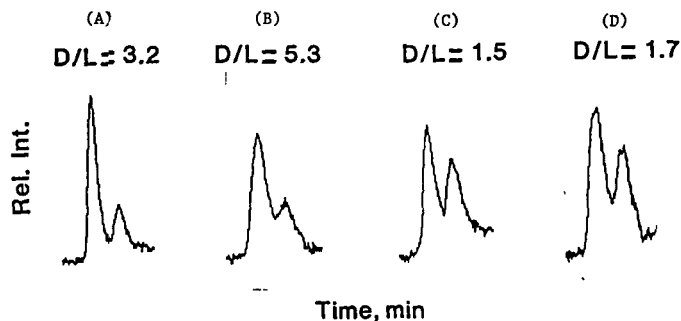


Fig. 4.

RELATIONSHIP OF THE STRUCTURE OF SUBUNIT CONTAINING PROTEINS TO THEIR ION EVAPORATION SPECTRA

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Ion Evaporation ionization is a process which has the capability to generate quasi-molecular singly and multiply charged ions from molecules in the tens of kilodaltons mass range(1,2). Pure Ion Evaporation spectra can be obtained from an Electrospray interface, an Ion Evaporation interface, or an Ion Spray interface. An Ion Spray interface was used with a Sciex TAGA 6000E MS/MS equipped with an Atmospheric Pressure Ion source to obtain the following data.

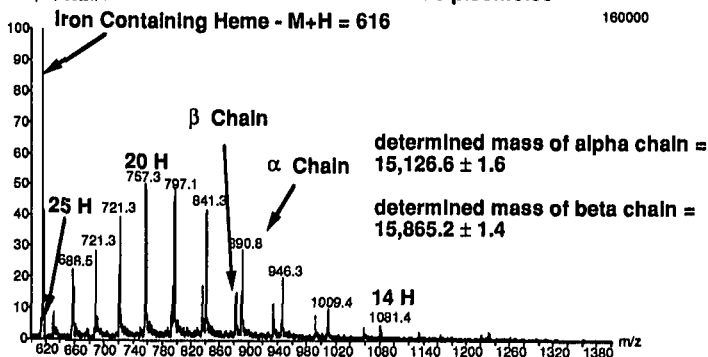
The spectra of several multiply charged high mass ions will be shown to provide considerable information about the nature of the molecular composition and structure of the molecules. Molecular weights of cyclic and linear proteins and peptides can be determined to within one dalton of the average mass with a simple calculation. The number of positive charges and the charge distribution provide information about the basicity of the molecule. Globular proteins generally have a subunit structure, the subunits being either identical or of different composition and bound together by hydrogen bonding, ionic interactions, and Van Der Waals forces. The spectra of subunit containing proteins provide the mass of the individual subunits. It appears that the molecules denature in the gas phase as a result of their highly charged state. Examples include hemoglobin of MW 61,983 composed of two pairs of identical chains (15,126 and 15,865 daltons) and a heme (616 daltons), and jacalin (a peanut agglutinin) composed of a 14,660 dalton protein and a 2058 dalton peptide. Under the correct conditions, co-factor and metal binding proteins also tend to release the ligand to allow for independent mass measurements of the protein backbone and the ligand. This can be observed in the spectra of hemoglobin when acquired in solvents containing a high content of organic modifier.

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Ion Evaporation Mass Spectrum of Human Hemoglobin *Non-Covalent Subunits and Co-Factors Dissociate* 2 Alpha Chains at 15,126; 2 Beta Chains at 15,865.2

hb103.TDT Apr 10,1989 15:41:00. 2 scans in 1 group: 06:58
Group 1, Scan: 1

20 picomoles



Influence of the Ratio of Glycerol to Analyte on FAB-MS Analyses

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Sample preparation is a critical factor in determining the quality of FAB-MS results. The mass spectrometrists would prefer to maximize the signal from the analyte and minimize the interfering spectrum from the liquid matrix. Various strategies have evolved to accomplish these goals. Many workers add acidic or cationic modifiers to their samples to enhance the analyte ion abundance in the positive ion mode; unfortunately, this method also increases the abundance or complexity of the matrix spectrum in many cases (1). Some laboratories apply the matrix from a solution of a particular concentration (e.g., 50% aqueous glycerol), while others deposit a neat portion of matrix on the sample pedestal. The present fundamental study documents the effects of the relative proportions of analyte, glycerol, and water upon the FAB-MS results for 5 different peptides and presents a model to explain these results.

This investigation was spurred by the following observation. 4 nmoles of the nonapeptide bradykinin provided significantly improved spectra when sampled from a mixture containing 0.10 mg glycerol and 1.92 mg water (a 2 μ l aliquot of 5% glycerol) over that sampled from a mixture containing 1.3 mg glycerol and 1.0 mg water (a 2 μ l aliquot of 56% glycerol). Both these compositions pertain to the sample prior to its exposure to the vacuum. The spectral improvement was pronounced in terms of increased visibility of analyte peaks with respect to matrix peaks. To characterize this effect, 2 μ l samples containing 4 nmoles of bradykinin in 56%, 21%, 5%, 2%, and 1% glycerol (prior to exposure to the vacuum) were analyzed repetitively. The first 10-second scan was evaluated in each case. Plots of peak intensity vs. weight % glycerol were constructed for both analyte and matrix ions. These plots illustrate a distinct increase in bradykinin peak intensities coupled with a significant decrease in glycerol peak intensities as the proportion of glycerol in the sample declined.

Because the spectral improvement coincided with a decrease in glycerol proportion and an increase in water proportion of the initial sample, experiments were conducted to identify the critical sample component. Samples containing the optimal portion of glycerol (0.10 mg) but widely varying quantities of water (along with the 4 nmoles of bradykinin) yielded indistinguishable results by FAB-MS. Gravimetric measurements of the optimal sample composition taken before and after exposure to the fore vacuum indicated that the vast majority of the aqueous component had been pumped away before the beginning of the analysis by FAB-MS. These results suggested that appreciable quantities of water played no direct role in the enhancement mechanism.

Calibration curves were constructed for several bradykinin ions from samples containing 1-12 nmoles of bradykinin in 1.3 mg of glycerol. These plots showed an approximately linear relationship between peak intensity and quantity of bradykinin. Another set of calibration curves was prepared from experiments employing a similar range of bradykinin quantities sampled from 0.10 mg of glycerol; this set showed a distinct tendency for bradykinin peak intensity to "level off" or to "plateau" for the larger amounts of analyte. Such curves have been observed by others involved in quantitation by FAB-MS (2); the plateau of the curves has been attributed to an optimum surface concentration of the analyte in the matrix. Response curves for the glycerol ions also were constructed from these experiments. The bradykinin samples containing 1.3 mg glycerol showed little change in matrix ion response for all the quantities of analyte employed. Analogous curves corresponding to the bradykinin samples containing only 0.10 mg glycerol showed a dramatic diminution of glycerol peak intensities with the increased portions of bradykinin. This observation also is interpreted as a surface effect, in which strong matrix signal suppression results from increasing analyte surface concentration.

The following model is proposed to explain the spectral enhancement. The molar ratio of glycerol to bradykinin in the 1.3 mg glycerol/4 nmol bradykinin samples is approximately 4,000:1. In the 0.10 mg glycerol/4 nmol bradykinin samples, this ratio reduces to 300:1. Depositing a constant amount of bradykinin into smaller portions of glycerol may be an effective means of forcing the analyte to the surface of the matrix.

Because this model emphasizes surface effects, one might expect differences in the enhancement behavior for molecules possessing different surface activities. Table 1 lists 5 peptides, the masses of their protonated molecules, and the Bull and Breese hydrophobicity index associated with each (3). Figure 1 illustrates the mass spectral enhancement for the protonated molecule of the most hydrophobic peptide, the most hydrophilic peptide, and the peptide of intermediate hydrophobicity when 4 nmoles of analyte are analyzed in the indicated amount of glycerol. The peak intensities correspond to the absolute intensity divided by the full scale value of the detector output. The trend illustrated in Figure 1 is consistent with the proposed model in that the peak intensities of the most hydrophobic peptide are maximized in larger portions of glycerol, while smaller portions of glycerol are necessary to observe the enhancement for the most hydrophilic peptide. Figure 2 documents the behavior of the protonated glycerol molecule for samples

containing each of these peptides. Interestingly, the region of the curves in which this matrix peak is significantly diminished coincides with the region in which the analyte peaks are significantly enhanced. This behavior also is consistent with the model which emphasizes the competition between analyte and matrix at the surface of the sample. Data from the experiments involving proenkephalin and fibronectin related peptide fit into the illustrated trend.

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Table 1

Peptide	Sequence	[M+H] ⁺	Bull and Breese Hydrophobicity Index
[Val ⁴]-Angiotensin III	Arg-Val-Tyr-Val-His-Pro-Phe	917	-463
Proenkephalin	Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu	900	-268
Bradykinin	Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg	1060	-104
Fibronectin Related Peptide	Cys-Gln-Asp-Ser-Glu-Thr-Arg-Thr-Phe-Tyr	1249	+119
NPNANPNANPNA	Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala	1207	+555

↑
Increasing Hydrophobicity

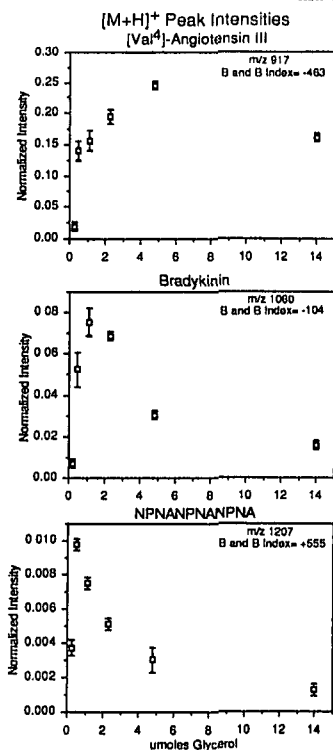


Figure 1. $[M+H]^+$ peak intensities for 4 nmoles of each peptide sampled from the indicated amount of glycerol.

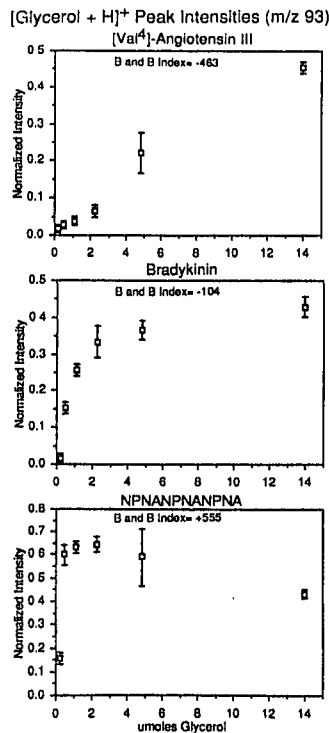


Figure 2. $[Glycerol+H]^+$ peak intensities (m/z 93) from samples containing 4 nmoles of peptide in the indicated amount of glycerol.

Differentiation of Leafy Spurge (*Euphorbia esula* L.) Accessions Based on Chemical Constitution Using Curie Point Pyrolysis-Mass Spectrometry-Pattern Recognition.

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Curie point pyrolysis-mass spectrometry, in combination with multivariate pattern recognition methodology (py-ms-pr), is shown to be an effective method for differentiating accessions of leafy spurge (*Euphorbia esula* L.) based on the chemical constitution of plant latex. Three multivariate statistical programs--hierarchical cluster analysis, factor analysis, and principal component analysis--correctly classified pyrolysis-mass spectra as to either *E. esula* or *E. cyparissias*. In addition, different accessions of *E. esula* corresponding to three different populations (Mandan, North Dakota; Cambridge, Idaho; and Baker, Oregon), were classified by this method. The features (m/z) responsible for each differentiation/classification were identified using Fuzzy c-Varieties Pattern Recognition (FCV). The results of this study were compared to results obtained in an earlier study using Curie point pyrolysis-gas chromatography-pattern recognition (py-gc-pr) to differentiate/classify the same set of leafy spurge plants.¹ Results from both techniques (py-ms-pr and py-gc-pr) elucidated various biotypes of leafy spurge, demonstrating the applicability of both methods for differentiating/classifying this troublesome weed.

Leafy spurge (*Euphorbia esula* L.) was introduced into North America from Europe in the 1800s, and has since spread throughout southern Canada and the northern United States.² Leafy spurge inflicts large economic losses for livestock producers because it reduces forage utilization and yield.³ Because leafy spurge is not indigenous to North America, natural enemies that would normally keep its population in check, are not present. Currently, a concerted effort is being made by American and Canadian researchers to develop effective biological controls using natural enemies such as phytophagous insects from native European spurge. Prerequisite to implementing successful biological control programs is understanding the biochemical similarities and/or differences among leafy spurge biotypes. Once relationships between biochemistry and host specificity are determined, success of a given biocontrol agent at a given site may be predicted.

Results Mass spectra from *E. esula* accessions were visually discernable from those of *E. cyparissias* spectra as shown in Figures 1 and 2. Pyrolysis-mass spectra from *E. cyparissias* plants could not be distinguished from one another by visual inspection. Mass spectra from four accessions of *E. esula* type spurge were less discernable from each other than from *E. cyparissias* spectra, but were more discernable from each other than were pyrograms resulting from py-gc analysis, which were indistinguishable from one another by visual inspection.¹ The results from hierarchical cluster analyses using UPGMA clustering and Euclidean distance measure, and Principal component analysis of the resulting data are shown in Figs 3-5. As can be seen in Figs 3 and 4, the pyrolysis-mass spectra from *E. esula* and *E. cyparissias* spurge were grouped into two major clusters by HCA and PCA, which is consistent with morphological evidence and is in agreement with previous results for the same samples using py-gc.¹ Subclustering the *E. esula* spectra (Figs 3 and 5) resulted in four distinct groups, corresponding to the different accessions of *E. esula*.

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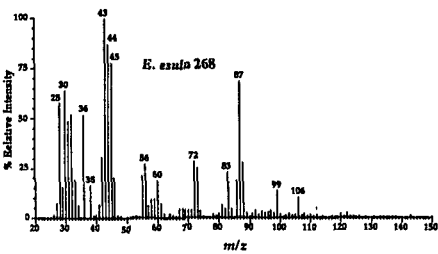


Figure 1

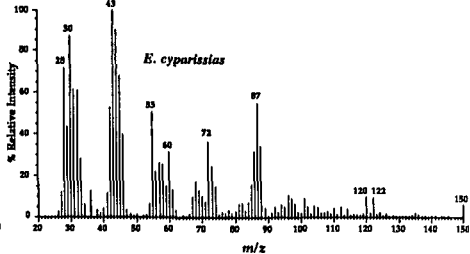


Figure 2

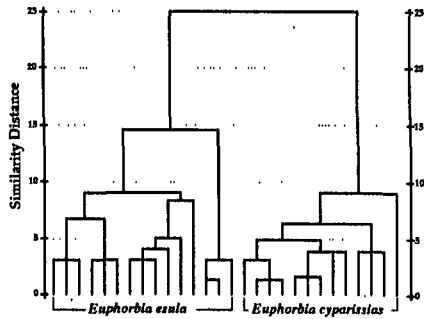


Figure 3

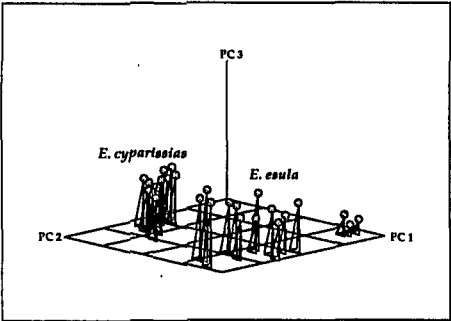


Figure 4

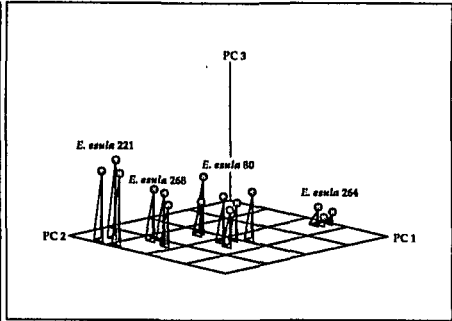


Figure 5

MATRIX ASSISTED UV LASER DESORPTION OF BIOLOGICALLY INTERESTING MOLECULES.

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Recent developments in the volatilization of large molecules using matrix assisted ultraviolet (UV) laser desorption have made it possible to produce intact quasimolecular ions from materials with molecular weights in excess of 100,000 using nicotinic acid matrices [1]. We have constructed a linear time-of-flight (TOF) mass spectrometer to be used for both basic research and rapid mass determinations of high mass molecules. Our results demonstrate that mass resolutions of $m/\Delta m \approx 500$ (FWHM definition) can be obtained for small proteins ($m/z < 20,000$), with sensitivities better than 1 picomole and mass range in excess of 100,000 u. Our results also indicate that the actual dimensions of the laser focus are not a critical factor in producing high mass ions. Our irradiation area of 0.1 mm x 0.3 mm produced sufficient numbers of ions so that detector saturation (tandem multichannel plates, 25 mm dia.) was a significant problem.

A survey of possible matrix materials has shown that several different types of compounds produce very similar results to those obtained from the original matrix material 3-pyridinecarboxylic acid (nicotinic acid). Materials were selected for trial on the following criteria: 1) strong UV absorption at 266 nm (the wavelength of our frequency quadrupled Nd-YAG laser); 2) water solubility (so that they could be co-dissolved with proteins); and 3) volatility (volatile/sublimable solids were chosen). Four matrix materials (in addition to nicotinic acid) were found to be useful. These were: 2-pyrazinecarboxylic acid (pyrazinoic acid); 3-methoxy, 4-hydroxybenzoic acid (vanillic acid); thymine; and thiourea. Surprisingly, the nicotinic acid isomers picolinic acid and isonicotinic acid did not work well as matrices, even though their UV absorption properties were very similar to nicotinic acid. Presumably the detailed interaction of the matrix molecules with the protein molecules and with each other is changed by the changing the position of the carboxylic acid group with respect to the nitrogen in the pyridine ring. The much less volatile and relatively insoluble dicarboxylic acid analogues of nicotinic acid did not work as matrices. Analogues of thiourea that did not have strong UV absorptions at 266 nm, urea and guanidine hydrochloride, were also ineffective as matrices.

Another important finding is that matrix assisted UV laser desorption produces both positive and negative quasimolecular ions, with the preferred intact species being singly charged. Figure 1 shows the complete positive and negative TOF mass spectra of insulin (MW = 5733.5 u). Both singly and doubly charged species are seen in both spectra, but the singly charged species is more abundant. This pattern has held true for all of the proteins and peptides so far examined (in excess of twenty different compounds).

Figure 2 is a detail of Fig. 1 (a), showing the positive, singly charged quasimolecular ion region. Fig. 2 clearly demonstrates the presence of several different adduct species. The most intense peak is the protonated insulin molecular ion. The second most intense peak is an adduct with a mass 80 u higher than the molecular weight. This ion can be explained by the addition of the matrix molecule's ring system (pyrazine, MW = 80 u) to the protein molecule. Pyrazine is an expected decarboxylation photochemical product formed by UV irradiation of the matrix (pyrazinoic acid, MW = 124 u). This type of adduct ion (i.e. the matrix molecule minus CO₂) is a feature common to all of the acidic matrices used. It is most significantly a major contribution to peak broadening for high mass proteins, where the resolution is not sufficient to separate the protonated and matrix adduct ion peaks.

The sensitivity of the technique is typically 1 picomole or less. Figure 3 shows the positive ion mass spectrum of *E. coli* β -galactosidase (MW = 116,336) showing the singly, doubly and triply charged quasimolecular ion species, obtained from 0.7 picomoles of protein using pyrazinoic acid as the matrix. The area of the sample probe is 3 mm² and less than 5% of this area was irradiated to produce this spectrum. Total sample probe loadings of 0.1 picomoles of insulin produce spectra with the protonated molecular ion as the base peak. More than 20 peptides, proteins and lipoproteins were examined, and each one produced strong quasimolecular ion signals from ≈ 1 picomole of applied protein.

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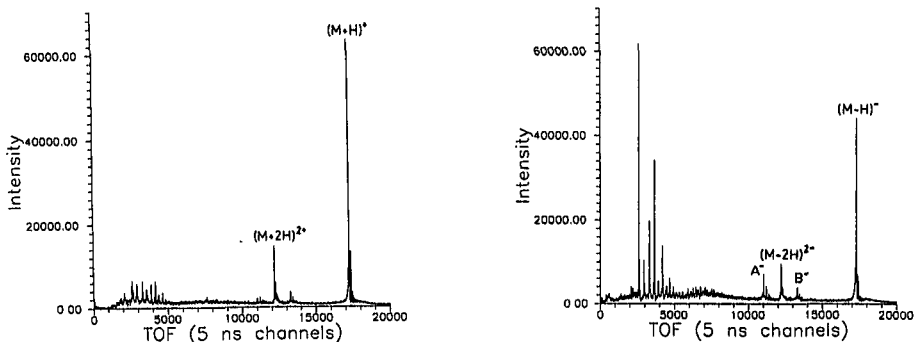


Figure 1. The positive (a) and negative (b) LD TOF mass spectrum of insulin (MW = 5733.5). Conditions: 0.5 μ l of protein/matrix solution loaded onto the probe (2 picomoles protein + 5 μ g pyrazinoic acid); total acceleration voltage (w.r.t. the detector) = + 17 kV; 200 laser shots accumulated; data recorded in 5 ns channels and displayed in 10 ns bins

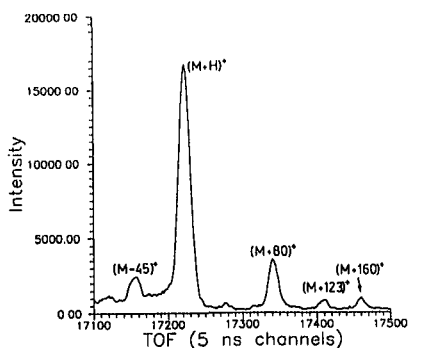


Figure 2 The quasimolecular ion region of the LD TOF mass spectrum given in Fig. 1 (a). This spectrum shows ions caused by the addition of by-products from matrix photochemical reactions (see text).

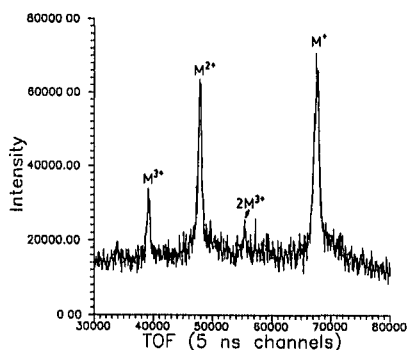


Figure 3. The high mass portion of a LD TOF mass spectrum of *E. coli* β -galactosidase (MW = 116,336). Conditions: 0.5 μ l of protein/matrix solution loaded onto the probe (0.7 picomoles protein + 5 μ g pyrazinoic acid); total acceleration voltage (w.r.t. the detector) = + 22 kV; 200 shots accumulated; data recorded in 5 ns channels and displayed in 250 ns bins.

STRUCTURAL ANALYSIS OF COMPLEX BACTERIAL OLIGOSACCHARIDES BY TWO- AND FOUR-SECTOR LSIMS

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Complete structural determination of the oligosaccharide components of the bacterial lipooligosaccharides (LOS) from *Neisseria gonorrhoeae* will further understanding of the role of the LOS in the pathogenicity and immunochemistry of the disease. LOS from different strains of *N. gonorrhoeae* have been shown to be highly variable in size and immunochemistry.¹ Therefore we are elucidating the structures of the oligosaccharides from several strains. Furthermore, five pyocin-resistant mutants of *N. gonorrhoeae* strain 1291, with altered LOS structures were produced by exposure to pyocin, a bacteriocin from *Pseudomonas aeruginosa*. LOS from these mutant strains have been suggested to contain progressive saccharide deletions.² Determination of the structures and properties of the altered LOS produced by the mutants should greatly aid the development of physical and immunochemical models of these complex and structurally variant LOS.

In general, relatively small quantities of material are available and thus sensitive and efficient procedures are needed for the analysis of LOS structures. Our strategy employs traditional methylation analysis, derivatization with 4-hydrazino-benzoic acid ethyl ester (HBEE),³ reverse phase HPLC purification, two- and four-sector LSIMS with high energy collision-induced-dissociation (CID). The HBEE derivatives are formed using 20 to 300 µg of oligosaccharide in 40 µl of methanol and 10 µl of H₂O with 1 µl of glacial acetic acid, and 1.5 equivalents of HBEE. The reaction condition typically used is 80°C for 30 min and no extraction is necessary due to the small amount of HBEE reagent used. The reaction mixture is dried and injected directly onto a C₁₈ reversed phase HPLC column with UV detection and eluted with an acetonitrile/H₂O gradient.

Figure 1 shows the two-sector negative ion LSIMS spectrum of HBEE derivatives of oligosaccharides from the LOS of 1291A, B, and C mutant strains. These oligosaccharides have so far all been found to contain phosphoethanolamine and O-acetate, which can be removed with aqueous HF treatment, and a common triantennary structure containing 3-deoxy-manno-2-keto-octulosonic acid (KDO) at the reducing terminus attached to a heptose disaccharide and a single N-acetylhexosamine.

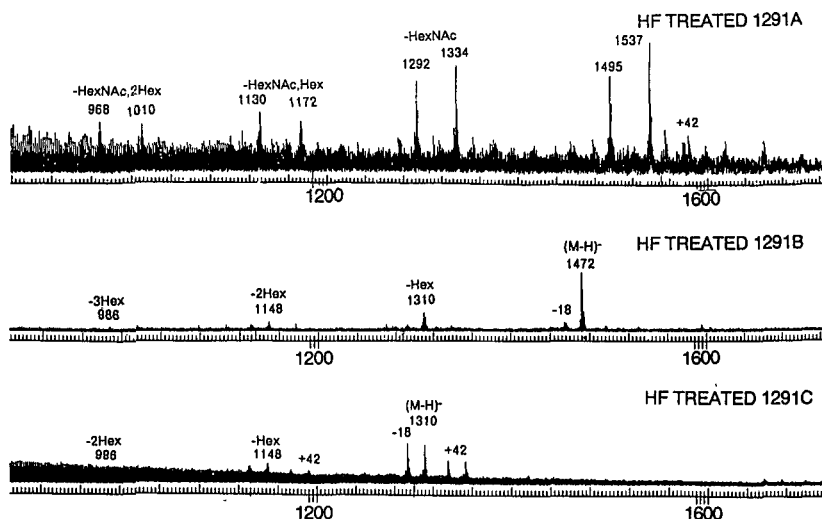


Figure 1. Negative ion two-sector LSIMS spectra of HF-treated oligosaccharide from mutant strains 1291 A,B, and C derivatized with HBEE. The 1291-A oligosaccharide is present as the anhydro derivative that is sometimes formed under the HBEE reaction conditions

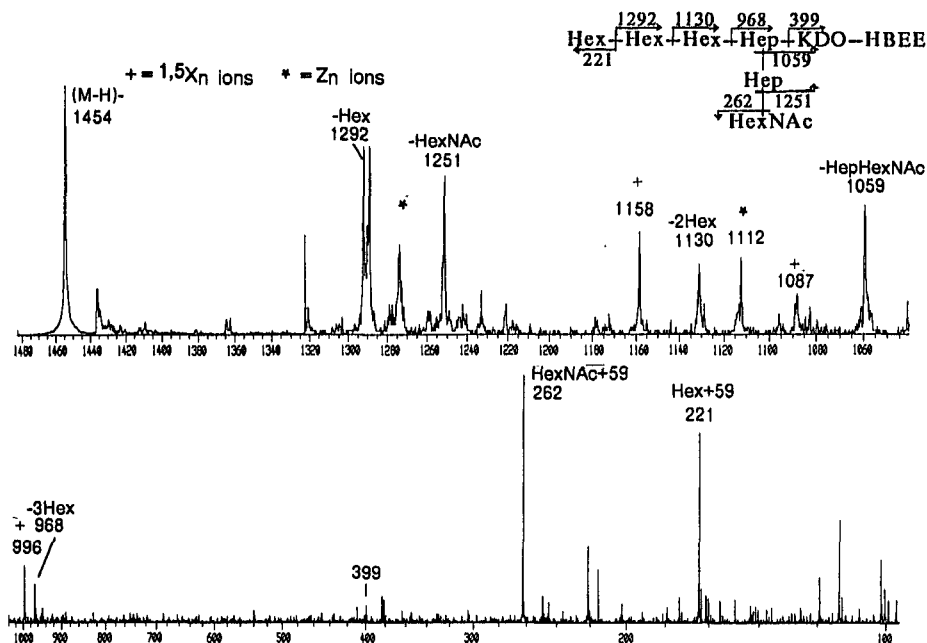


Figure 2. Negative ion four-sector spectrum with CID LSIMS of HF-treated oligosaccharide from mutant strain 1291B derivatized with HBEE.

A four sector MS (Kratos Concept II HH) with an optically-coupled diode array detector on MSII was used to acquire the MS/MS data. LSIMS spectra were obtained in MS-I in negative ion mode using a Cs⁺ beam and CID was performed in a He collision cell. Figure 2 is a negative ion MS/MS spectrum of HF-treated 1291B oligosaccharide derivatized with HBEE. A systematic nomenclature for the observed fragmentation has been recently described.⁴ Under CID conditions, fragments were observed resulting from glycosidic bond cleavage at every possible linkage including the Hep-Hep bond which was not found in the conventional two-sector LSIMS spectrum (see Figure 1). Valuable branching and sequence information is contained in this MS/MS spectrum as supported by methylation analysis and work on known standard oligosaccharides.

In conclusion, by derivatization of the reducing terminal KDO with HBEE increased sensitivity and fragmentation in LSIMS can be obtained. This fragmentation is mainly directed with charge retention on the derivatized reducing terminus containing the HBEE moiety. More complete fragmentation of the molecule can be obtained by four-sector LSIMS with CID than two-sector LSIMS. Non-reducing terminal fragments perhaps obscured by matrix ions or altogether absent in the two-sector spectrum can be observed in the MS/MS spectrum. The sequence and branching of the molecule can be determined from the fragments formed to some extent in two-sector LSIMS and completely in four-sector LSIMS with CID.

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HIGH ENERGY CID (TANDEM MS) OF OLIGOSACCHARIDES USING MULTICHANNEL ARRAY DETECTION

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Identification of oligosaccharides, whether attached to lipids or proteins, is central to the interpretation of the function of glycoconjugates. In order to elucidate the structures of glycans and conjugates we have enhanced the information content available from pmol amounts of oligosaccharides by enhancing the sputtering yield and fragmentation of glycans. This is accomplished by derivatization and tandem mass spectrometry.

In their native state oligosaccharides can be protonated or deprotonated in MS1 and their high energy CID spectra obtained. The sputtering yield for underivatized materials is low (1-10 nmol required), particularly for positive ions. Fragmentation of deprotonated species in the negative mode is dominated by ring cleavages of the type $1,5A,1$. Increasing the hydrophobicity of glycans by derivatization improves sputtering yields and directs the fragmentation by providing preferred sites of protonation, deprotonation and charge retention. All hydroxyls may be capped by permethylation or peracetylation, increasing the sensitivity by a factor of ten. Alternatively, free reducing termini may be coupled to a lipid-like tail. If tetradecylaniline is used the sensitivity has been shown to increase by a factor of 50. The positive ion CID spectra of these derivatives are dominated by $1,5X, Y, Y+2$, and Z fragments. The masses of the Y ions are shown in Figure 1 while the $1,5X, Y+2$ and Z ions are designated $+$, $!$ and $*$ respectively. The $Y+2$ fragmentation pathway involves glycosidic bond cleavage with a hydrogen rearrangement onto the nonreducing terminal neutral fragment. Ten picomoles of the p -tetradecylaniline produced fragments in the tandem spectrum of sufficient intensity to allow structural assignment.

The coupling to aniline derivatives may be combined with peracetylation. For example, the ethyl p -aminobenzoate derivative of a high mannose oligosaccharide underwent partial acetolysis and the positive ion CID spectrum, of a $Man_6GlcNAc$ cleavage product is shown in figure 2. B fragments (oxonium ions) for 1, 2 or 3 hexose residues were the only abundant fragments in the spectrum demonstrating the directing effect of permethylation and peracetylation. The fragments are consistent with the predicted longest hex sequence in the structure containing three mannose residues.

The sputtering yield of glycolipids may be enhanced by permethylation since no reducing terminus is available. We have analyzed a novel ganglioside whose molecular weight is approximately 2500u. The CID spectrum of an abundant fragment m/z 1087 contained B and Z ions which identified a Hex-(Fuc-3)HexNAc terminus.

- 1) Domon, B. and Costello, C.E., *Glycoconj. J.* (1988) 5: 397-409

This work was supported by grants from NIH (RR 01614, A.L. Burlingame) and NSF (DIR 8700766, A.L. Burlingame)

Maltoheptaose Tetradecylaniline
Positive Ion CID

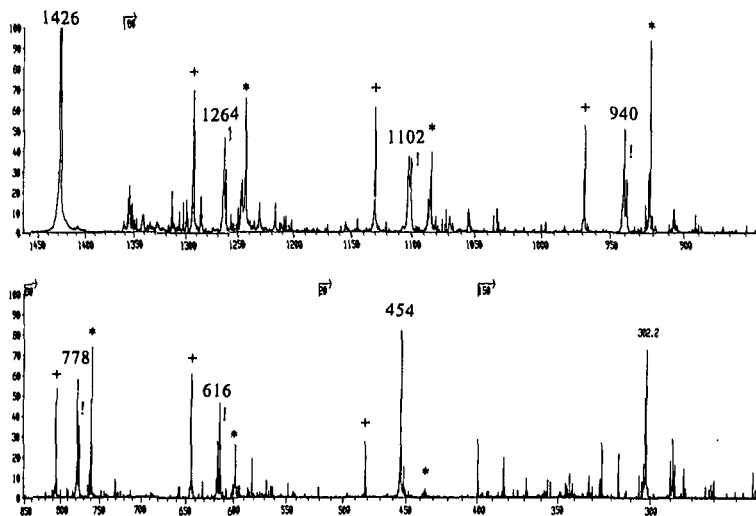


Figure 1. Positive ion high energy CID spectrum of maltoheptaose reductively coupled to tetradecylaniline.

Partial Acetolysis Product
Peracetyl ABEE Man₆GlcNAc

(M+H)⁺ 2308

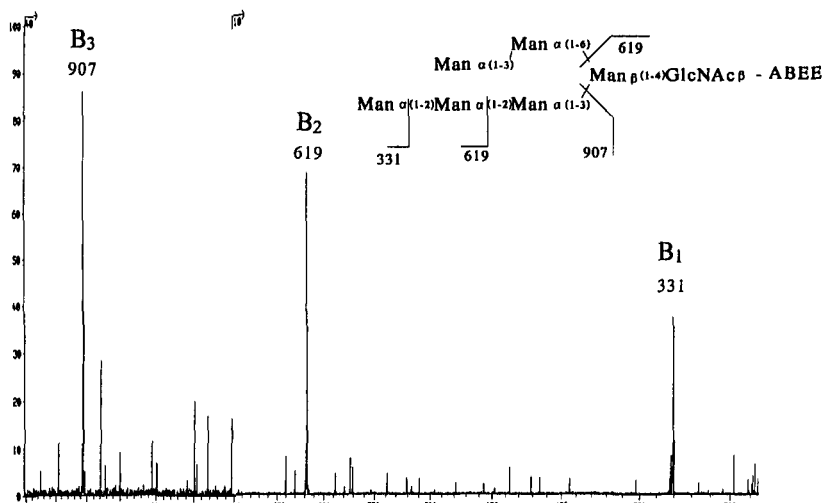


Figure 2. Positive ion CID spectrum of Man₆GlcNAc-ABEE peracetylated. This sample is one product of the partial acetolysis of a Man₆GlcNAc oligosaccharide isolated from the IgM of a patient with Waldenstrom's macroglobulinemia.

Chromatographic Separation of High Mannose Oligosaccharide Isomers and
Their Characterization by Collision Induced Dissociation

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Tandem mass spectrometers have been shown to be effective instruments for resolving mixtures of peptides. Moreover, sequence for these linear biomolecules can be directly deduced from their CID spectra. Comparable approaches, unfortunately, are not applicable to oligosaccharides, which frequently consist of isomeric monomers with branched sequence arrays. Thus, for the analysis of glycoconjugates there is a greater reliance on chromatographic methods to resolve the structural details inherent in these biopolymers. Collision (or FAB) spectra in the absence of data on isomeric purity should not be considered as an appropriate answer to questions on chain branching. To address this branching-CID relationship, we have utilized a two-dimensional chromatographic approach (1) employing an amino column, and a reverse phase C₁₈ column for the isolation of N-linked high mannose isomers. The samples were obtained from Endo H treatment of a soybean lectin, and from the urine of sheep suffering from mannosidosis. For sensitive HPLC detection, the mixtures were prepared with either pyridinylamine (PA) or aminobenzyl ethylester (ABEE) labels on the reducing-end. The purified fractions were salt-free and used directly for MS studies.

In this study, an aminopropyl column was used for size fractionation. These fractions were then injected onto a uBondapak C₁₈ column, (Waters Assoc, Milford, MA) using a gradient of 6-12% acetonitrile in 30 minutes. It was found that for high mannose oligosaccharides the resolution provided by the ABEE derivative on an amino column was as good or marginally better than either the reduced PA or the unreduced PA glycosyl amine. The reduced PA derivative is suitable for C₁₈ separations of isomers containing the more hydrophobic chitobiose reducing end (GlcNAc-GlcNAc); however, PA labeled oligosaccharides with single GlcNAc reducing ends are not retained on the column (using isocratic water) for a sufficient period of time to affect separation. With the more hydrophobic ABEE label this is not a problem. The alpha and beta anomers of the unreduced PA glycosylamine complicate the C₁₈ chromatograms; only the reduced derivatives are therefore recommended in this procedure.

There is little difference between the mass spectra of the two sugar derivatives. Both PA and ABEE labeled oligosaccharides gave abundant (M+H)⁺ or (M+Na)⁺ ions with little fragmentation in the positive mode. The negative FAB spectra exhibit (M-H)⁻ ions as well as abundant fragment ions for both derivatives. The fragment ions in the negative FAB spectra are of the same mass as (M-H)⁻ ions of lower molecular weight oligomers, so positive FABMS is best for determining molecular weight heterogeneity.

ABEE derivatized soybean lectin oligomers were fractionated on an amino column. The fraction containing two Man₇GlcNAc-ABEE derivatives was collected and separated on a C₁₈ column. The negative ion CID spectra, (EB-CID-EB), of these two fractions were recorded on a four-sector mass spectrometer, (HX-110), (Fig. 1). Both terminal end (A) and reducing end (Y) fragments are observed (2). Figure 1a, (C₁₈ fraction 1), clearly shows the losses of one (m/z 1341), two (m/z 1179), and four (m/z 855)

hexoses, but no fragment for a loss of three hexoses (m/z 1017). Similarly, terminal end fragment ions are found containing one (m/z 221), two (m/z 383), and four (m/z 707) hexoses, but nothing corresponding to a terminal fragment containing three hexoses (m/z 545). Comparison with Figure 1b, (C₁₈ fraction 2), reveals that these two fractions contain different isomers; the known structures are easily matched with the spectra as shown. Of significance is the observation that the so-called "double cleavage" ions, i.e., ions resulting from the rupture of more than one bond, are apparently absent. It remains an open question as to whether the absence of "double cleavages" is due only to obtaining samples that are free of isomeric contaminants, or if, in addition, the reducing end label suppresses the detection of multiple cleavages.

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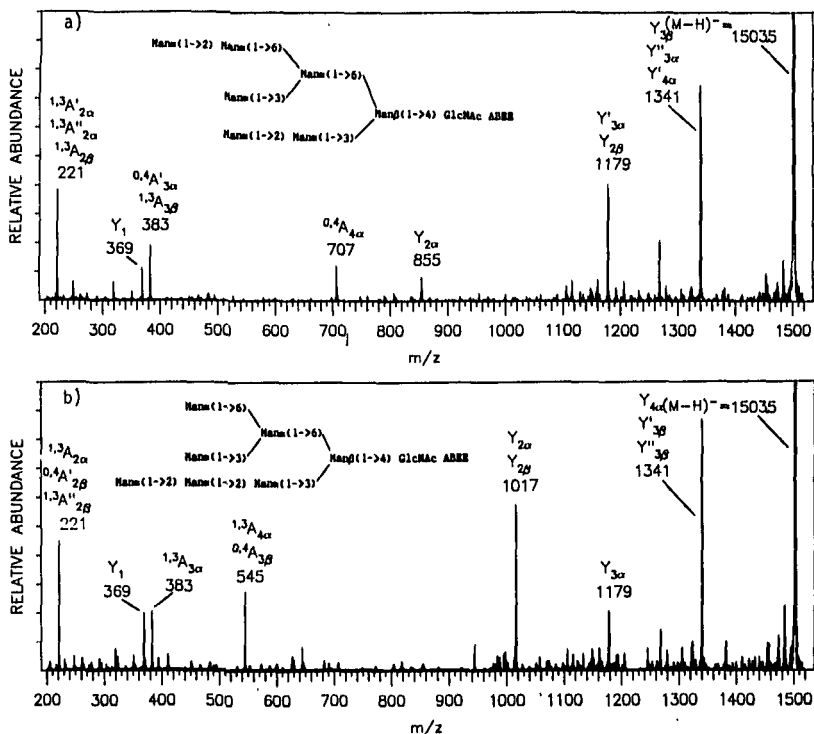


Figure 1: The negative CID spectra, (EB-CID-EB), of the Man₇GlcNAc-ABEE isomers from C₁₈ fraction 1, (Fig. 1a), and fraction 2, (Fig. 1b).

LDI-FT-ICR MASS SPECTROMETRY OF OLIGOSACCHARIDES.

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Our research effort has been directed towards LDI-FT-ICR of bacterial capsular polysaccharides (CPS) by hydrolysis using viral enzymes^{1,2}. The oligosaccharides obtained by this procedure correspond to the repeating units of CPS³. Such oligomers are commonly tri- to hepta-saccharides, substituted with labile non-carbohydrate moieties, such as, O- and N-acetate, pyruvic acid acetal, which determines the antigenicity of the CPS. The structural elucidation of these carbohydrate biomolecules involves mostly wet chemical techniques, with FAB and DCI coming into prominence for their role in sequencing. FAB spectra usually show only glycosidic bond cleavages, and the oligosaccharide has to be reduced prior to mass spectrometric analysis for unambiguous sequencing. Furthermore, derivatization (permethylation or peracetylation) is normally required. Unfortunately, both methods may remove base- or acid-labile substituents that may be present in the oligosaccharide⁴.

The LDI-FT-ICR fragmentation patterns seen in oligosaccharides¹⁻² appear to be characteristic of carbohydrates in that these patterns can be used⁵ to assign structural features to fingerprint mass spectra of polysaccharides⁶.

The following examples illustrate the use of positive and negative ion LDI-FT-ICR for the sequencing of underivatized oligosaccharides derived from bacteriophage degradation of *Klebsiella* serotype K3 and *E. coli* serotype K9 bacterial CPS^{7,8}. LDI-FT-ICR spectra of the oligosaccharides showed intense fragmentation resulting from glycosidic bond cleavage and ring rupture. The presence of both ring cleavage ions and glycosidic bond cleavage ions allows, without the necessity of a prior reduction step, unambiguous sequencing of oligosaccharides.

The low resolution mass spectral data of the positive ion LDI-FT-ICR of *Klebsiella* K3 repeating oligosaccharide indicated that i) the CPS is made up of a pentasaccharide repeating unit, consisting of four hexoses, one hexuronic acid and one pyruvic acid acetal; ii) three hexose units are linked together; iii) the hexuronic acid is linked to the three hexose unit; iv) the pyruvate is not linked to the three hexose unit. Thus, the low resolution glycosidic bond cleavage data suggested two possible sequences for the oligosaccharide: Hex-HexA-Hex-Hex-Hex or Hex-Hex-Hex-HexA-Hex. The high resolution ring cleavage data indicated that i) the pyruvate is linked to the terminal single hexose unit; ii) the reducing end terminal hexose is linked via either position 3 or 4; iii) a two hexose unit is sandwiched between two ring cleavage fragments (m/z 553.1760: $[(2\text{Hex})+\text{C}_4\text{H}_7\text{O}_4+\text{C}_4\text{H}_7\text{O}_3+\text{Na}]^+$). The fragment $-\text{C}_4\text{H}_7\text{O}_3$ can only be derived from a hexose unit, thus, the sequence of this m/z 553.1760 fragment is $\text{C}_4\text{H}_7\text{O}_4-(2\text{Hex})-\text{C}_4\text{H}_7\text{O}_3$. The reverse of this sequence will imply a four hexose unit, which is ruled out by the low resolution glycosidic bond data. Therefore the sequence of the pentasaccharide is Hex(pyr)(1→?)HexA(1→?)Hex(1→?)Hex(1→3/4)Hex.

The low resolution mass spectral data of the negative ion LDI-FT-ICR of *E. coli* K9 repeating oligosaccharide indicated that i) the CPS is made up of a repeating tetrasaccharide, consisting of two hexoses, one acetyl hexosamine and one acetyl neuraminic acid; ii) the two hexoses are not linked to each other; iii) the hexosamine unit is sandwiched between the two hexoses; iv) the neuraminic acid is linked to a hexose unit. Thus, the low resolution glycosidic bond cleavage data suggested two possible sequences for the oligosaccharide: Hex-HexNAc-Hex-NeuNAc or NeuNAc-Hex-HexNAc-Hex. The high resolution ring cleavage data indicated that i) one hexose residue is either 3- or 4- linked to the hexosamine (m/z 170.0448: $[(C_3H_4O_2N + C_4H_7O_3) - H_2O - H]^-$); ii) the neuraminic acid gave five fragmentation products, indicating that it is 4- linked to the HexNAc-Hex unit. Therefore the sequence of the tetrasaccharide is Hex(1→?)HexNAc(1→3/4)Hex(1→4)NeuNAc.

In conclusion, high resolution LDI-FT-ICR mass spectrometry can unambiguously sequence underivatized oligosaccharides, including location of an acid-labile pyruvic acid acetal. Tentative position-of-linkage information can also be obtained from both the positive and negative ion spectra. Our earlier work indicated that negative ion LDI-FT-ICR may be able to distinguish the anomeric stereochemistry of carbohydrate molecules, however, this does not seem to be a general phenomena and further work is being carried out to establish the analytical aspect of this empirical observation.

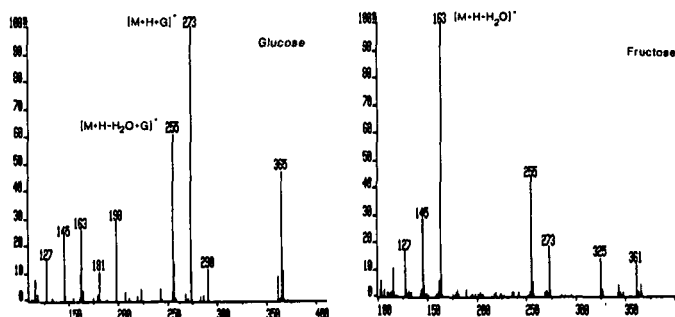
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**FAST ATOM BOMBARDMENT MASS SPECTRAL BEHAVIOR OF SACCHARIDES
AND NUCLEOSIDES: MATRIX EFFECT.**

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The positive ion fast atom bombardment mass spectral behavior of five sugars and five nucleosides was investigated using glycerol, diethanolamine, and propanolamine as the matrix compounds.

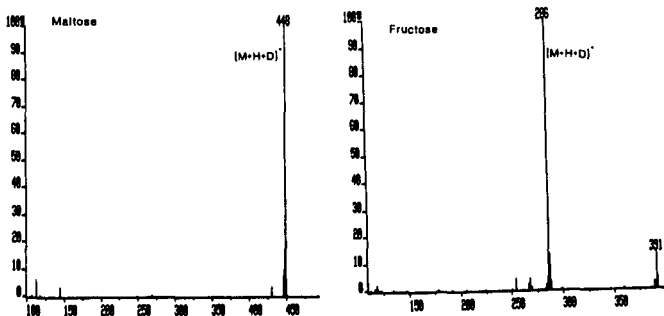
Saccharides. In glycerol, the monosaccharides give very weak or no quasi-molecular ion. The predominant process is dehydration for fructose; adduct formation for glucose.



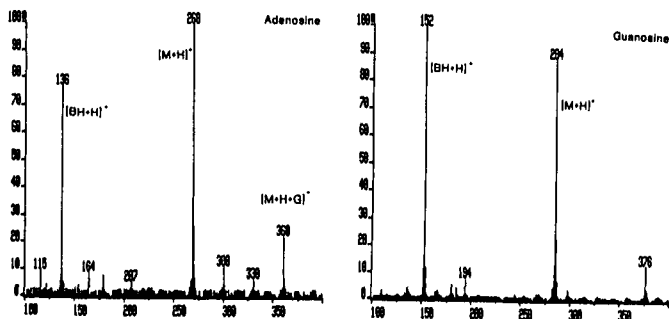
The disaccharides show abundant dehydrated quasi-molecular ions as well as adduct ions between glycerol and the quasi-molecular ions. Sucrose gives abundant ion at m/z 163 which is also the most abundant ion in fructose. The efficient dehydration of fructose is well known and it is attributed to the presence of two CH_2OH groups in the furanose ring.

In propanolamine all the saccharides show extensive dehydration which leads to the adduct ion from propanolamine and the dehydrated quasi-molecular ions.

All five saccharides show no dehydration in diethanolamine. Rather they give the adduct ions formed between the quasi-molecular ion and the matrix.

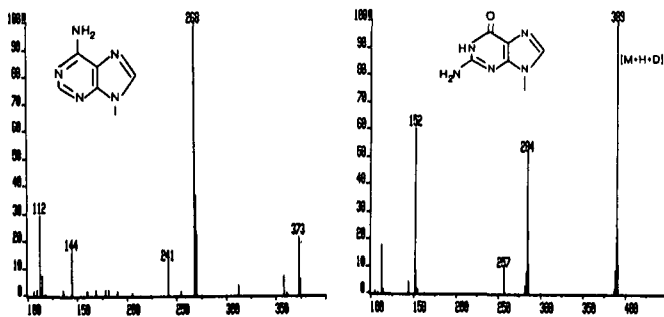


Nucleosides. Dehydration of the nucleosides is absent. They give abundant $[M+H]^+$ and $[B+H]^+$ ions in glycerol. In addition to these adduct ions resulting from addition of one or more glycerol molecules to $[M+H]^+$ are observed.



Propanolamine has one major influence on the compounds; they are fragmented to give $[B+H]^+$ as the major ion. No adduct ions are observed in this matrix.

There is a graded behavior of the compounds in diethanolamine. With adenosine which has the amino group as the only function in the base, $[M+H]^+$ is the most abundant ion. Guanosine and cytidine have similar spectral features. Both have an amino and an oxo functions in the base and they give more abundant adduct ion formed from the quasi-molecular ion and the matrix. Uridine and 5-methyluridine which have two oxo groups in the base do not give any $[M+H]^+$ ions. The adduct ion becomes the only predominant ion. The matrix and the nucleobases appear to play important roles in the stability of the compounds.



CHEMISTRY OF IONS DERIVED FROM METAL CARBONYLS

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Electron impact on metal carbonyls produce a variety of species including atomic metal ions, bare metal clusters and coordinatively unsaturated metal carbonyl ions. These species have been found to have a rich and varied chemistry. The study of this chemistry has grown into a substantial activity yielding both technological and scientific returns. We give attention here to two types of ions. First we discuss the chemistry of 17 electron radical anions with organic molecules of varying electron affinity. The variation of reactivity with ligand electron affinity suggests that charge transfer plays a role in the mechanism for ligand substitution. Then we consider the chemistry of clusters of 4 metal atoms with cyclohexane. The reactivity of these clusters depends on the number of carbonyl ligands they have. This variation in reactivity suggests a specific electronic requirement for cluster reactivity.

The total rate constants for reactions of $\text{Cr}(\text{CO})_5^{-(1)}$, $\text{Fe}(\text{CO})_4^{-(1)}$ and $\text{Ni}(\text{CO})_3^{-(1)}$ with a series of organic ligands were measured using FTICR techniques on a Nicolet FTMS-2000 instrument. The anion reactants were formed by electron attachment to $\text{Cr}(\text{CO})_6$, $\text{Fe}(\text{CO})_5$ and $\text{Ni}(\text{CO})_4$. The reactant neutral was introduced to a pressure between 2×10^{-8} Torr and 2×10^{-7} Torr. Mass spectra of the trapped ions were obtained at varying delay times following an initial ionizing electron beam pulse. Decays of the reactant ion signal were exponential over at least 3 half-lives for reactive ligands suggesting that the results are unaffected by hot ions. With few exceptions the observed products corresponded to ligand substitution. Total rate constants are plotted vs. EA in Figure 1.

The reactions of species with large electron affinities proceed with near unit efficiency. At an electron affinity near 1 eV but slightly different for each metal reaction efficiency drops sharply with decreasing electron affinity. At larger electron affinities there is a tendency to replace more than one CO ligand. The products of these multiple substitutions tend to react further producing species with two or three reactant ligands bound to the metal. All exothermic charge transfer reactions of $\text{Ni}(\text{CO})_3^{-(1)}$ are observed. Only tetracyanoethylene accepted charge from $\text{Fe}(\text{CO})_4^{-(1)}$ and $\text{Cr}(\text{CO})_4^{-(1)}$. The failure of 2,3,5,6-tetrachloroquinone to react by charge transfer from those two ions suggests the corresponding neutrals have electron affinities greater than 2.78 eV or that there is a kinetic barrier to charge transfer.

The reactions are interpreted in terms of a potential surface represented schematically as a double well in Figure 2. The first well represents an electrostatically bound complex stable by ΔE_1 relative to reactants. At the top of the barrier between the wells (up $\Delta E'$ from the electrostatic well) is a transition state in which the electron is shared between the metal and the ligand. Whereas the radical anion reactant is a 17 electron species, transfer of charge to the ligand leaves a 16 electron species which might be expected to form a strong bond the ligand anion (bond strength = $\Delta E'$) resulting in the second well. This assumes that both the ligand and the ligand anion serve as a two electron donors. The complex in the second well might be called an inner sphere complex. If the bond between the metal and a CO ($\Delta E''$) in this complex is such that CO displacement is overall exothermic then that process will be the observed. If the electron affinity of the ligand is such that ΔE^0 is negative then charge transfer should complete with ligand substitution.

According to this model two quantities are critical in determining reactivities. If ΔE^0 decreases (dashed line in figure) the product surface and its intersection with the reactant surface, i. e. the transition state, drop. Conversely, if ΔE^0 is sufficiently large the energy of the transition state will exceed that of the reactants and reaction will not proceed efficiently. This is consistent with the observed correlation between ligand electron affinity and reactivity. Changes in $\Delta E'$ also effect the energy of the transition state. Stronger bonding between $\text{M}(\text{CO})_n$ and L^- will tend to lower the energy of the transition state. This may account

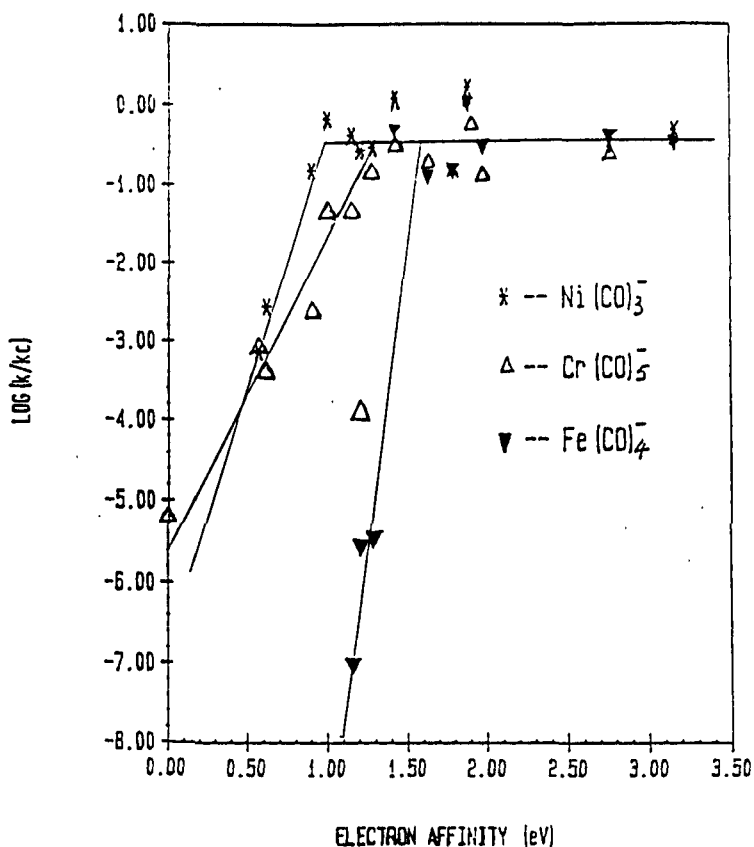


FIGURE 1

for the differences between the metals. For Ni ligand substitution occurs only if ΔE^0 is zero or negative while for Cr and Fe ligand substitution occurs when ΔE^0 is as large as 1 eV. This suggests that $\text{Ni}(\text{CO})_3$ binds L^- more weakly than do $\text{Cr}(\text{CO})_5$ and $\text{Fe}(\text{CO})_5$. The Ni complex is d^{10} and should be a relatively weak electrophile.

$\text{M}_4(\text{CO})_n$ complexes where M is Re can be prepared by ion molecule reactions in $\text{Re}_2(\text{CO})_{10}$ and by electron impact tetrametal dodecacarbonyls for M = Ir or Co. The chemistry of the Re and Ir clusters with cyclohexane have been described in the literature(2). Clusters of both metals dehydrogenate cyclohexane for sufficiently small n. For Re the n must be 11 or less, and for Ir n must be 6 or less. This was rationalized in terms of the electronic structure of a four atom cluster. Such a cluster will have 20 MOs derived from metal d orbitals and 4 MOs derived from metal s orbitals. It requires 48 electrons to fill these 24 orbitals in a low spin configuration. Since $\text{Ir}_4(\text{CO})_6^+$ has 47 electrons and $\text{Re}_4(\text{CO})_{11}^+$ has 49 electrons, it is suggested that a vacancy in the "s band" of orbitals much enhances the reactivity of the clusters. This is consistent with a

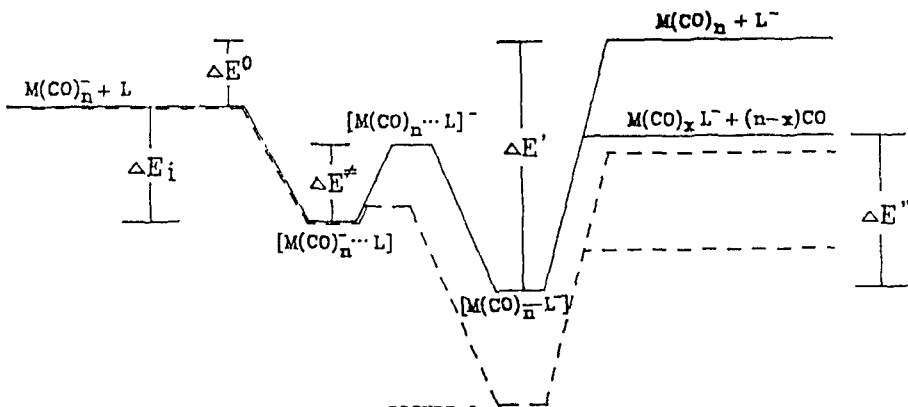
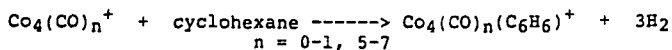


FIGURE 2

mechanism in which the metal acts as an electrophile towards the electron pair in a C-H bond.

The following reactions are observed for cobalt clusters:



This suggests an effect similar to that observed in Ir and Re for $n = 5-7$ since $\text{Co}_4(\text{CO})_7^+$ has 49 valence electrons. For $n = 2-4$, however, the results pose something of a puzzle. This puzzle can be solved using our orbital occupancy picture by supposing that the least substituted Co clusters have high spin ground states. This can lead to occupied s band orbitals for more than one CO substituent. With enough CO substituents a low spin configuration becomes favorable. This leaves more vacancies in the s and d bands so that electrophilic attack again becomes possible. Possible occupations of the s and d bands are indicated below (Scheme I).

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SCHEME I
 Co_4^+ Molecular Orbitals

High Spin		Low Spin	
4s		4s	
	0 vacant		4 vacant
3d	9 singly occupied	3d	1 singly occupied
	11 doubly occupied		15 doubly occupied
	20 orbitals		20 orbitals

"DAMAGE CONTROL" IN CHARACTERIZING COMPLEXES

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University of Tennessee, Knoxville, TN 37996-1600.

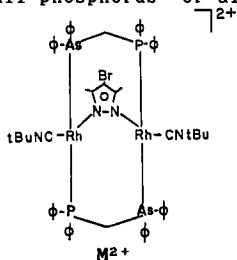
There are several means whereby artifacts can be introduced into the desorption ionization mass spectrum of an organometallic. Particularly pernicious are the possibilities of reduction and/or ligand exchange, which may arise from intrinsic sample chemistry in the DI matrix, or may be induced by the desorption ionization/sampling process. In the analysis of true "unknowns," such artifacts may complicate spectral interpretation or, worse yet, may lead to erroneous conclusions.

Electrohydrodynamic (EH) MS can provide valuable insight into potential problems specifically associated with the DI matrix, because non-invasive field evaporation is the only means of sampling in EH MS. For example, the EH mass spectrum of $\text{Ru}(\text{bpy})_3^{3+}$ sampled from glycerol is identical to that obtained from the $\text{Ru}(\text{II})$ analog, comprising only the $\text{Ru}(\text{bpy})_3^{2+}$ ion [1]. It can be shown by optical spectroscopy that the reduction of $\text{Ru}(\text{III})$ to $\text{Ru}(\text{II})$ is promoted by glycerol, independent of any mass spectrometric perturbations. By contrast, the further reduction of $\text{Ru}(\text{II})$ to $\text{Ru}(\text{I})$, and the fragmentation observed in the corresponding FAB mass spectrum are entirely bombardment-induced, and may be mitigated to varying extents by reducing the primary atom flux, by increasing the primary atom energy, and/or by resorting to a less reductive matrix solvent (e.g., m-nitrobenzyl alcohol, MNBA) [2]. The appearance of the ion pair $[\text{Ru}(\text{bpy})_3\text{ClO}_4]^+$ in the FAB spectrum may arise from the uncertain and varying analyte concentration at the bombardment site. The low abundance of M^{2+} in even the best FAB spectra of the $\text{Ru}(\text{II})$ complex may reflect the need for high fields in the region of desorption [3] for good sampling; M^{2+} is sampled with excellent sensitivity in EH MS [4].

Strong interactions with the extracting field do not always ensure good sampling efficiency in EH MS; when charge is "exposed" to strong solvent interactions, rather than surrounded by relatively non-polar ligands (as in the $\text{Ru}(\text{II})$ complex), sampling efficiency can drop precipitously [4]. Even 18-crown-6 does not appear able to protect coordinated metals from interactions with counterions and solvent [5]; sampling efficiencies therefore vary markedly depending on the metal's solvation and propensity to pair with counterions.

Other aspects of analyte chemistry may also affect the appearance of spectra. Labile complexes may exchange ligands for solvent or other anions, as was observed in EH spectra of $\text{Cr}(\text{bpy})_3^{2+}$ [6]. Condensation reactions with solvent are even possible in the presence of strong Lewis acids, like the Fe^{3+} -catalyzed esterification of EDTA with glycerol [7].

Despite these potential pitfalls, MS can provide substantial information useful for the characterization of organometallic complexes. In the most ambitious such undertaking in our laboratory to date, we have examined a family of three bridged dirhodium A-frame complexes, including M^{2+} , shown below, and two analogs with all phosphorus- or all arsenic-containing bridging ligands:



These complexes (MW 1605-1781) exceed the mass range of our EH-equipped mass spectrometer (a MS-902). Although the m/z of their molecular dications (657-745) would be accessible to the MS-902, the experiment has yet to be attempted. However, the FAB mass spectra (obtained with a ZAB-EQ) have been quite informative, as have been MIKES spectra obtained with the same instrument. (While the better resolution attainable with Q-scans on our hybrid instrument would relieve some uncertainty from spectral interpretation, the instrument is as yet incapable of performing effective linked E-Q scans over a broad mass range, as needed in this application. Simple Q-scans cannot accommodate the high collision energies necessary for effective CID of these large, stable molecules).

Although the FAB spectra of M^{2+} and its homologs are quite complex (e.g., Figure 1), several features stand out, especially with clarification from the MIKES spectra. Even under "optimized" bombardment conditions (low flux and high bombardment energy, with MNBA matrix), the prominent analyte ion in each case is the product of one-electron reduction, M^+ . Since this is the starting material from which M^{2+} is synthesized, its abundance could lead to an erroneous conclusion that the synthesis had failed; EH MS of this complex (with its freedom from beam-induced reduction) will be a valuable adjunct. Fortunately, the intact dication can be detected in the FAB spectrum (e.g., Figure 2), with the half-mass isotope spacing representative of doubly charged ions, and with isotope cluster intensities in good agreement with expectations. An ion pair (containing M^{2+} and one PF_6^- counterion) is also detected, confirming that at least some of the analyte is in the expected oxidation state.

The MIKES spectra of the dirhodium complexes are especially suggestive of the strength of the various ligand-metal interactions. CID of the ion pairs yields only one prominent ion, corresponding in each case to the loss of PF_6^- plus one *t*-butyl group. This surprising result may indicate localization of the counterion, at least in the gas phase; further study will be needed to confirm this speculation. CID spectra of the reduced material (M^+) (e.g., Figure 3) are richer in fragmentation. Whereas phenyl-containing moieties are detected only in the MIKES spectra of As-containing ions, the Br-containing ligand is lost only from the complex containing four phosphorus atoms. Both of these observations are consistent with the otherwise unconfirmed expectation that the P-Rh interactions should be stronger than the corresponding As-Rh interactions. They have even prompted speculation that the Br-containing (anionic) ligand may transfer an electron to Rh in the complex containing no As, generating a more easily detached neutral (radical) ligand.

Much more study (including MS/MS of the M^{2+} cation) will be needed before these and similar speculations can be confirmed or refuted. However, they serve to illustrate both the potential and the pitfalls of organometallic DI MS. As instrumentation and experience improve, this will clearly provide a powerful characterization tool.

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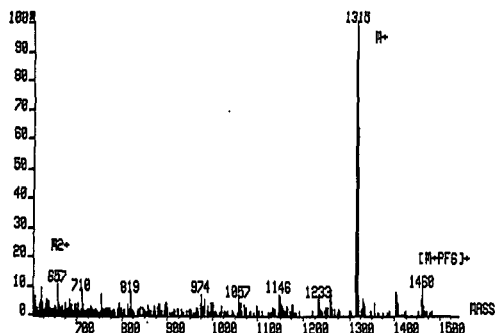


Fig. 1. FAB Spectrum of the Analog of M_2^+ Containing 4 P Atoms.

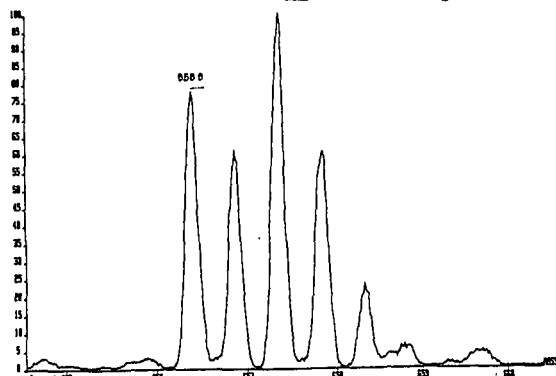


Fig. 2. Portion of an Averaged FAB Spectrum Showing the Isotope Cluster at M_2^+ of the Complex Containing 4 P Atoms. (Note half-mass isotope spacing.)

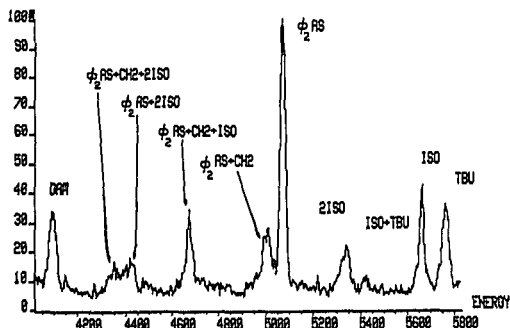


Fig. 3. MIKES Spectrum of the M^+ (Reduced) Ion Derived from the Complex Containing 4 As Atoms. (Labels correspond to ligands lost. TBU = t-Butyl; ISO = t-butyl isocyanate.)

STRUCTURE AND CHEMISTRY REVEALED BY THE MS AND MS/MS
SPECTRA OF INORGANICS AND ORGANOMETALLICS.

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LSIMS and FDMS have significantly widened the range of mass spectral analyses for inorganic, organometallic and polymer chemists, just as they have done for organic and biochemists. Usually, one or both ionization methods produce molecular ions for the analytes. However, LSIMS spectra of inorganics and organometallics are often complicated by the presence of ions that arise by reactions with the matrix. Positive and negative ion MS and MS/MS spectra are useful for structure determination, and for the insight they provide about the chemistry of the compounds and the nature of impurities. In general, the mass spectral sensitivities of these materials are excellent, and the product ion yields upon collision higher than those observed for large organic molecules.

We have obtained positive and negative FD and LSIMS spectra of neutral and ionic complexes of Tc and similar metals, and metastable and collision induced decomposition MS/MS spectra to determine effects of varying the ligand, the metal atom and the oxidation state of the metal. Relationships between mass spectral and chemical behavior have been demonstrated.

FD mass spectra of metallocene polymers (avg MW 2000-6000) show distributions of M^+ , and sometimes M^{2+} and/or M^{3+} as well. The combination of MS and MS/MS information facilitates the identification of synthetic byproducts. Fig. 1A-C show the m/z 50-1000 region of the FD MS/MS spectra obtained for molecular ions representative of three different series of components present in a metallocene polymer mixture: Components A, the intended product, and Components B and C, polymeric species in which the end group is incorrect.

FDMS and LSIMS and CID MS/MS spectra of large inorganic compounds with molecular weights in the 1500-5000 dalton range have been obtained, and include product ions due to both ligand and metal atom losses. Since these compounds contain numerous atoms with multiple isotopes, the isotope patterns of molecular, fragment and product ions can be interpreted to give compositional information. The two ionization methods are often complementary. For example, although for a very concentrated solution, the molecular ion cluster had extremely low abundance in the LSIMS spectrum of the bicyclic compound Sn_nR_nBu ($R = 2,6$ -diethylphenyl, $Bu = n$ -butyl), M , 1968 daltons, a unit resolution FD mass spectrum (which showed only molecular ions, no fragments) could be obtained from a dilute solution. Fig. 2 shows the LSIMS/MS spectrum obtained for the fragment ion at m/z 1198 in the cluster Sn_nR_nBu of the LSIMS spectrum of this compound. It can be noted in this spectrum that the cluster distribution of product ions is related to the number of multi-isotopic atoms lost from the precursor.

LSIMS mass spectra of heteropolytungstates vary greatly depending on the structures of the clusters. For some, the spectra are simple, containing only singly and doubly-charged molecular ions and few fragments. For others, facile losses of structural units are observed in both the normal and CID spectra. The spectrum of a new fluorine-containing compound is shown in Fig. 3A and the upper mass region of the CID spectrum of its major high-mass peak is shown in Fig. 3B. These illustrate that some types of large inorganic clusters undergo extensive, stepwise fragmentation and that CID can be quite informative even for compounds of this size.

Experimental. All spectra were obtained with a JEOL HX110/HX110 tandem mass spectrometer, operated at ± 10 kV accelerating voltage. Collisions took place in a cell in the field free region between MS-1 and MS-2. Helium or xenon was used as the collision gas, at a pressure sufficient to reduce the precursor ion abundance to 20% of its initial value. The collision cell was maintained at ± 3 kV from ground potential, so that collision energy was 7 kV. For LSIMS, the 6 keV primary beam was either Xe^+ or Cs^+ . For FDMS, sample solutions were applied to the activated carbon emitters with a syringe. The extraction potential was ~ 2.5 kV.

Fig. 1. Low mass region of the (+)FD CID MS/MS spectra of the M⁺ ions of polymeric metallocenes, n=6, present in a mixture where n = 2-25.

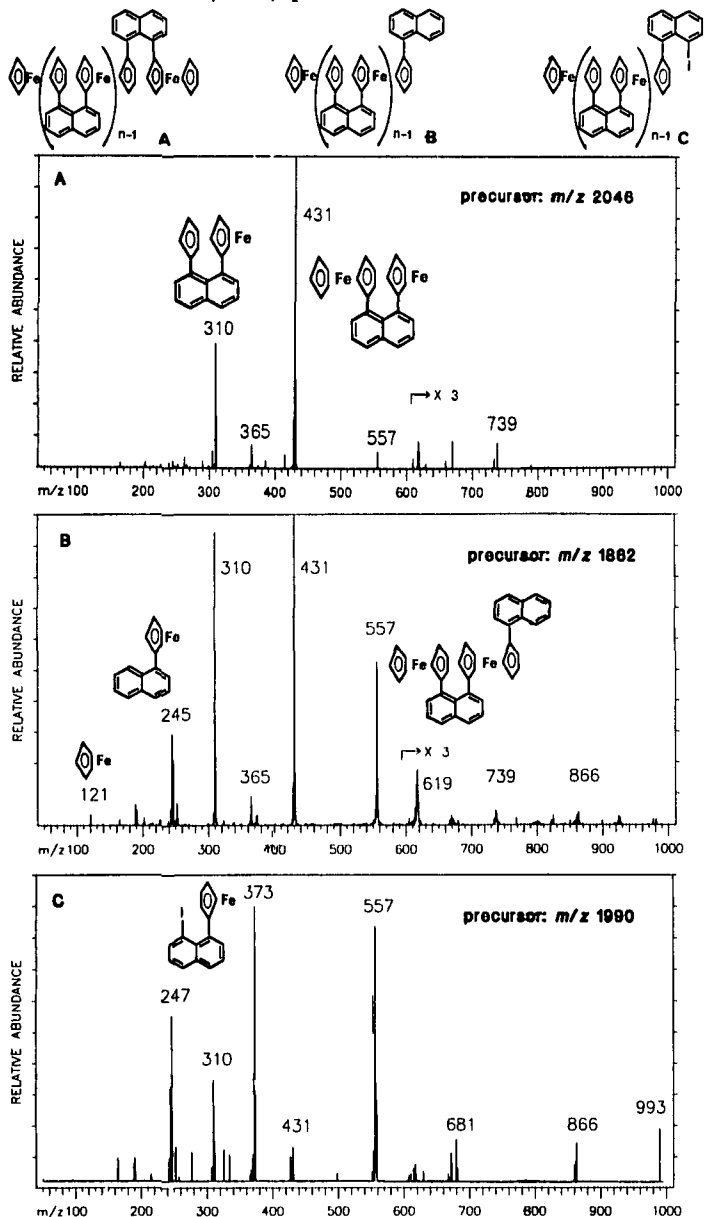


Fig. 2. CID MS/MS spectrum of the fragment ion at m/z 1198 in the (+)LSIMS spectrum of $\text{Sn}_6\text{R}_6\text{Bu}$ ($\text{R}=2,6\text{-diethylphenyl}$) dissolved in 1:1 benzene/ $m\text{-NBA}$.

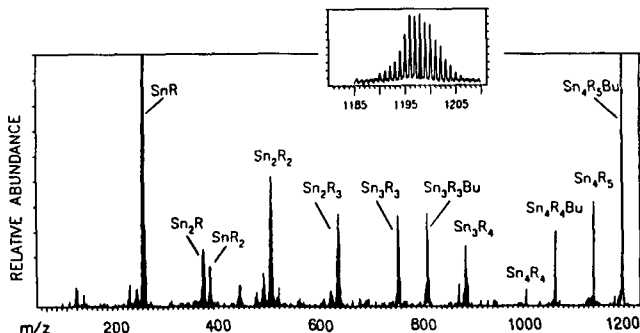
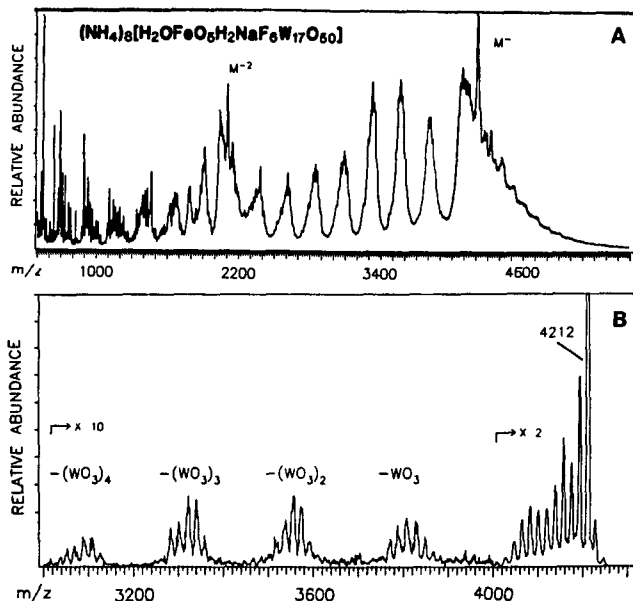


Fig. 3. (A) (-)LSIMS spectrum of a new heteropolytungstate dissolved in 1:1 water/glycerol. (B) CID MS/MS spectrum of the ion at m/z 4212.



Acknowledgements. The MIT Mass Spectrometry Facility is supported by the NIH Division of Research Resources (Grant No. RR00317, to K. Biemann). Tc MS studies are also supported by the US. Dept. of Energy. Samples for these studies were provided by A. Davison, MIT, and A. G. Jones, Harvard Medical School, (technetium); M. Rosenblum, Brandeis University, (metallocenes); L. R. Sita, Carnegie-Mellon University, (tin); and S. H. Wasfi, Delaware State College (heteropolytungstates).

MASS SPECTROMETRY OF METAL-LINKED DIMERS AND COVALENT HEME PRODUCTS

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The mass spectral analysis of organometallic complexes has been an interest of this laboratory for some years. Laser desorption (LD), fast atom bombardment (FAB) and plasma desorption mass spectrometry (PDMS) have all been employed for such studies.

In 1983 we reported the construction of a unique laser desorption mass spectrometer based upon a Tachisto (Needham, MA) 215G pulsed carbon dioxide laser and a CVC Products (Rochester, NY) model 2000 time-of-flight (1). The instrument had two interesting properties. First, the sample ions were formed in a field-free region and could be extracted up to 100 microseconds later to enable time-resolved studies. Second, an electron beam could be pulsed on just prior to ion extraction as a means of observing the desorbed neutrals. Spectra were generally obtained at about 20 us, where the major ions observed were cationized species. With this instrument we were able to obtain mass spectra of porphyrin dimers with and without Co atoms (2). In both cases the molecular ions observed were MK⁺ ions, when KCl was added to the sample.

The time delay feature was then used to study the metastable decomposition of chlorophyll-a from 5 to 30 us after the laser pulse (3)). These studies clearly revealed a two step fragmentation process involving loss of the phytol chain followed by extensive fragmentation of the ring structure. We also studied two derivatives: methylchlorophyllide-a (in which the phytol chain is replaced by a methyl group) and pyrochlorophyll (in which the carbomethoxy group is replaced with a proton). Interestingly, the absence of the phytol group resulted in the formation of molecular ions that were stable to further fragmentation, while the absence of the carbomethoxy group produced mass spectra showing prompt loss of the phytol group.

Fast atom bombardment mass spectrometry has been used for the study of reactions between myoglobin and BrCCl₃ as a model system for the production of radical-mediated heme-adducts of cytochrome P-450 (4). The analyses were carried out on a Kratos (Manchester, UK) MS-50 double focussing mass spectrometer with a high-field magnet and an Ion Tech (Teddington, UK) saddle field fast atom gun. HPLC separation of the products (Figure 1) revealed several modified hemes (peaks 2, 3 and 6) which were analyzed by FAB mass spectrometry. Isotopic patterns (Figure 2) were used to determine the number of Cl atoms incorporated into each of the modified hemes and to elucidate the structures shown in Figure 3. A final product (peak 5 in Figure 1) has been shown to be the result of covalent attachment of a modified heme to the peptide. The elastase digest of this product was separated by reversed phase HPLC, and the FAB mass spectrum indicated that it is attached to the elastase peptide with the sequence: AQSHAT. The structure of the modified heme forming the adduct was determined by comparison of the measured and theoretical isotopic distributions of the modified heme

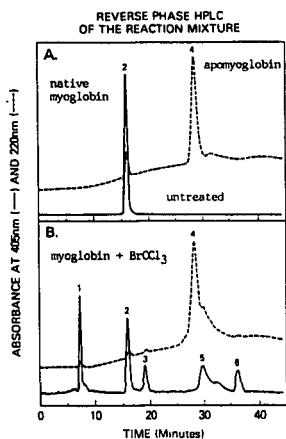
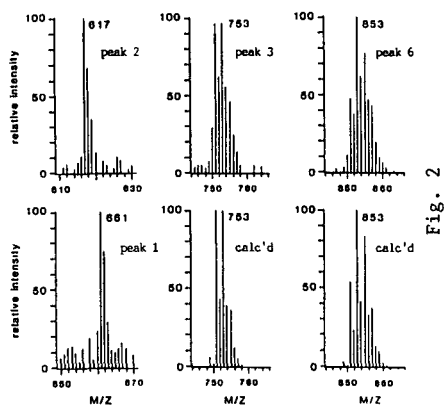


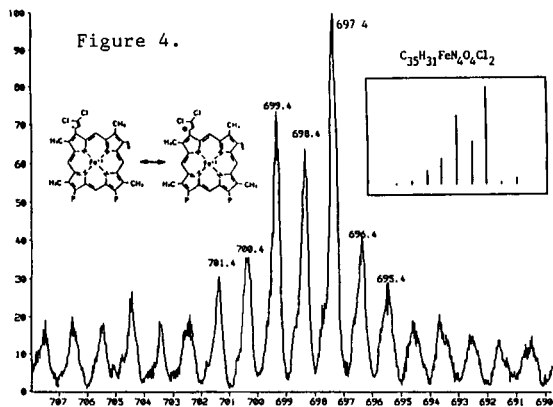
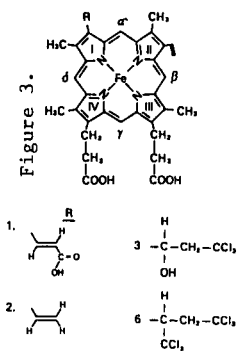
Fig. 1



fragment ion as shown in Figure 4. Further digestion of the elastase peptide fragment with carboxypeptidase A and aminopeptidase-M resulted in isolation of a heme product bound to histidine.

Plasma desorption mass spectrometry was used to study the dimerization of the tat protein from human immunodeficiency virus (HIV) shown in Figure 5 (5). The analyses were

STRUCTURES OF
MODIFIED HEMES ISOLATED
BY REVERSE PHASE HPLC



carried out on a BIO-ION (Uppsala, Sweden) BIN-10K mass spectrometer. The 18-amino acid that contains the cysteine-rich region of the tat protein was synthesized. The plasma desorption mass spectrum of this peptide revealed (almost exclusively) molecular ions corresponding to the monomer. However, when the peptide solution was mixed stoichiometrically with two Cd^{+2} ions per monomer, the plasma desorption mass spectrum (Figure 6) revealed extensive dimerization involving four Cd^{+2} ions (Figure 7).

ALA CYS THR ASN CYS TYR CYS LYS LYS —
— CYS CYS PHE HIS CYS GLN VAL CYS PHE

Fig. 5

Met Glu Pro Val Asp Pro Arg Leu Glu Pro
Trp Lys His Pro Gly Ser Gln Pro Lys Thr
Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys
Cys Phe His Cys Gln Val Cys Phe Ile Thr
Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys
Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln
Gly Ser Gln Thr His Gln Val Ser Leu Ser
Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp
Pro Thr Gly Pro Lys Glu

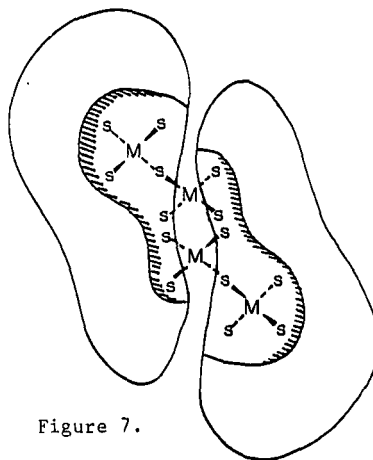
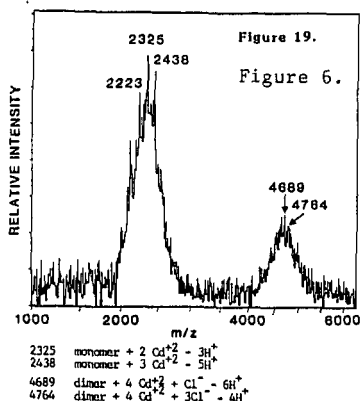


Figure 7.

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**ADVANCED FEATURES OF A FAST BUFFER BOARD FT-ICR
INTERFACE TO A PERSONAL COMPUTER**

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The basic operations of the interface circuits of a personal-computer based broadband FT-ICR have been illustrated [1]. The advantages of using a personal computer to host the FT-ICR instrument include: (1) very low cost of the computer; (2) extensive graphic and mathematic capabilities; (3) easy performance alteration or expansion; (4) an abundance of application software; and, (5) support for a wide range of output devices. Real-time ICR operations also require high speed and accurate timing control. A dedicated digital hardware interface has been designed and constructed to solve the timing problems. The digital circuits of the spectrometer were assembled on an IBM PC AT extension board. The extension board, or "fast buffer board," is used to replace the frequency synthesizers and waveform recorders normally used for arbitrary waveform generation and data acquisition operating at a speed of several mega Hertz. This design has not only led to great reduction in construction cost but has increased the flexibility of the instrument to perform complex experiments as well.

The digital hardware design focused on meeting critical timing requirements and all the other signals are left to be generated by software. This strategy reduces the complexity of the hardware design and allows one to utilize more of the capabilities of the computer.

Design features:

1. Common memory for both excitation and data acquisition. Since ion excitation and ion-transient signal acquisition always take place sequentially, it is possible to use a common buffer memory to store the excitation waveform and ion transient data. Using common memory reduces the memory requirement for both excitation and data acquisition by half. This memory-stored-ion-excitation method also gives the operator great freedom in choosing from a variety of excitation signals. Because the excitation waveform is transmitted by clocking out the data stored in the buffer memory, the control circuit design is simplified. In this instrument, twelve 16k X 1 bit static RAM is used. This means that 16K 12 bit-words of data can be stored in the buffer memory. However, the word length can be easily expanded to 16 bits.

2. Control logic for critical timing steps. In the chemical cycle of FT-ICR experiments, the timing events can be divided into segments. There are only a few segments in which absolute timing controls are required (Figure 1). For this configuration, the control logic of the buffer interface is only active during these crucial time periods. The control logic consists of three 8-to-1 multiplexers, three flip-flops, and a 3-to-8 decoder. The control inputs are provided by software through a COMMAND register. The supported functions include: (1) basic ICR operations (transmitting an ion-excitation waveform, waiting a time period, and recording the corresponding ion transient); (2) two-dimensional excitation operation (transmitting an ion excitation waveform, waiting for a time period, and transmitting the same waveform again); (3) excitation only mode (transmitting a waveform or repeating the transmission of the waveform); and, (4) data acquisition (storing a digitized signal

in the buffer memory). The combination of these functions allows one to perform almost all FT-ICR experiments.

3. Counters and latches for address counting. During excitation-data transmission and data acquisition, the address of the data is provided by address counters. The address counters also control the length of time for delay periods. In this instrument, four 4-bit binary counters are synchronously connected for 16K data counting. However, the counters can be cascaded for many more stages for larger counting data. This means that the buffer memory size is not limited by the overall design.

4. Address mapping for fast data transfer. For some FT-ICR experiments, i.e. tandem MS, several excitation waveforms have to be transmitted and at least one ion transient has to be recorded in a single chemical cycle. Since only a single bank of memory is used for storing all the waveforms and ion transient, the data transfer rate between the buffer memory and the computer's system memory has great influence on the time efficiency of buffer interface. The problem is minimized by mapping the buffer memory directly into the computer's system-memory space. For a typical 80286 based personal computer, the best block data transfer rate can be obtained by the "repeat following" string operations provided by the processor's instruction set. It takes less than 10 ms to transfer all the 16K words of data to or from the buffer memory. The data transfer rate is several times faster than the direct memory access (DMA) operations provided by the computer's hardware.

5. Multi-board operations. For some ICR experiments, signal generation and data acquisition have to operate at the same time, as in heterodyne-mode operation. These types of experiments can be realized by using more than one buffer board. The only modification needed is to change the address decoding. For multiple-cell instruments, the use of parallel buffer boards could allow monitoring ions in more than one cell simultaneously.

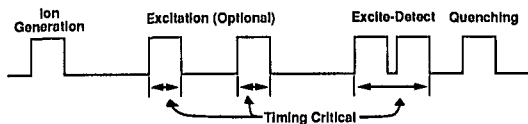
Reference:

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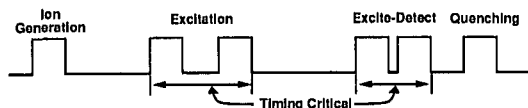
ICR Operation Real Time Control

1. Absolute Timing
2. Fast Data Transfer Rate

Conventional FT - ICR Experiments



2D FT - ICR Experiments



**ENHANCED MASS RESOLUTION OF BROADBAND FT-ICR MASS SPECTRA
BY THE MAXIMUM ENTROPY METHOD (MEM)**

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The maximum entropy method [1] has been demonstrated to be an attractive spectral analysis tool for improving mass resolution of narrow band FT-ICR mass spectra. We have found that more data points must be used to extend the method for broad-band spectral analysis. In our laboratory, the method has been adapted for use on our home-built FT-ICR mass spectrometer [2] which is based on an IBM PC AT compatible computer. The computer has a 6 MHz 80287 math coprocessor and 640 kByte of system RAM. The program, written in Microsoft Fortran, can produce 16384 error filter coefficients (EFCOF) and output the resulting spectral data into AutoCAD binary interchange files which can be further processed by AutoCAD programs to produce hard copies of publication quality spectra.

The subroutine which calculates error filter coefficients using Burg's algorithm was adapted from the literature [3]. When more time-domain data are analyzed, the data cannot be considered as stationary. The signal decay effect on the spectral analysis method is examined first with synthetic signals. The peak width of a single frequency signal continuously decreases to approach the theoretical line width with an increase in the number of data points used. When more data points are used, the spectral shape is found less sensitive to the initial phase.

The program has been used to calculate a propiophenone spectrum. The MEM and FFT spectra are shown in Figure (1) and Figure (2) respectively. The results of the two methods are summarized in Table (1). A resolution improvement of 40 times for most ion signals between 50 kHz to 250 kHz is achieved by using 4000 time-domain data and 2000 error-filter coefficients. In this case, there is no peak splitting found and difference of peak frequencies between FFT and MEM spectra is within the frequency interval of the FFT procedure.

The large computation time required for the method is well-known. However, this inconvenience will be relieved by the continued rapid development of powerful computers. The computation time for each calculation reported in this work is listed in Table (2). The computation time for the error-filter coefficient calculations increases quadratically with the number of data points when the ratio of data points and EFCOF is kept at the value 1/2. For comparison, a time domain ion transient signal with 120 data points and 90 EFCOF has been analyzed. It takes only 3.2 seconds for EFCOF calculation which is almost ten times faster than that cited for the previous work [1].

References:

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2. S. Guan and P. R. Jones, *Rev. Sci. Instrum.*, **59**, 2573 (1988).
3. W. H. Press, *et al.*, in Numerical Recipes, Cambridge University Press, 1986, p. 433.

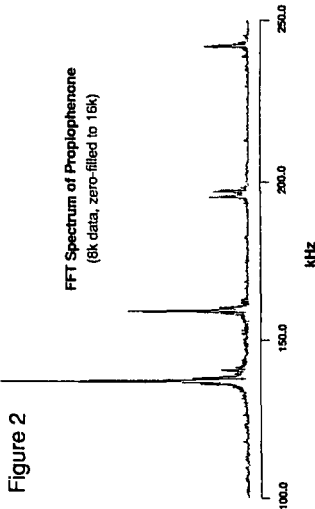
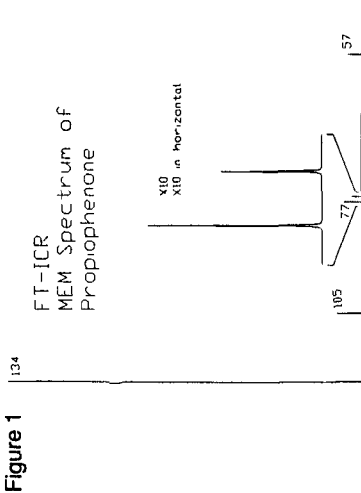
Table 1.
Comparison of FFT and MEM Spectra of Propiophenone

Normal Mass (au)	Peak Frequency (kHz)		Resolution	
	FFT*	MEM**	FFT	MEM
57	238.66	238.870	277	11619
76	178.10	178.185	226	9191
77	175.82	175.908	205	8110
105	128.50	128.275	206	1264
134	99.50	98.528	156	7050

*The FFT frequency interval is 244.14 Hz.
**The MEM frequency interval is 2.5 Hz.

Table 2.
Computation Time for EFCOF

No. of EFCOF / No. of Data Points	= 1/2				
No. of Data Points	500	1000	2000	4000	8000
Computation Time	35.8s	2min 23s	9min 30s	41min 52s	2h 48min



AN ADVANCED DATA SYSTEM FOR FT/ICR

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Introduction – The Cyanamid external ion source FT/ICR mass spectrometer¹ was designed to provide high mass accuracy over a wide mass range², and to be compatible with a wide range of sample inlet and ionization techniques, especially Thermospray LC/MS³. In all modes of operation the goal is to obtain unambiguous elemental composition information. In order to meet this goal we have constructed a data system which enables routine acquisition, processing and storage of 512k point transients. The system also controls our spectrometer and performs complete off-line analysis of data.

Primary Objective – To build a flexible open architecture data system capable of: 1. Acquiring a large number (524,288+) of data points. 2. Acquisition rates to 4 million samples per second. 3. Real-time storage of raw data on disk. 4. On-line signal processing for display and decision making. 5. Obtaining accurate mass information from raw data. 6. Using accurate mass data to determine elemental compositions.

Hardware – The primary components include a VME bus host computer, a high speed floating point array processor with a private bus, several disk drives, and a variety of standard and special interfaces to the outside world.

A Motorola MVME147 board is the host processor for the system. This board includes a 20 MHz 68030 microprocessor with a 68882 floating point co-processor, 4 MB of memory, four RS-232 serial ports, a parallel printer port, a SCSI bus interface for disk and tape, and an Ethernet port. A pair of Fujitsu M2361 ESMD 689 MB, 2.5 MB/s transfer rate disk drives are used for on-line storage of transient data. Because of software limitations these drives have a formatted capacity of 480 MB and an actual transfer rate of about 500 kB/s. Control of ion optics and RF quadrupole electronics is through an Opto-22 Optomux industrial control system. This system provides multiple channel, optically isolated, analog outputs using a RS-422 data link from the host computer.

For fast data acquisition and vector processing a Mercury Computer Systems ZIP 3232+ array processor with 10 MB of memory is used. The array processor is capable of operating at 10 or 20 MFLOPS and is user programmable via a "C like" language. A Burr-Brown ADC600 12 bit analog-to-digital converter is interfaced to the array processor through a Cyanamid built interface. Acquisition rates of up to 4 million samples per second are currently supported by the hardware. The number of data points that can be acquired is limited only by the total memory installed and the number of buffers required for the desired signal processing algorithms. One million point data acquisition and processing has been demonstrated, but the largest data record in routine use is 524,288 points.

Table 1 lists Fourier Transform times for several data table lengths. Due to hardware limitations in the array processor, 32k and larger Fourier transforms can use only one of the two arithmetic pipelines. Consequently, these functions operate at only 10 MFLOPS.

Table 1. ZIP3232 Real FFT Time, Single Pipeline (10 MFLOPS)

Points	Time, ms
32,768	194
65,536	304
131,072	831
262,144	1704
524,288	3540
1,048,576	7272

Data Acquisition Software – The host computer uses Motorola's VERSAdos real-time, multi-tasking operating system. This full service operating system provides a range of utilities and software development tools.

The acquisition and control software consists of six separate tasks which communicate with each other through shared memory and inter-task message passing. This multi-tasking scheme allows separation of high priority real-time tasks from those which must interact with the operator. The host computer programs are written in Pascal and assembly language and the array processor programs are written in Zip-C and microcode. Most of the signal processing routines are part of the standard algorithm library supplied with the array processor. The data acquisition microcode and several supplementary array processor programs were written specifically for this project.

All user interaction with the data system is done through two programs. CyAcq handles data acquisition and reduction and CnOp handles the user interface to the ion optics control system. These two programs communicate with several real-time tasks which run at a higher priority.

Table 2 outlines a typical LC/MS data acquisition sequence. From the table it is apparent that the most time consuming steps are the 128k Fourier transform and the transfer of 16 bit integer data to disk.

Table 2. Acquire and store 524,288 points, process 131,072 points on-line

Flush ions from cyclotron cell, Inject and trap ions in cell	
Acceleration Sweep	1-5 ms
Delay for pre-amp recovery	1 ms
Acquire 524,288 points, 12 bit integer data, 4 MHz	131 ms
Process 131,072 points for display/decision making	
Convert 16 bit integer to 32 bit floating point	26 ms
Real Fourier Transform	831 ms
Scale	34 ms
Power Spectrum	24 ms
Square Root (dual pipeline, 20 MFLOPS)	46 ms
Put Spectrum into increasing mass order	21 ms
Upload spectrum to host (256 kB)	73 ms
Display spectrum	90 ms
Check chromatographic window for peak (5k points)	8 ms
If peak is above threshold then	
Upload transient data to host for disk dump (1 MB)	288 ms
Dump 524,288 raw data points to disk	2014 ms
Total for Acquisition Sequence	3587 ms

Off Line Analysis – Peak centroids are normally obtained by CyAcq using a frequency interpolation technique². The peak centroid data is then analyzed by CyMass, a separate accurate mass program. CyMass provides calibration, elemental composition searches, chromatogram generation, pattern searching, stick plots and other services. It is possible for the entire set of acquisition, control, and analysis software to run on the data system simultaneously so that data quality can be quickly checked to the elemental composition level in a tune-up mode. CyMass and the mass spectrometry tool programs also run on MS-DOS computers and are being ported to Sun workstations.

The use of this data system has been an important factor in our ability to obtain high mass accuracy over a wide mass range^{2,3}.

1. J.T. Meek and G.W. Stockton, United States Patent No. 4,686,365.
2. J.T. Meek, W.G. Millen, T.J. Franci, M.L. Thomson, G.W. Stockton and R.S. Wayne, Proceedings of the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, Colorado, May 1987.
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A COMPARISON OF SEVERAL APODIZATION FUNCTIONS FOR SWIFT WAVEFORMS

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Stored Waveform Inverse Fourier Transform (SWIFT)¹ has been used to improve the selectivity of the excitation process in FT ICR mass spectrometry. In FT ICR mass spectrometry, apodization of the received signal before calculation of the mass spectrum reduces the sidelobe height at the expense of resolution. A similar tradeoff exists in the excitation process of FT ICR when using SWIFT excitation. The finite duration of an excitation waveform causes masses outside the defined mass windows to be excited in addition to masses of interest. A reduction in the power input into these sidelobes through apodization of a SWIFT time domain waveform is accompanied by broadening of the originally defined mass windows.

For applications requiring the highest selectivity of the excitation waveform, a rectangular window (no apodization) is suggested. This window has the disadvantage of producing rather large sidelobes. For most applications, ultimate mass selectivity is not needed, so an apodization function such as the Blackman-Harris 3-term window² is suggested, which has slightly lower mass selectivity than the rectangular window but has much lower sidelobes. Due to the smooth response provided by the Blackman-Harris window, it provides an excitation waveform more insensitive to frequency changes such as due to changes in pressure, trapping voltage, or magnetic field drift. This is in contrast to a SWIFT waveform with no apodization, which may have a large difference in the excitation response with a small change in frequency.

Calculation of the expected response of a SWIFT excitation waveform allows one to determine if the excitation conditions can provide the selectivity needed. Due to the discrete nature of the sampling and the inverse dependence of mass on frequency, the desired mass windows never fall exactly at an array point in the frequency domain, so roundoff errors are present in defining the original frequency domain spectrum for the excitation. Evaluation of the expected response gives one an idea of how well the excitation deviates from that desired, which can be significant at high masses. Finally, plotting the expected response linear in mass (rather than linear in frequency, as is usually shown), presents the data in a manner more useful to the mass spectrometrists.

Figure 1 shows the initial mass windows defined for a SWIFT waveform. Figures 2 and 3 are the expected response calculated using no apodization and a Blackman-Harris window, respectively. Note the greatly reduced sidelobe height for the Blackman-Harris window at the expense of slightly lower mass selectivity along the edges of the excitation window.

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²Harris, F.J. *IEEE Proc.*, 1978, 66, 51.

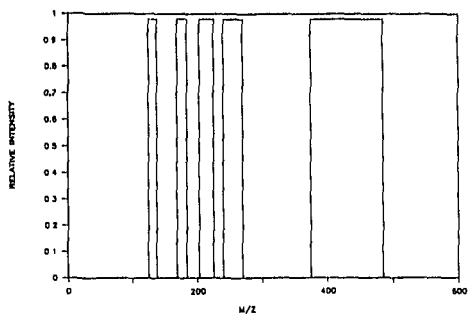


Figure 1: Original mass windows for a SWIFT waveform.

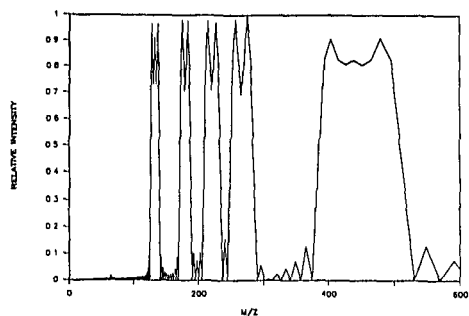


Figure 2: The expected response for a SWIFT waveform calculated for the mass windows of Figure 1 using no apodization.

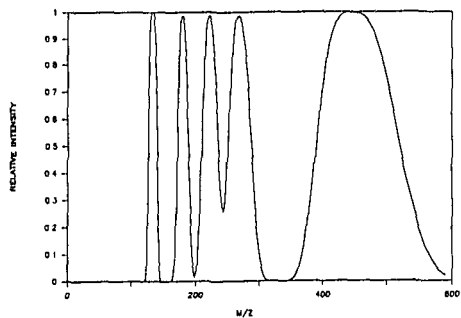


Figure 3: The expected response for a SWIFT waveform calculated for the mass windows of Figure 1 using a Blackman-Harris 3-term apodization window. Note the slightly reduced mass selectivity and the greatly reduced sidelobes.

A NEW METHOD FOR GENERATING SWIFT WAVEFORMS FOR FTMS

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Control of ion excitation in Fourier transform mass spectrometers has been greatly enhanced by the stored-waveform inverse Fourier transform (SWIFT) technique. The goal of the SWIFT technique is to achieve excitation selectivity superior to the detection selectivity of FTMS and to excite selected ions to known radii or kinetic energy. Here, we present a signal processing technique that overcomes some inherent problems and produces the desired excitation spectra.

In the original SWIFT technique^{1,2}, we convert the desired ion excitation profile into an excite amplitude spectrum, take the inverse Fourier transform, then apply the resulting waveform to a DAC which produces an analog voltage for the excite power amplifier. This produces waveforms with much higher selectivity than burst or chirp excitation, but can result in waveforms that require thousands of volts to produce ion excitation over a wide mass range. Phase scrambling has been proposed to reduce the peak excitation voltage. This causes distortion that results in poor control of ion radii, particularly in the case of broad band excite spectra that require large amounts of phase scrambling.

To simultaneously excite an ion to a chosen radius while leaving a neighboring ion with an arbitrarily low kinetic energy, that is, to maintain high selectivity, it is essential to window (apodize) the SWIFT waveform. This is necessary to remove the Gibb's oscillation that occur whenever a continuous spectrum is represented with a finite length waveform. However, windowing a SWIFT waveform that has been phase scrambled results in very severe distortion.

The distortion problem of phase scrambling followed by windowing is caused by time shifts introduced by the phase scrambling function. The group delay (time shift) of the phase function shifts the energy at each frequency past the end of the data set at one end. Windowing the result takes more energy away from some frequencies than others, depending on the amount of their time shift, and makes the distortion worse. We propose "expanded windowing" to achieve distortionless phase scrambling: window, adding enough zeros to each end of the window function to accommodate the maximum group delay of the phase function, then phase scramble. The resulting actual excite spectrum is shown in Figure 1. Note that the absence of Gibb's oscillations and the well-behaved band edges. Just as in the FTMS detection case, the side lobe ripples can be made arbitrarily low and the resolution arbitrarily high by the choice of window function and number of data points.

We can derive an optimal phase function for any arbitrary excite amplitude spectrum by using this knowledge. Starting with the definition of group delay as the negative of the derivative of phase with respect to frequency, and spreading the excite power uniformly in the time domain, we arrive at the following relation, which is general for any excite amplitude spectrum.

where $\theta(n\omega_s/N) = \text{phase (radians)}$

$$\text{Equation 1. } \theta(n\omega_s/N) = \frac{\pi}{N} \sum_{k=0}^{N-1} \frac{\omega_k}{\omega_s} \frac{d}{d\omega_k} \left(\frac{1}{\omega_k} \right) - \frac{\pi}{N} \sum_{k=0}^{N-1} \frac{d}{d\omega_k} \left(\frac{1}{\omega_k} \right)$$

$n = \text{integer frequency index}$

$\omega_s = \text{sampling frequency (radians/sec)}$

$N = \text{total number of data points}$

$n_d = \text{number of group delay points each end}$

$$S_d(X) = |F_d(jkX)|^2$$

$F_d(jkX) = \text{discrete excite voltage spectral density (V/Hz)}$

An excite amplitude spectrum phase-scrambled with this function approaches a sweep (chirp) function in its ability to deliver excite energy efficiently. Note that for a single rectangular excite band, the optimal phase function is a quadratic, confirming empirical results. The center (non-zero) part of the expanded window function consists of any standard window scaled to the appropriate length. We choose the length of this center section as the minimum length required to achieve the desired excite resolution. Figure 2 shows the SWIFT waveform resulting from the use of the optimal phase function Equation 1 on the spectrum of Figure 1 after expanded windowing. Note the extremely even power distribution across the waveform.

Two more problems remain: how do we scale a SWIFT waveform to excite ions to a given radius, and how do we know how much phase scrambling to use to reduce the peak voltage required to a given level? Since the SWIFT waveform is a voltage waveform, the excite amplitude spectrum is a voltage spectral density (Volts/Hz). We derive the relation between voltage spectral density and radius by analogy with the fixed frequency sine wave (burst) excite case. The result is,

Equation 2. $F_d(jk\Omega) = \frac{rBd}{-2T}$ where $F_d(jk\Omega)$ = discrete excite voltage spectral density (V/Hz)

$j = (-1)^{1/2}$
 k = integer index value
 Ω = frequency point spacing (rad/sec)
 r = orbital radius (meters)
 B = magnetic field flux density (Webers/meter²)
 d = distance between excite plates (meters)
 T = time point spacing (sec)

The effect of the optimal phase function (Eqn. 1) on the peak voltage of the resulting SWIFT waveform may be derived by assuming that energy is conserved during phase scrambling and nearly conserved during expanded windowing. Using Parseval's energy relation, we achieve a very good approximation of the amount of phase scrambling for a given amount of peak voltage reduction. Putting this all together, we arrive at the following process for producing a SWIFT waveform conforming to any arbitrary requested excite spectrum.

1. Specify the requested excite spectrum in terms of mass, radius (or kinetic energy) and resolution
2. Convert mass to frequency, convert radius (energy) to voltage spectral density
3. Inverse Fourier transform
4. Estimate phase scrambling required and window end segment length for distortionless phase scrambling; calculate window center segment length to achieve requested resolution; perform expanded windowing
5. Forward Fourier transform
6. Calculate optimal phase function, apply to spectrum
7. Inverse Fourier transform

CONCLUSIONS: Using the above process, we can produce SWIFT waveforms that satisfy arbitrary requested excite spectra. Any amount of peak voltage reduction can be achieved while maintaining any desired resolution. The only limitation is the number of data points and the total excite time required. The method works equally well for heterodyne and direct mode SWIFT excite.

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ACTUAL EXCITE SPECTRUM

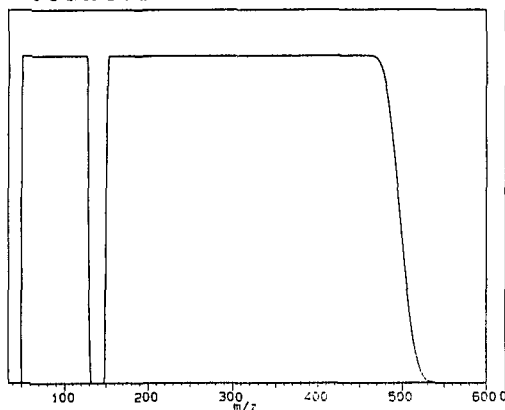


Figure 1. Actual excite spectrum of SWIFT waveform

SWIFT WAVEFORM

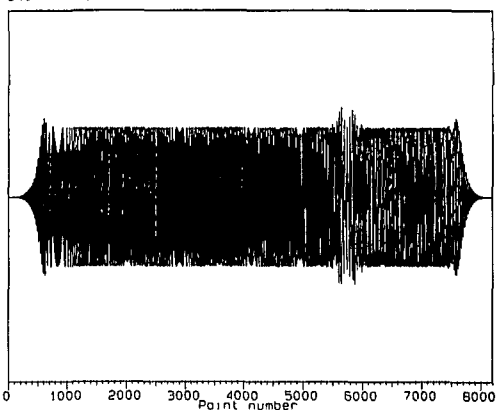


Figure 2. Optimally phase-scrambled SWIFT waveform

THEORY OF IMPULSE EXCITATION FOR FOURIER TRANSFORM MASS SPECTROMETRY

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Most Fourier transform mass spectrometers utilize a rapid radiofrequency sweep (chirp) to accelerate ions for detection. A mass spectrum is then obtained by monitoring the image current signals that are produced by the coherent motion of the ions. In impulse excitation Fourier transform mass spectrometry, ions are accelerated simultaneously by a sub-microsecond high voltage pulse. Unlike the rf-burst and the rf-chirp methods used previously, impulse excitation is not a resonance method, because all ions receive the same nonselective jolt from the high voltage pulse.

The equation of motion for a charged particle of mass m and charge q in electric (E) and magnetic (B) fields

$$m (d\mathbf{v}/dt) = q \mathbf{E} + q \mathbf{v} \times \mathbf{B}$$

can be solved using the impulse electric field in x -direction

$$E^{on} = (2U/L)(1 - e^{-at}) \quad (0 < t < T)$$

$$E^{off} = (2U/L)(1 - e^{-aT}) e^{-a(t-T)} \quad (t > 0)$$

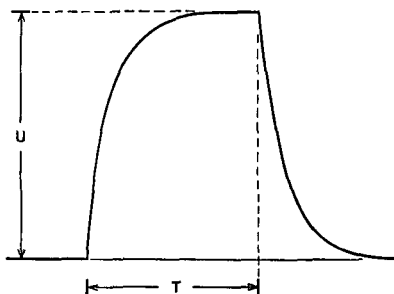


Fig. 1

for the turn-on and turn-off period of the pulse (Fig. 1) respectively. Here is U the potential applied to one transmitter plate, L the distance between the transmitter plates, a the risetime constant of the impulse and T the pulse width. Solutions of the differential equations of motion can be obtained using the continuity of the ion motion at the time of the turn-off ($t=T$) and the initial condition $v=0$ at the time $t=0$. Velocities and position coordinates of the ions for turn-on and turn-off times are calculated to be

$$v_x^{on}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w) \sin wt - \cos wt + e^{-at} \}$$

$$v_y^{on}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w) \cos wt + \sin wt + (w/a) e^{-at} - 2qU/(mLw) \}$$

$$x^{on}(t) = 2qUa/[mL(a^2 + w^2)] \{ -(a/w^2) \cos wt - (1/w) \sin wt - (1/a) e^{-at} + 2qU/(mLw^2) \}$$

$$y^{on}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w^2) \sin wt - (1/w) \cos wt - (w/a^2) e^{-at} - 2qU/(mLw) + 2qU/(mLwa) \}$$

for $0 \leq t \leq T$, and

$$v_x^{off}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w) [\sin wt - \sin w(t-T)] - \cos wt + \cos w(t-T) + e^{-at} - e^{-a(t-T)} \}$$

$$v_y^{off}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w) [\cos wt - \cos w(t-T)] + \sin wt - \sin w(t-T) + (w/a) [e^{-at} - e^{-a(t-T)}] \}$$

$$x^{off}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w^2) [\cos w(t-T) - \cos wt] - (1/w) [\sin wt - \sin w(t-T)] - (1/a) [e^{-at} - e^{-a(t-T)}] \}$$

$$y^{off}(t) = 2qUa/[mL(a^2 + w^2)] \{ (a/w^2) [\sin wt - \sin w(t-T)] - (1/w) [\cos wt - \cos w(t-T)] - (w/a^2) [e^{-at} - e^{-a(t-T)}] - 2qU/(mLw) \}$$

for $t > T$.

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The cyclotron radius of the ion after the impulse excitation can be obtained from the turn-off time velocities using the equation $r^{off}(t) = [(v_x^{off})^2 + (v_y^{off})^2]^{1/2} / w$ and the final radius of gyration can be calculated from its limit value for $t \rightarrow \infty$:

$$r_{final} = 2qUa/[mLw^2] \{(2 - 2\cos wT)/(a^2 + w^2)\}^{1/2}$$

The radius of gyration depends on the mass m , the cyclotron frequency w of the ion, the impulse peak potential U , risetime of the impulse signal $1/a$, its pulse width T and the distance L between the transmitter plates. If the period of the cyclotron motion is shorter than the pulse width of the impulse waveform, the ion will be first accelerated but then decelerated as it starts moving against the field. This can cause a collapse of the cyclotron motion, which can easily be seen from the final radius equation. The radius becomes zero if the term $2 - 2\cos wT = 0$, that is:

$$m_n = qBT/2n\pi \quad (n=1,2,3,\dots)$$

Fig. 2 is a diagram of the final radius of gyration versus the mass at $B = 1T$, $U = 120V$ and $a = 6.67 \times 10^7 \text{ s}^{-1}$. It also shows the masses, of which the cyclotron motion collapses. After the largest collapsing mass, $m = qBT/2\pi$, the curve approaches to an asymptotic value. This approach occurs at much lower masses, if the pulse width can be kept short enough. A limit case of the impulse excitation is that both the risetime of the pulse and the pulse width are very short. The equation for the final radius of gyration becomes $r = 2qUT/mLw$. By replacing w with qB/m and $2U/L$ with E , one obtains

$$r_{final} = ET/B.$$

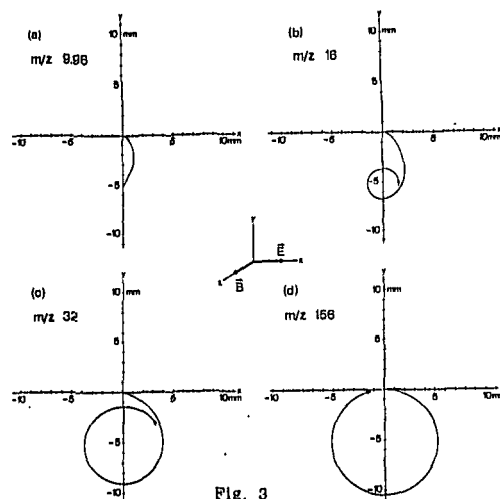


Fig. 3

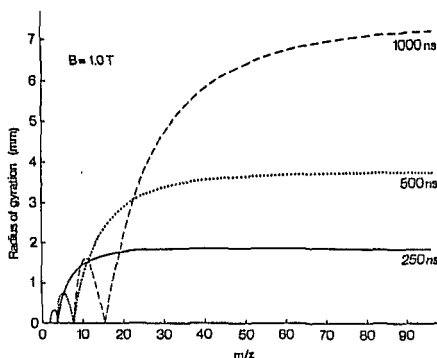


Fig. 2

Fig. 3 shows ion trajectories during impulse excitation at $T = 650 \text{ ns}$. The ion's center of gyration appears to be shifted to the negative y -direction, while it is excited by impulse. The constant term $-2qU/mLw$ in the equation of the velocity v_y^{off} helps understanding the shift of the center of gyration into the negative y -direction. For the time period T , this translation can be calculated as $y_T = -2qUT/mLw$. For ions with high m/z ratios, that is, with low cyclotron frequencies, the final radius of gyration can be approximated as $r_{final} = 2qUT/mLw$, which is the same value as the displacement of the center of gyration.

Trajectory calculations show, that all ions are accelerated to the same orbit, if the risetime of the impulse is very short and its duration less than the period of the ion's revolution in the cyclotron orbit. One of the limitations of impulse excitation is that double resonance ejections and sweep-out experiments, which are very useful in studying ion/molecule reactions, cannot be done, because it lacks the necessary selectivity. Yet, these selective resonance experiments can still be done in conventional manner, while impulse excitation is used for detection of ions. Experiments done with impulse excitation have shown, that it gives much more stable peak heights and does not require much "tuning" to obtain good quality transients.

INCORPORATION OF AVERAGED ION-ION INTERACTION INTO A MODEL FOR PEAK SHAPES IN FTMS

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Successful accurate exact mass measurement in Fourier transform mass spectrometry (FTMS) derives from a complete understanding of the ion resonant frequency's dependence on mass. The lack of total success in mass measurement through the use of the current mass calibration law¹ is apparently due to slight inaccuracies in the frequency-mass relationship upon which the current calibration law is based. These inaccuracies manifest themselves as ion number dependent mass measurement errors that change with excitation parameters, e^- beam parameters, and trap electric field shape.²

Computational models that give peak shape as a function of postulated conditions in the ion trap are useful in gaining insight into the mechanisms that affect an ion's apparent resonant frequency. This is because a distribution of ions of the same mass-to-charge ratio contribute a distribution of frequencies to a complex peak shape in actual practice. Such a model was used to explain the experimental observation of split peaks for a cubic trap and how compensating the cubic trap electric field removes this effect.³ This model has been revised to include the averaged interactions of ions.

Model assumptions include:

- No collisions during excitation and detection periods. The finite observation time, however, is included in the computation of the peak shape.
- Ions with the same z-mode amplitude are excited to the same cyclotron radius. The z-mode amplitude remains unchanged by the excitation.
- Each ion moves harmonically over a cylinder coaxial to the z axis with incommensurate frequencies (magnetron radius = 0.0).
- The z component of the forces between the ions is zero.
- Ions of the same species with the same cyclotron radius do not interact radially.
- The force between two ions with different cyclotron radii can be replaced with the force obtained by distributing the charge of one ion over its cylinder of motion so that the charge in an area of the cylinder is proportional to the time spent there.

A .0254 m, 304 SS cubic trap was used at $V_T = 1.0$ V. The magnetic induction was 1.2 Tesla. The experimental peak shapes were measured from the molecular ion of benzene, which was introduced at 5×10^{-8} Torr. Ionizing electron energy was 11.5 eV. The electron current was 160 nA and the beam time was varied from 15 ms to 1 s to change the ion number. An RF burst waveform with amplitude of .345 V_{b-p} and time of 700 μ s was applied to the molecular ion of benzene. The excitation was delayed 100 ms after the ionization to allow the ion cloud to approximate a thermal state.

Results obtained from the revised computational model show qualitative, experimentally observed features that have been attributed to ion-ion interaction. These features include-

- The elimination of peak splitting as the ion number is increased: compare, in Fig. 1, the peaks for 10000 and 140000 ions.
- The optimization and subsequent degradation of resolution as the ion number is increased. For the calculated peaks, the maximum amplitude on the graph is given per 1000 ions. For the measured peaks, the relative amplitude reads 1.0, 3.5, 4.96. Note that the experimental peak continues to increase in contrast to the calculation.
- The exaggeration of the high mass peak skirt for high ion number. Note that the degradation of the high mass skirt occurs more quickly for the calculated peak.

In addition, the calculation shows a shift in the ion population fraction that contributes to the main portion of the peak.

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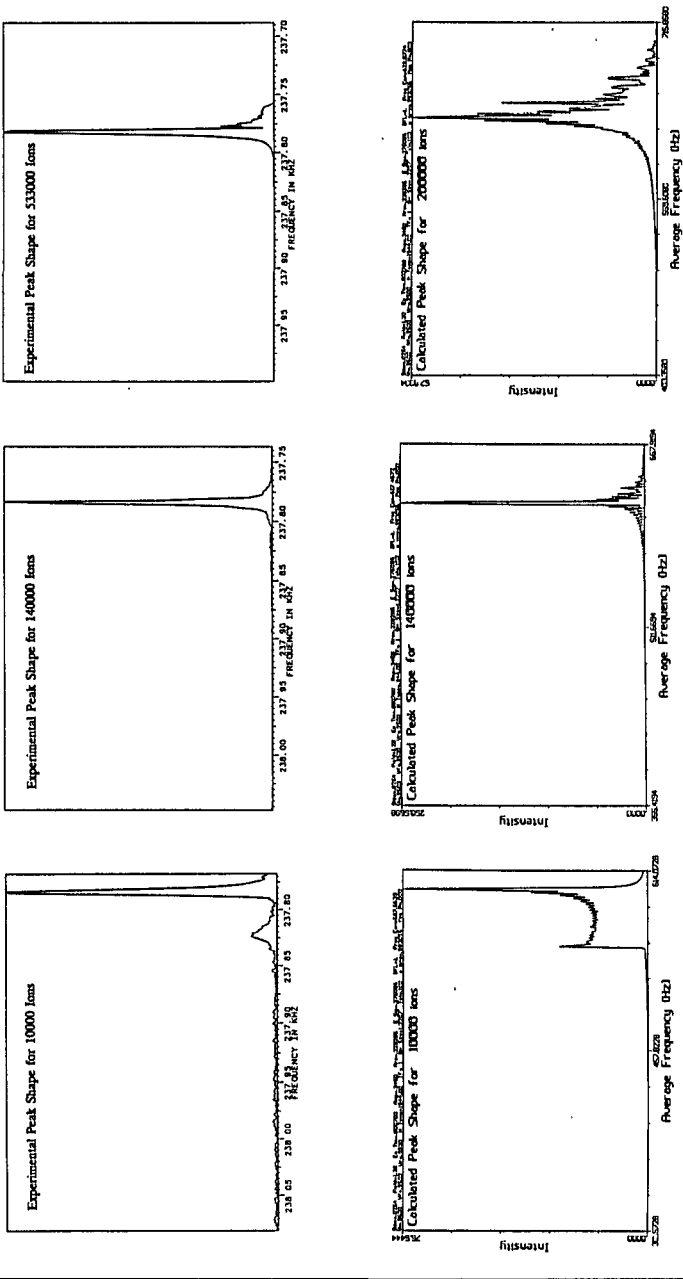


Figure 1. Comparison of experimental and computed peak shapes.

A MODEL FOR THE REDUCTION OF MASS MEASUREMENT ERRORS DUE TO ION-ION INTERACTION IN FTMS

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The current calibration law created by Ledford et al. (1) which produces ~ppm level mass measurement errors for low ion number shows systematic error with increasing ion number. Experimental evidence suggests that relative ion abundance should be incorporated into the calibration procedure (2). By using the theory of Jeffries, Barlow and Dunn (JBD) (3), corrections for the current calibration law have been derived from the assumption of a relationship between relative ion number and peak intensity with a calibration constant K.

Experiment parameters were: .0254 m of 304 SS compensated cubic trap -- V_T (inner segment) = 1.007 V, V_R (outer segment) = 1.705 V; magnetic field = 1.2 T; beam current = 100 nA; beam voltage -- 70 eV on acetophenone for mass calibrations, 12.5 eV on benzene and ethylbenzene for frequency shift experiments; rf burst -- amplitude = .345 V_{b-p} , excitation time = 700 us.

It is postulated that $G_{\rho i}$ (as in ref. 3) satisfies the propositions: G_{ρ} is proportional to N (beam time X beam current X pressure) and relative ratio of G_{ρ} = relative ratio of A (peak intensity). Hence, $G_{\rho i} = A_i N K$.

From JBD theory, the first order frequency perturbation by space charge is $\partial \omega_i / \partial G_{\rho i} = -q / B \epsilon_0$, if $i \neq j$; or $\alpha (-q / B \epsilon_0)$, if $i = j$. Experiment result from Fig. 1 shows that α is about 0.83 instead of 0.0 (by ref. 4).

Inputting the masses and frequencies of two calibrant ions to calibration equation: $m = a / w + b / w^2$ (1) would solve a and b. Then the calibrated mass of test ion is obtained by the equation with test ion frequency. Relative error (ξ) = $(M_{\text{calib.}} - M_{\text{ext.}}) / M_{\text{ext.}}$.

The slope (as in table I) of the curve (ξ vs. N) (as Fig. 2) can be shown as: $d\xi / dN = (\partial \xi / \partial \omega)(\partial \omega / \partial G_{\rho})(dG_{\rho} / dN)$. Since $dG_{\rho} / dN = AK$, calibration constant K is:

$$K = \frac{\left(\frac{d\xi}{dN}\right) m_{\text{ext.}} \omega_T^3 (\omega_1 - \omega_2)^2}{f(m_1, m_2, m_{\text{ext.}}, \omega_1, \omega_2, \omega_T, A_1, A_2, A_T) \left(-\frac{q}{B \epsilon_0}\right)}$$

Here, subscripts 1,2 and T are represented for calibrant ion 1, 2 and test ion, respectively. Also, f which replaces a complicated expression from derivation is a function of masses, frequencies and peak intensities of the two calibrant ions and the test ion.

Once K is obtained, G_{ρ} is determined, frequency shift from space charge could be calculated. And, frequencies unperturbed by space charge would be obtained. Then space-charge-unperturbed calibrated mass is obtained by recalibration with these frequencies. In table II, the overall average value of K for the four sets is $0.911 \times 10^{17} \pm 0.189 \times 10^{17}$ (20.7 percent). With the result of K varying by 15 to 25 percent, it is expected that a factor of 4 to 6 reduction in ion-ion-interaction-caused errors could be obtained.

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Fig. 1

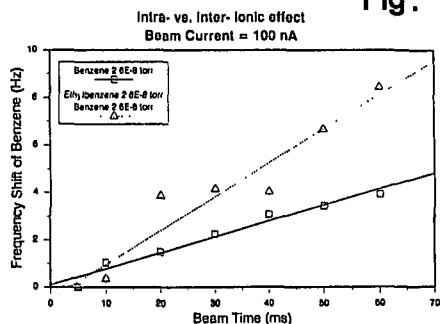


Fig. 2

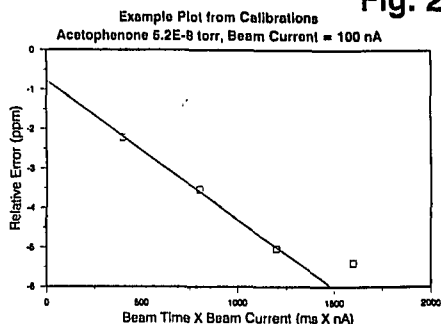


Table I

Combination	Slope ($\times 10^{-3}$)	Average ($\times 10^{-3}$)	Zero intercept	Average
[51 77 105]	-0.130	-2.087 ± 1.090	-0.587	-1.514 ± 0.958
	-2.961		-0.324	
	-1.516		-2.920	
	-2.744		-2.000	
	-2.804		-1.822	
[51 77 120]	-2.364	-4.485 ± 0.938	-1.431	-3.440 ± 2.247
	-3.513		-0.787	
	-5.913		-6.608	
	-4.188		-3.152	
	-3.940		-4.522	
[51 105 120]	-4.873	-7.761 ± 1.114	-2.100	0.295 ± 3.570
	-6.816		-6.109	
	-8.491		2.237	
	-8.433		0.961	
	-6.353		-0.252	
[77 105 120]	-7.265	-4.800 ± 1.516	0.372	-0.064 ± 2.357
	-9.208		4.559	
	-6.300		-3.834	
	-5.018		2.311	
	-3.574		-0.573	
	-2.930		0.501	
	-6.178		1.276	

Table II

Combination	K	Average ($\times 10^{17}$)
[51 77 105]	1.138×10^{17}	1.194 ± 0.297
	1.455×10^{17}	
	1.508×10^{17}	
	0.780×10^{17}	
	1.091×10^{17}	
[51 77 120]	0.836×10^{17}	0.822 ± 0.098
	0.910×10^{17}	
	0.682×10^{17}	
	0.859×10^{17}	
[51 105 120]	0.821×10^{17}	0.804 ± 0.144
	0.812×10^{17}	
	1.029×10^{17}	
	0.702×10^{17}	
	0.658×10^{17}	
[77 105 120]	0.581×10^{17}	0.824 ± 0.261
	0.606×10^{17}	
	1.030×10^{17}	
	1.162×10^{17}	
	0.974×10^{17}	
	0.589×10^{17}	

Elimination of z-Ejection in FT/ICR by rf Electric Field Shimming

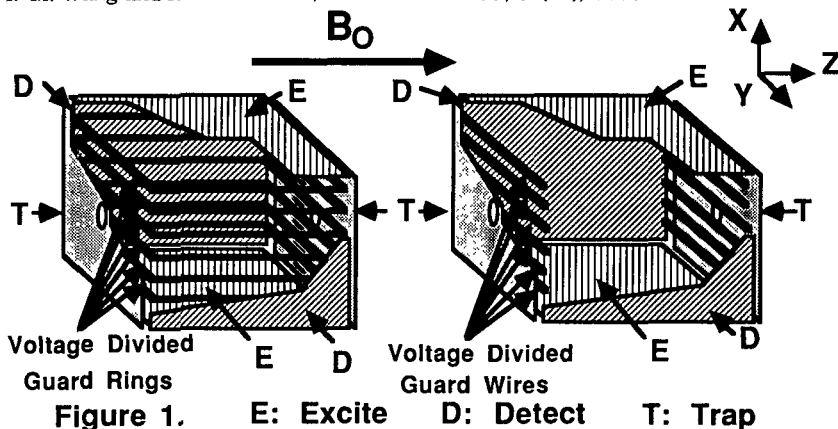
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In FT/ICR mass spectrometry, coherent ion cyclotron orbital motion is produced by resonant rf excitation. However, because the excitation electrodes are of finite dimensions, the desired transverse (to the applied magnetic field) rf field is accompanied by an rf field along the z- (magnetic field) direction, resulting in mass-dependent z-ejection and mass-dependent relative FT/ICR peak relative magnitudes. Addition of several "guard wires" of voltage-divided rf amplitude¹⁻³ allows the rf electric field to be "shimmed" to near-perfect uniformity (right).

Here we present two types of rf-shimmed ion traps. In the first type (Fig. 1, right), "guard wires" are placed only in front of the trapping electrodes. In the second type (Fig. 1, left), "guard wire rings" are placed inside the detector and trapping electrodes. For either arrangement, SIMION simulations were used to adjust the rf voltages applied (by voltage division) to the guard wires or rings so as to produce optimally uniform rf field within the trap. The virtual elimination of z-excitation is confirmed by the plots of magnitude-mode relative peak height vs. ICR orbital radius in Figure 2. Because the guard wires (or rings) tend to shield the ions from the trapping electrode potential, the shift in ICR frequency with trapping voltage is also reduced (Figure 3), but not as well as with a screened trap.⁴ In side-by-side tests of conventional and rf-shimmed traps mounted colinearly along the z-axis, the ICR signal strength was reduced to ~87% and ~25% by the addition of guard wires or guard rings, respectively. The authors thank Dr. Russell for sharing his unpublished results. This work was supported by NSF (CHE-8721498) and The Ohio State University.

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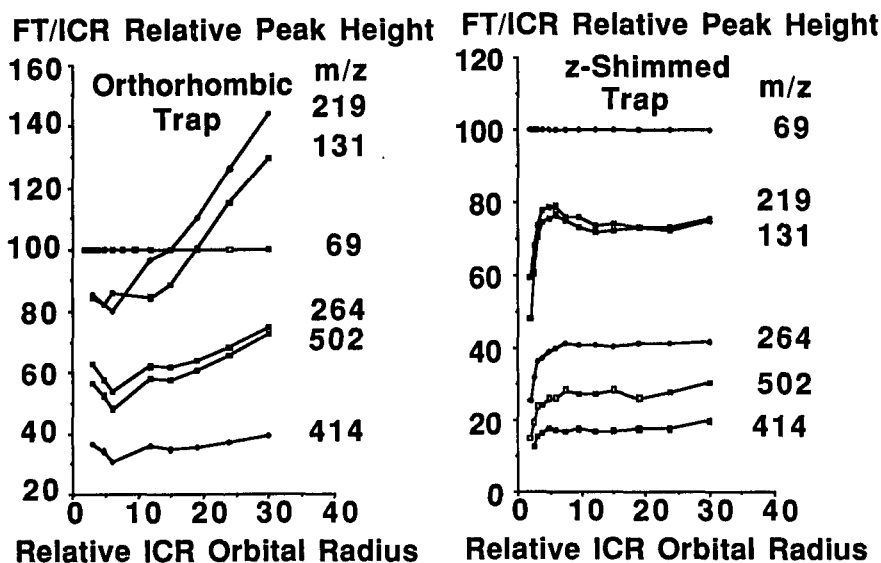


Figure 2.

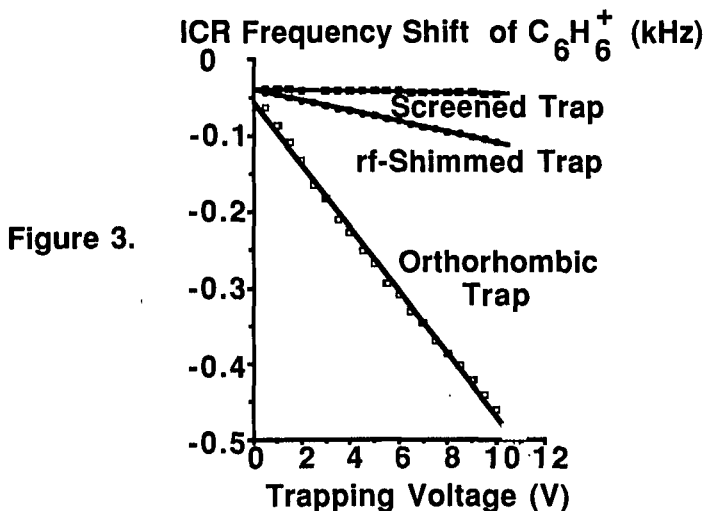


Figure 3.

**A "Screened" Electrostatic Ion Trap for Enhanced Mass Resolution,
Mass Accuracy, Reproducibility, MS/MS, and Upper Mass Limit in
Fourier Transform Ion Cyclotron Resonance Mass Spectrometry**

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Until now, it was thought that the optimal static electromagnetic ion trap for Fourier transform ion cyclotron resonance (FT/ICR) mass spectrometry should be designed to produce a *quadrupolar* electrical potential, for which the ion cyclotron frequency is independent of the ion's pre-excitation location within the trap. However, a quadrupolar potential results in a transverse (to the magnetic field) electric field which increases linearly with distance from the center of the trap. That radially linear electric field shifts the observed ICR frequency, increases the ICR orbital radius, and ultimately limits the highest mass-to-charge ratio ion which can be contained within the trap.

In this abstract, we present a new static electromagnetic ion "trap" in which screens which are grounded (or set to a specified voltage) placed just inside the usual "trapping" plates produce an electric potential which is *near-zero* almost everywhere within the trap.¹ The static electric field within the trap is reduced by more than an order of magnitude, as shown by the SIMION isopotential contours of Figure 2.

For experimental comparisons, we constructed a two-section trap (Figure 1) consisting of screened and unscreened (but otherwise identical) orthorhombic traps mounted colinearly end-to-end along the magnetic field axis.

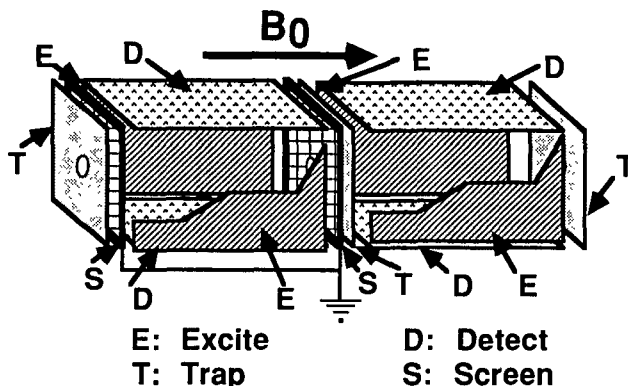


Figure 1. Dual Screened and Unscreened Ion Traps

For the screened orthorhombic (2.5" x 2" x 2") trap, the experimental ICR frequency shift due to trapping voltage is reduced by a factor of ~60 compared to an unscreened conventional orthorhombic (2.5" x 2" x 2") ion trap (see Figure 3). The ICR signal induced on the detector electrodes of a screened trap is reduced to about 72% of that for the same-size unscreened trap. (Those measurements represent an average of the results obtained with the two traps placed either as shown in Figure 1 or interchanged left-to-right, to make sure that the effect was not due simply to the location of a given trap in the magnet.) As will be documented elsewhere,¹ the new screened trap offers higher mass resolution (and accuracy) and reproducibility during *detection* (due to more uniform electric field); higher mass resolution during *excitation* (since ICR frequency no longer varies significantly with ICR orbital radius) for improved MS/MS; and potentially much higher upper mass limit (since the radial electric field has been eliminated) than prior electromagnetic ion traps.

We acknowledge P. B. Grosshans for independent development of a similar idea. This work was supported by the National Science Foundation (CHE-8721498) and The Ohio State University.

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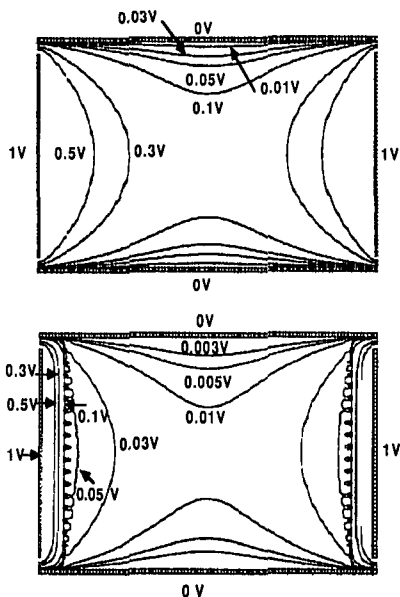


Figure 2. Isopotential Contours of Unscreened (Top) and Screened (Bottom) Traps

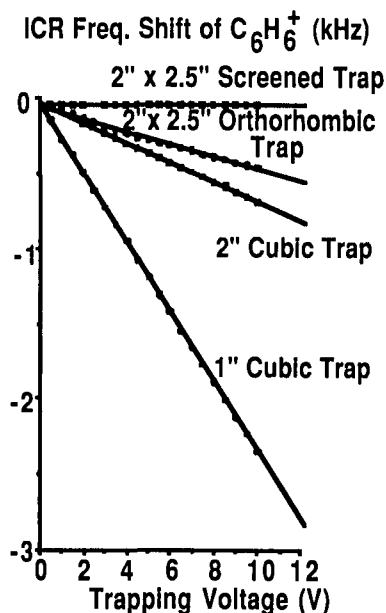


Figure 3.

Solution of the Equations of Ion Motion During the Ion Cyclotron Resonance Excitation Event

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In the past, the rate at which an ion's radius increases during the excitation event has been calculated as if the excitation plates were infinite in extent; this approximation overestimates the power input to the ion's motion owing to the presence of the detection and trapping plates. Here we have solved Laplace's Equation for the electrostatic (in the low frequency limit) potential exactly during the excitation event and expanded this solution in a Taylor series about the center of the ion trap. From this approximate potential, the equations of motion are then solved exactly with quite general initial conditions. The expression for the rate at which the cyclotron radius increases is of the same form as in the earlier model (the infinite plate model) with the exception of a correction factor. We conclude that the earlier model overstates the rate at which an ion's radius increases by 39% for a cubic ion trap.

We assume that the time-dependent potential field, $V(x,y,z,t)$, propagates instantaneously throughout the cell—a very accurate approximation for non-relativistic ion velocities. Let the magnetic field, $\mathbf{B}$, define the z -axis and let the trap have a square cross-section transverse to this axis; with electrodes at $x = \pm a/2$, $y = \pm a/2$, and $z = \pm L/2$; we then solve Laplace's Equation for $V(x,y,z,t)$:

$$\nabla^2 V(x,y,z,t) = 0 \quad \text{where} \quad \nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \quad (1)$$

with the boundary conditions:

$$V(\pm \frac{a}{2}, y, z, t) = 0 \quad V(x, \pm \frac{a}{2}, z, t) = \pm \frac{V_{pp}}{2} \sin \omega_+ t \quad V(x, y, \pm \frac{L}{2}, t) = V_t$$

We have chosen the plates at $y = \pm a/2$ to be the excitation plates and we employ a single frequency excitation waveform at the modified cyclotron frequency, ω_+ , with peak-to-peak voltage, V_{pp} . Because ∇^2 is a linear operator, any linear combination of solutions to Eq. 1 is also a solution. Therefore, we can break this problem into two much simpler problems as summarized by the following equations:

$$V(x, y, z, t) = V_1(x, y, z) + V_2(x, y, z, t)$$

$$\nabla^2 V_1 = 0 \quad V_1(\pm \frac{a}{2}, y, z) = V_1(x, \pm \frac{a}{2}, z) = 0 \quad V_1(x, y, \pm \frac{L}{2}) = V_t \quad (2)$$

$$\nabla^2 V_2 = 0 \quad V_2(\pm \frac{a}{2}, y, z, t) = V_2(x, y, \pm \frac{L}{2}, t) = 0 \quad V_2(x, \pm \frac{a}{2}, z, t) = \pm \frac{V_{pp}}{2} \sin \omega_+ t$$

The solutions are:

$$V_1 = \frac{16V_t}{\pi^2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n} \cos\left[\frac{(2m+1)\pi x}{a}\right] \cos\left[\frac{(2n+1)\pi y}{a}\right] \cosh\left[\frac{\kappa_{mn}\pi z}{a}\right]}{(2m+1)(2n+1) \cosh\left[\frac{\kappa_{mn}\pi L}{2a}\right]}$$

$$V_2 = \frac{8V_{pp} \sin \omega_+ t}{\pi^2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n} \cos\left[\frac{(2m+1)\pi x}{a}\right] \cos\left[\frac{(2n+1)\pi z}{L}\right] \sinh\left[\frac{\kappa_{mn}\pi y}{a}\right]}{(2m+1)(2n+1) \sinh\left[\frac{\kappa_{mn}\pi}{2}\right]} \quad (3)$$

where $k_{mn} \equiv \sqrt{(2m+1)^2 + (2n+1)^2}$ and $\kappa_{mn} \equiv \sqrt{(2m+1)^2 + \frac{a^2}{L^2} (2n+1)^2}$

$V(x,y,z,t)$, from Equations 2 and 3, may be expanded about the center of the trap by use of the three variable Taylor series; neglecting terms of order 3 and higher, we have:

$$V(x,y,z,t) \approx V_1 \left[\gamma - \frac{\alpha}{2a^2} (x^2 + y^2 - 2zz^2) \right] + \frac{\beta V_{pp} \sin \omega_+ t}{a} y \quad (4)$$

$$\text{where } \alpha \equiv 16 \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n} (2m+1)}{(2n+1) \cosh \left[\frac{k_{mn} \pi L}{2a} \right]} \quad \beta \equiv \frac{8}{\pi} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n} \kappa_{mn}}{(2m+1)(2n+1) \sinh \left[\frac{\kappa_{mn} \pi}{2} \right]}$$

$$\text{and } \gamma \equiv \frac{16}{\pi^2} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{(-1)^{m+n}}{(2m+1)(2n+1) \cosh \left[\frac{k_{mn} \pi L}{2a} \right]}$$

These series for α , β , and γ converge rapidly; their functional dependence on the aspect ratio, L/a , of the trap is plotted in the figure below. The electric field, $\mathbf{E}$, is derived from this approximate potential and is then substituted into the Lorentz Force Equation

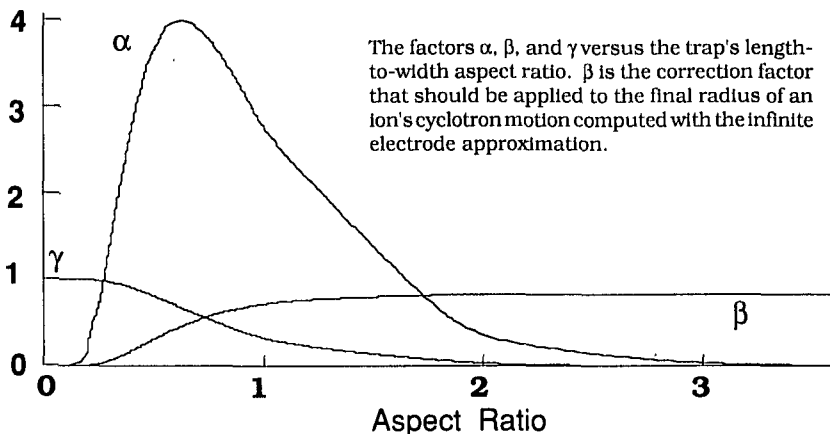
$$\mathbf{F} = m\mathbf{a} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B}) \quad (5)$$

The differential equations of motion may then be solved by the Laplace Transform Method; we find that the cyclotron radius, ρ_c , increases at a rate given by:

$$\frac{\partial \rho_c}{\partial t} \approx \beta \frac{V_{pp}}{aB} \quad \text{whereas the infinite electrode approximation has} \quad \frac{\partial \rho_c}{\partial t} \approx \frac{V_{pp}}{aB} \quad (7)$$

The factor β is the only difference. It has a maximum value of 0.835 and a minimum value of 0; for a cubic trap, this factor is 0.72. Thus, the older formula overestimates the rate of radius increase by at least 20%, and by 39% for a cubic trap.

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A New Method for Calculating the Signal Induced on ICR Detector Plates

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An analytic solution for the signal induced in on the detection plates of an FT/ICR cell can be derived for both rectangular and cylindrical geometries from basic electrostatics. With the assumption that the motion of the ions is non-relativistic, the potential within the cell is given by¹:

$$\Phi(\mathbf{r}) = \int_{V_c} \frac{\rho_f(\mathbf{r}') G(\mathbf{r}, \mathbf{r}')}{4\pi\epsilon_0} d\mathbf{r}' + \int_{S_c} \frac{d\mathbf{a}'}{4\pi} \left[G(\mathbf{r}, \mathbf{r}') \frac{\partial \Phi(\mathbf{r}')}{\partial n'} - \Phi(\mathbf{r}') \frac{\partial G(\mathbf{r}, \mathbf{r}')}{\partial n'} \right] \quad (1)$$

where V_c is the volume of the cell, S_c is the surface defined by the inner faces of the electrodes, ρ_f is the ionic charge density, ϵ_0 is the permittivity of free space, n' is the outward normal coordinate at the cell boundary, and the primed coordinates are integration variables. If we further assume that the potential on the plates is known (Dirichlet boundary conditions), then the Green function, $G(\mathbf{r}, \mathbf{r}')$, appropriate for the rectangular cell with plates at $x = 0, a$; $y = 0, b$; and $z = 0, c$; is given by:

$$G(\mathbf{r}, \mathbf{r}') = \frac{32}{\pi abc} \sum_{l,m,n=1}^{\infty} \frac{\sin\left(\frac{l\pi x}{a}\right) \sin\left(\frac{l\pi x'}{a}\right) \sin\left(\frac{m\pi y}{b}\right) \sin\left(\frac{m\pi y'}{b}\right) \sin\left(\frac{n\pi z}{c}\right) \sin\left(\frac{n\pi z'}{c}\right)}{\left(\frac{l}{a}\right)^2 + \left(\frac{m}{b}\right)^2 + \left(\frac{n}{c}\right)^2} \quad (2)$$

For the cylindrical cell the Green function is given by:

$$G(\mathbf{r}, \mathbf{r}') = \frac{8}{a^2 L} \sum_{m=0}^{\infty} \sum_{l,n=1}^{\infty} \frac{\epsilon_m \cos[m(\phi - \phi')] J_m\left(\frac{x_{ml}\rho}{a}\right) J_m\left(\frac{x_{ml}\rho'}{a}\right) \sin\left(\frac{n\pi z}{L}\right) \sin\left(\frac{n\pi z'}{L}\right)}{J_{m+1}^2(x_{ml}) \left[\left(\frac{x_{ml}}{a}\right)^2 + \left(\frac{n\pi}{L}\right)^2 \right]} \quad (3)$$

$\epsilon_m \equiv \begin{cases} 1 & \text{for } m=0 \\ 2 & \text{for } m \neq 0 \end{cases}$ and x_{ml} is the l^{th} zero of J_m , the Bessel function of order m .

One is usually interested in the differential charge induced between opposed detection plates by an ion of charge q and position, $\mathbf{r}_1$. For each detection plate one computes the induced surface charge density, σ , from

$$\sigma(\mathbf{r}, \mathbf{r}_1) = \epsilon_0 \frac{\partial \Phi(\mathbf{r}, \mathbf{r}_1)}{\partial n} = -\epsilon_0 E_n(\mathbf{r}, \mathbf{r}_1) \quad (4)$$

This charge density is then integrated over the surface of each detection plate to yield the total induced charge. The difference in induced charge between these plates is the required differential charge, ΔQ_1 . If the detection plates are those located at $y = 0$ and b , the result for the rectangular cell is:

$$\Delta Q_1(\mathbf{r}_1) = \frac{128q}{\pi^3 b^2} \sum_{l,m,n=1}^{\infty} \frac{m \sin\left[\frac{(2l-1)\pi x_1}{a}\right] \sin\left[\frac{2m\pi y_1}{b}\right] \sin\left[\frac{(2n-1)\pi z_1}{c}\right]}{(2l-1)(2n-1) \left[\left(\frac{(2l-1)}{a}\right)^2 + \left(\frac{2m}{b}\right)^2 + \left(\frac{(2n-1)}{c}\right)^2 \right]} \quad (5)$$

Similarly, for a cylindrical cell with trapping plates at $z = 0$ and L and radius, a , and with the ring electrode segmented into four equal quadrants:

$$\Delta Q(r_l) = \frac{-16q}{a^2 \pi^2} \sum_{l,m,n=1}^{\infty} \frac{\{(-1)^m \cos(m'\phi_l) - \sin(m'\phi_l)\} x_{m',l} J_{m'}\left(\frac{x_{m',l} \rho_l}{a}\right) \sin\left[\frac{(2n-1)\pi z_l}{L}\right]}{m'(2n-1)J_{m'+1}(x_{m',l}) \left[\left(\frac{x_{m',l}}{a}\right)^2 + \left(\frac{(2n-1)\pi}{L}\right)^2\right]} \quad (6)$$

where $m' \equiv 2m+1$. For N ions, the net differential charge induced is simply the linear superposition of the individual induced charges. Assuming that the detection circuit is essentially capacitive in the frequency range of interest with equivalent capacitance C_{eq} , the induced voltage, $V(t)$, between the detection plates is simply the differential charge divided by C_{eq} . Comisarow³ has derived an expression for the charge induced on one of the detection plates as a function of ion position; he finds

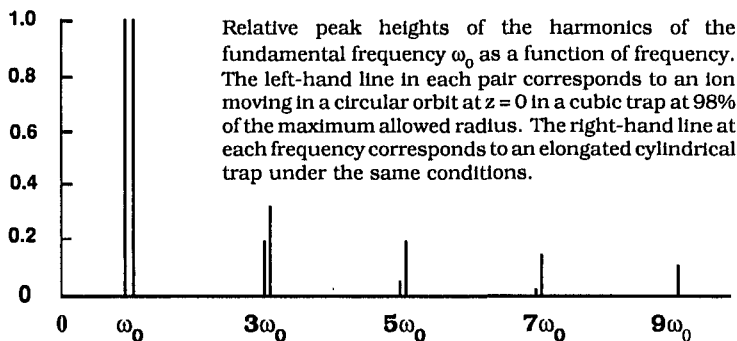
$$\Delta Q(r_l) = \frac{qy_l}{b} \quad (7)$$

where y_l is the y -coordinate of the ion's position and b is the spacing between the detection plates as above. Upon comparing Equation (7) to Equation (5), we see that Comisarow's approximation oversimplifies the problem. Indeed, when this result is put into the form of Equation (7), it is easy to see that it is equivalent to the infinite electrode approximation. Finally, we note that for an ion moving in a circular orbit of angular frequency, ω , and radius, r , from the z -axis, both Equations (5) and (6) can be put into the following form:

$$\Delta Q(t) = q \sum_{j=1}^{\infty} A_j \cos[(2j-1)\omega t + \phi_j] \quad (8)$$

Thus, the appearance of signals at odd harmonics of the fundamental frequency is predicted in a natural way by our theory (see figure below). This work was supported by the National Science Foundation (CHE-8721498) and The Ohio State University.

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Precision in Determination of Relative Ion Abundances from FT/ICR Mass Spectra: Absorption Peak Area is Superior to Peak Height or Complex Area

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In FT/ICR mass spectra, phase-corrected absorption-mode peak area is a direct measure of the number of detected ions of that ICR frequency, provided that the time-domain signal acquisition period, $T \gg \tau$, where τ is the time-domain damping constant. In this abstract, we discuss four methods for estimation of absorption-mode peak area: (a) direct integration or peak-fitting to the (phase-corrected) absorption-mode spectrum; (b) sum of the areas of the real and imaginary components of the complex FFT spectrum ("complex area");¹ (c) absorption-mode peak area obtained as a parameter from non-linear least-squares fit to the magnitude-mode spectrum; and (d) absorption-mode peak area obtained as a parameter from non-linear least-squares fit to the (phase-corrected) absorption-mode spectrum. We find that method (c) is best.

Precision as a function of signal-to-noise ratio and number of data points

It has previously been shown that the precision, $P(a)$, of spectral parameter, a (a = peak height, peak width, or peak center frequency), determined by non-linear least-squares fit to a single frequency-domain spectrum, is given by:^{2,3}

$$P(a) = c(a) \text{ SNR} \sqrt{K} \quad (1)$$

in which SNR is frequency-domain spectral peak height-to-noise ratio, K is the number of data points per (full) line width at half-maximum peak height, and $c(a)$ is a line shape-dependent constant.

Frequency-domain spectral precision

In Figures 1-2, we show that Eq. 1 can be extended to the determination of absorption-mode peak area, for various area-measuring means. When phase correction is difficult (as in FT/ICR), magnitude-mode peak height or even magnitude-mode peak area (mathematically non-integrable for Lorentzian peak shape) are often used in place of absorption-mode peak area.

We have discovered that absorption-mode peak area can be recovered from magnitude-mode as well as from (phase-corrected) absorption-mode spectra by least-squares fit. Interestingly (see Figures 1,2), higher precision is obtained by determination of absorption-mode peak area from non-linear least-squares fit to the magnitude-mode FT spectrum than by similar direct fits to the absorption-mode (or complex) FT spectrum.

Time-domain precision

Frequency-domain absorption-mode peak area is a measure of time-domain signal initial magnitude (which in turn is directly related to the number of detected ions in an FT/ICR experiment. Eq. 1 may be extended to the determination of time-domain signal initial magnitude, in which SNR and K are replaced by time-domain signal-to-noise ratio, snr , and number of data points, k , per time-domain damping period, τ .

Relation between time- and frequency-domain precision: effect of zero-filling

From computer-simulated data, we find that the precision of spectral parameters determined by least squares fit to a noisy single-frequency *time-domain* signal is higher than that determined by least squares fit to the corresponding *frequency domain* spectrum. One time-domain zero-fill before FFT increases the precision of the frequency domain fit to the same level as that from the time-domain data. Further zero-fills do *not* improve frequency-domain precision.

Experimental tests of theory

Figures 3 and 4 offer experimental FT/ICR mass spectral examples of the computer-simulated results shown in Figures 1 and 2. Figure 3 also includes precision based on magnitude-mode peak height (obtained from least-squares fit to magnitude-mode spectrum). Clearly, the theoretical predictions tend to overestimate the precision realized experimentally. The results nevertheless qualitatively support our theoretical predictions and show that absorption peak area (computed from best-fit to a magnitude-mode spectrum) offers the most precise measure of relative ion abundances from unphased FT/ICR mass spectra. This work was supported by N.S.F. (CHE-8721498) and The Ohio State University.

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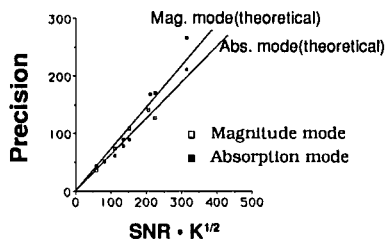


Figure 1

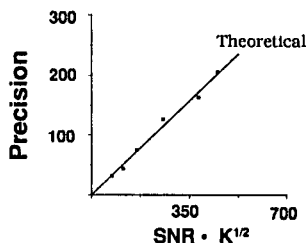


Figure 2

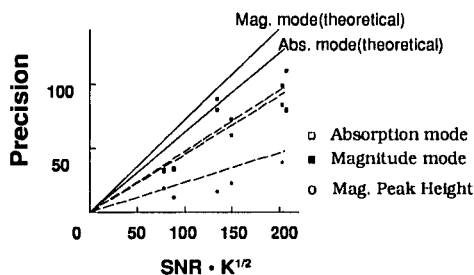


Figure 3

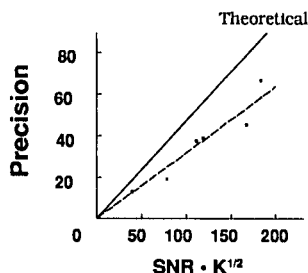


Figure 4

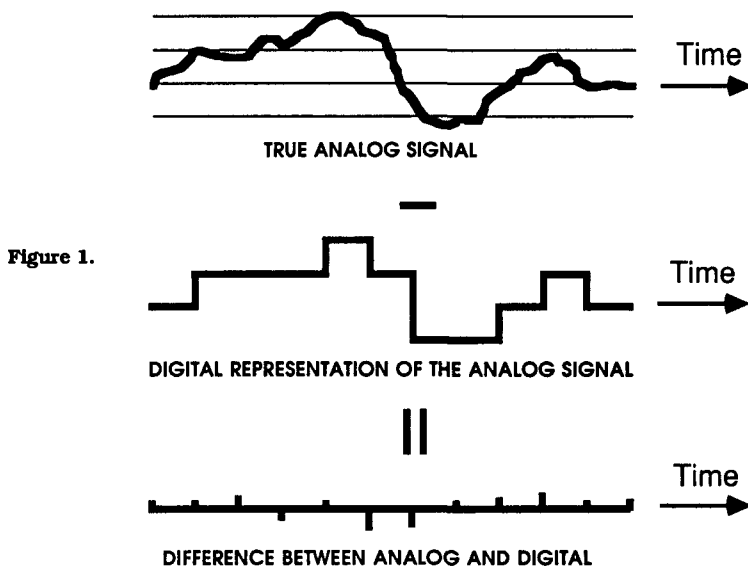
EFFECT OF SAMPLING RATE ON FT/ICR MASS SPECTRA: IS OVERSAMPLING OVERRATED?

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In Fourier transform spectrometry, an analog time-domain signal is sampled at equally spaced intervals and subjected to a discrete Fourier transform to yield a discrete frequency-domain spectrum. The Nyquist theorem requires that the time-domain signal be sampled at least twice per cycle of the highest frequency in the spectral bandwidth. It is well known that *under-sampling* (i.e., sampling at less than the Nyquist frequency) results in "foldover" ("aliasing") of signals whose frequencies exceed the Nyquist limit. Undersampling can in fact be used to advantage for simultaneous detection of ions of widely separated m/z values at ultrahigh resolution, by use of multiple foldover (1). However, *over-sampling* (i.e., sampling faster than twice the spectral bandwidth) has received much less attention. In this work, we discuss the effects and advantages of oversampling for FT/ICR mass spectra (2).

First, it is important to understand that the sampling process itself produces time-domain amplitude errors which appear as frequency-domain "quantization noise", as shown in Figure 1. An analog-to-digital converter (ADC) must assign each analog time-domain value to one of a finite number of (discrete) amplitude values. Thus, the ADC effectively "rounds off" the true analog signal to the nearest available discrete ADC



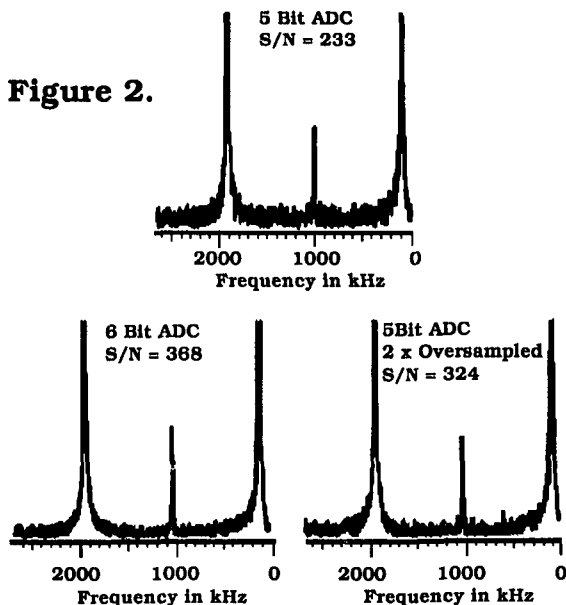
amplitude. The differences (errors) between discrete and analog values define a time-domain error ("quantization noise") signal.

Figure 2 (top) shows the frequency-domain spectrum produced by discrete FT of a Nyquist-sampled *noiseless* analog signal. The vertical scale has been expanded to show the quantization noise. Quantization noise may be reduced in two ways. First, one can (say) double the number of bits per ADC word, so that the average time-domain amplitude sampling error from the same analog time-domain signal is reduced, and the corresponding frequency-domain spectral signal-to-noise ratio increases by a factor of about $\sqrt{2}$ (see Figure 2, bottom left). Alternatively, one can *oversample* the time-domain signal by (say) a factor of two. Although the average time-domain amplitude sampling error for each time-domain data point is the same as before, the time-domain signal is sampled twice as often, so that the frequency-domain spectral bandwidth after FT is doubled. In effect, the quantization noise is "spread out" over twice the frequency range, and its rms magnitude in the original bandwidth of interest is reduced by a factor of about $\sqrt{2}$ (Figure 2, bottom right).

In practice, the *absolute* quantization error magnitude generated is very small. (≤ 1 part in $\sim 8,000$ for a 12-bit ADC). However, a practical advantage of oversampling is that the analog low-pass filter bandwidth can be made larger than the desired spectral bandwidth (since oversampling eliminates foldover from signals above the desired bandwidth), thereby reducing amplitude and phase distortion in the spectral bandwidth of interest.

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ISOTOPE-RATIO MEASUREMENTS BY FT/ICR: ESTIMATING INITIAL
AMPLITUDES AND DAMPING CONSTANTS FROM SEGMENTED TIME SERIES

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Isotope geochemists routinely correct for instrumental fractionation effects in thermal ionization mass spectrometry and SIMS by a simple mass-dependent model. For Kr isotopes, for example, the model would predict that an isotope-ratio plot such as Fig. 1 should be linear according to:

$$\frac{(^{82}\text{Kr}/^{84}\text{Kr})_m - (^{82}\text{Kr}/^{84}\text{Kr})_t}{(^{82}\text{Kr}/^{84}\text{Kr})_m - (^{82}\text{Kr}/^{84}\text{Kr})_t} = \frac{(^{82}\text{Kr}/^{82}\text{Kr})_t}{82 - 84}$$

where the subscripts m and t refer to measured and true (or corrected) ratios, respectively, and the second term on the right is the ratio of the mass differences. For atmospheric Kr, $(^{82}\text{Kr}/^{82}\text{Kr})_t \approx 1$, so that an array of fractionated measurements should be linear with slope 1/2 and pass through the point $[(^{82}\text{Kr}/^{84}\text{Kr})_t, (^{82}\text{Kr}/^{84}\text{Kr})_t]$.

The FTMS data in Fig. 1 were collected at two different pressures by successively increasing the duration of the electron beam (BEM), so that the arrow marks an increasing number of ions. Obviously at low ion number the isotope ratios fall at or near the true values and move away with increasing BEM, with behavior becoming non-linear at the higher pressure presumably due to space-charge effects. The slope of the linear portions of these plots is ~1, however, rather than 1/2 as predicted for mass fractionation. We have observed similar behavior for laser desorption/ionization of solids, e.g., Ti metal, in which the isotope fractionation pattern linearly favors the most abundant mass. This suggests a damping mechanism in which the dominant isotopes survive longest in the transient and are thus favored in peak amplitudes. Empirically, this fractionation approximately follows $[I_1/I_2]_m = [I_1/I_2]_t * [I_2/I_2]_m$, where I_2 is the concentration of the most abundant isotope.

We can estimate for each mass the decay constants and amplitudes (at end of excitation) by segmentation of the signal. By dividing the original transient into shorter time series as a function of the time of the first data point of each segment, T_i , a plot of the log of the FT magnitude peak height, $\ln[M]$, vs. T_i is linear assuming that the transient is an exponentially decaying sinusoid. It can then be shown that

$$\ln[M] = -DT_1 + \ln\left\{ (A/2) \int_0^T g(t) \exp(-Dt) dt \right\}, \quad (1)$$

where M, A, D, τ , and $g(t)$ are the FT magnitude peak height, amplitude (at $T_i = 0$), decay constant, segment length, and apodization function, respectively.

We have applied this segmenting procedure to Kr in a set of experiments where BEM was varied from 2.5 ms to 25.0 ms in increments of 2.5 ms at a background pressures of 3.3×10^{-7} T with trapping voltage of 9.0 V. Other experimental parameters include: number of data points=32K, number of sweeps=1000, beam voltage=-50 V, beam current=1 A, sweep rate=3.2 KHz,

excitation voltage=75 V_{pp} (full bandwidth), bandwidth= 2.667 MHz. The transient was divided into 20 overlapping segments each 8K data points long and separated by 1000 points. Each segment was apodized by the Blackman-Harris 3-term window before transformation. Plots of ln[M] vs. T_i were fitted to a straight line whose slopes and intercepts were used with eq. (1) to find D and A for each isotope. Figure 2 shows the results of the extrapolation procedure for the same Kr isotope ratios displayed in Figure 1 and demonstrates that the observed fractionation is dominated by abundance-dependent differences in decay constants.

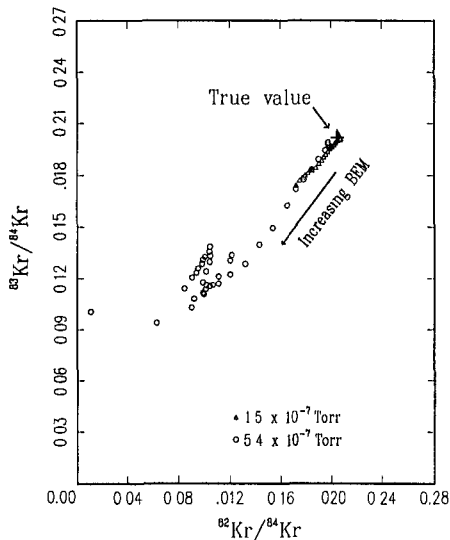


Figure 1. Kr isotope ratios measured on FTMS-2000 at two nominal pressures and trapping voltage = 7.5V. No segmentation of transients.

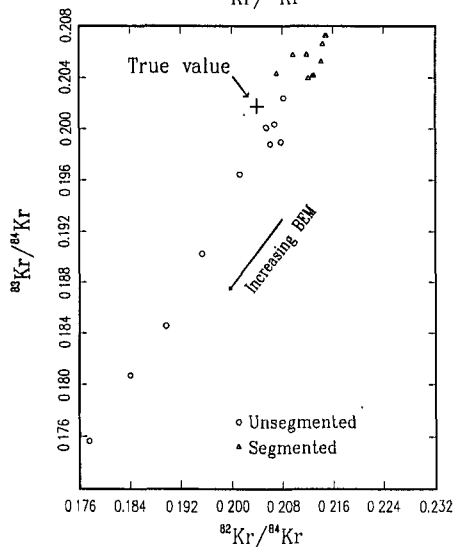


Figure 2. Comparison of Kr isotope ratios for full transients (unsegmented) vs. same ten experiments segmented and extrapolated to end of excite period. Trapping voltage = 9.0 V. Note scale expansion compared to Figure 1.

ICR EXPERIMENTS ON SHORT-LIVED ISOTOPES AND CLUSTERS: NEW THEORETICAL AND EXPERIMENTAL RESULTS

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Mass spectrometers based on ICR techniques have been set up at CERN/Geneva and at the University of Mainz. The experiments have different aims, and therefore different methods are used for ion creation, excitation, and for the detection of the cyclotron resonances. In the following these techniques are described and the advantages and disadvantages are discussed.

After development at Mainz, an ICR apparatus has been set up at CERN/Geneva in order to perform high-resolution precise mass measurements of short-lived isotopes. These isotopes are produced by bombarding suitable targets with 600 MeV protons from a synchro cyclotron. They are available to the ICR device as a continuous beam of 60-keV ions to the ICR device delivered by the on-line isotope separator ISOLDE. The ions are stopped on a foil, surface-ionized and trapped in a first Penning trap (located in an electromagnet). Subsequently they are transferred[1] to a precision trap inside a superconducting magnet, where the mass measurement takes place. This is done by a narrow-band radio frequency (RF) field exciting the cyclotron motion of the stored ions. In resonance the ions absorb energy from the RF field. Afterwards the ions are kicked out of the trap and drift to a channel plate detector. In the inhomogeneous magnetic field the energy of the cyclotron motion is transformed into axial kinetic energy which shows up as a decrease of the time of flight (TOF) to the ion detector[2]. A resonance curve is obtained scanning the RF and measuring the TOF for each frequency point. So far a resolution of more than 2,000,000 has been achieved for mass 133. In a first application to short-lived isotopes evidence has been obtained for a first mass spectrometrical discrimination of two isomers in the same nucleus, namely in the case of ¹²²Cs[3].

It should be emphasized that the resonance observed is not at the reduced cyclotron frequency ν_+ but at an interharmonic of ν_+ and the magnetron frequency ν_- . Both frequencies add up for a perfect Penning trap to $\nu_+ + \nu_- = \nu_c$, where $\nu_c = (eB)/(2\pi M)$ is the cyclotron frequency of an ion of mass M and charge e in a homogeneous magnetic field B in the absence of any trapping potential. Extensive numerical calculations as well as analytical investigations show that a resonance ($\nu_{RF} = \nu_c$) can best be observed by catching the ions with a large magnetron but small cyclotron radius and by using a azimuthal quadrupole electric field for driving the RF transitions. The calculations are confirmed by experiments with a four-segment ring electrode[4]. The amplitude of this resonance is comparable to that of the reduced cyclotron frequency ν_+ using a dipole excitation via a ring split into two halves. The quadrupole excitation with

$\nu_+ + \nu_-$ makes the ions alternate between the cyclotron and the magnetron motion. The beating frequency between these motions depends on the amplitude of the driving RF field. Using these technique a new cooling scheme for ions stored in a Penning trap has been developed[4].

The apparatus at Mainz will be used for laser spectroscopy of cluster ions[5]. Here the trap is not just filled with one ion species as in the Geneva experiment. It is used for the preparation of the ion ensemble of interest, for storage while the interaction with the laser takes place and for the analysis of the products. While the resonance detection technique by TOF, applied at Geneva, is very sensitive (one ion per measurement cycle is enough to perform the experiments) it is also time consuming and becomes cumbersome, if different ion species of an unknown ion cloud have to be identified. In this case FT-ICR is the method of choice. For test experiments an apparatus using a Penning trap inside an electromagnet has been set up at Mainz. The conventional FT-ICR detection scheme has been tested and new detection schemes have been developed. For example by exciting the ions via one end cap and using the other as a pick-up electrode it is possible to directly detect the trapping frequencies of the ion ensembles [6]. The frequencies of these resonances do not involve the magnetic field like the cyclotron frequency does. Therefore it seems feasible to extend these experiments to RF traps.

It has also been shown that resonances at combinations of the reduced cyclotron frequency and the magnetron frequency, namely $\nu_+ + \nu_-$ and $\nu_+ - \nu_-$ can be observed directly without the resonance at ν_+ , which is the most strongest in conventional FT-ICR. This is done by adding the signals of two opposite ring segments instead of subtracting them [7].

With the help of these experiences a new Penning trap has been constructed for a superconducting magnet at Mainz. The new trap has a twelve-electrode configuration, eight of which form the ring electrode. With this scheme different types of excitation and detection can be performed. From the observation of the resonances at interharmonics of ν_+ and ν_- by use of two opposite electrodes it can easily be shown that a quadrupole detection arrangement should show only the resonance at $\nu_+ + \nu_-$ while the one at $\nu_+ - \nu_-$ must vanish. This has been confirmed experimentally[4]. The new detection scheme can be used in narrow-band excitation as well as in the broad-band mode. The experiments have been performed with ions produced by electron impact ionization of the rest gas in the trap as well as with carbon cluster ions produced by laser evaporation of a target placed at one of the endcaps. The experimental investigations will be continued using externally created cluster ions.

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IN-SITU PYROLYSIS AND THERMAL DESORPTION IN AN
ION TRAP MASS SPECTROMETER

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Normally, the Finnigan ITD is designed to accept only gas phase samples introduced through the GC transfer line. While a number of inlet variations have been reported, including some that allow solid sample analysis, they all generate a gas phase sample external to the ion trap. In the present experiment, a small loop of 0.005" diameter Chromel wire was located within one of the 0.045" diameter ion exit holes provided on the detector end cap. Electrical connection was made by a spring contact to a BNC feedthru that was added to the standard vacuum manifold. A tantalum shield was placed over the wire loop on the detector side to prevent photons emitted by the hot filament from reaching the multiplier directly. Solid samples were loaded onto the wire loop, either directly or dissolved in a suitable solvent. The trap assembly was then loaded into the vacuum manifold and pumped down.

Thermal desorption/pyrolysis mass spectra were then obtained by acquiring data in the usual manner while slowly ramping the current passing through the wire loop. Despite the use of the photon shield, heating currents were limited to the point where significant optical emission saturated the detector. Within the accessible temperature range (estimated to be up to 500 deg C), a number of solids including sucrose, 1,3-dimethylthymine, and cellulose fibers gave strong desorption and/or pyrolysis spectra.

In order to allow for a shorter cycling time for inorganic compounds, and to reduce the background interferences from a hot filament near the multiplier, an external vaporization chamber was built of 2-3/4" conflat parts. It was connected to the trap inlet via 3-1/2" of 1/4" o.d. stainless steel tubing. Since the conductance of this tube is 0.12 l/s and the exit conductance of the trap is 2 l/s, the trap operated at 6 mTorr with the vaporization chamber at 100 mTorr and a 1 ml/min flow rate of carrier gas. The heater filament in the vaporization chamber was 5/8" long, and either .030" x .001" rhenium or .100" x .0005" tungsten. Samples placed on the heater were ramped from room temperature to 1500+ deg C in about 6 minutes.

Samples such as cadmium and zinc evaporate over a narrow temperature range, giving fairly sharp "peaks" in the total ion chromatogram. Measured isotopic ratios agree well with standard values, as shown in Figure 1 for cadmium. As₂O₃ gave, at first, mixed oxides, then as the temperature increased, arsenic clusters appeared, as shown in Figure 2. Results using other compounds are listed in Table 1.

When the carrier gas used to entrain the evaporated materials was switched from helium to hydrogen, thermal desorption of As₂O₃ produced oxides, clusters, and hydrides of arsenic (see Table 1). The former appeared at lower temperatures than the latter. Zinc hydride was also observed during the thermal desorption of ZnCl₂ using hydrogen as a carrier gas.

This technique should prove useful for the analysis of heavy metals and inorganic compounds using the ITD.

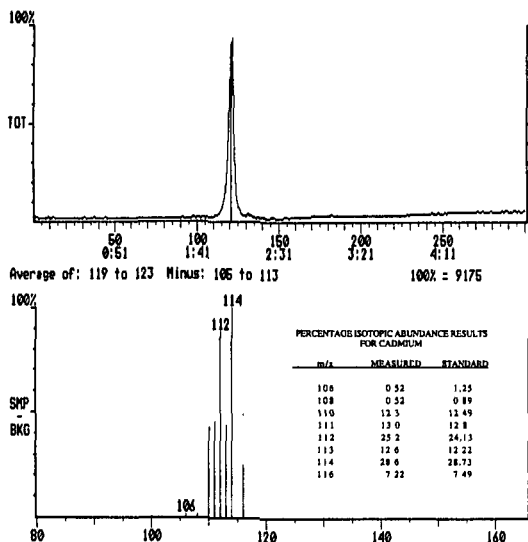
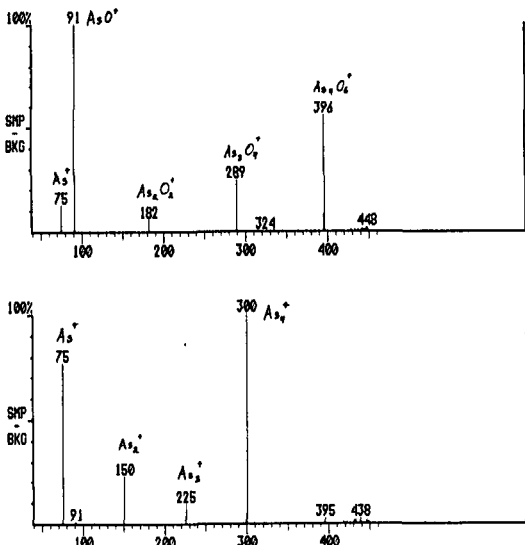


Figure 1. Upper panel (left) shows the total ion chromatogram for the thermal desorption of cadmium. The lower panel shows the mass spectrum obtained by subtracting the average of scans 105 to 113 from the average of scans 119 to 123. Also shown is a table of measured isotope ratios.

Figure 2. The upper panel (below) shows a mass spectrum of the initial materials desorbed when As_2O_3 is evaporated from a hot rhenium filament. The lower panel is the spectrum taken later in the heating cycle, corresponding to higher temperatures.

TABLE 1
SUMMARY OF DATA OBTAINED
BY THERMAL DESORPTION OF VARIOUS
SAMPLES

SAMPLE	CARRIER GAS	OBSERVED IONS
$NaCO_3$	He	CO_2^+
Zn	He	Zn^+
Cd	He	Cd^+
Pb	He	none
Zn_3As_2	He	As^+ , AsO^+ , $As_2O_3^+$, $As_3O_4^+$, $As_4O_6^+$, As_2^+ , As_3^+ , As_4^+
YbTe	He	Tc^+
Te	He	Tc^+
WSe	He	Se^+
$HAuCl_4 \cdot H_2O$	He	Cl_2^+
Au	He	none
$BaCl_2$	He	none
$ZnSO_4 \cdot H_2O$	He	Zn^+ , SO^+ , SO_2^+
$ZnCl_2$	He	Zn^+
As_2O_3	He	As^+ , AsO^+ , $As_2O_3^+$, $As_3O_4^+$, $As_4O_6^+$, As_2^+ , As_3^+ , As_4^+
$ZnCl_2$	H_2	Zn^+ , ZnH^+
$BaCl_2$	H_2	none
As_2O_3	H_2	As^+ , AsO^+ , $As_3O_4^+$, $As_4O_6^+$, $As_2H_4^+$, $As_2H_5^+$, $As_3H_6^+$, As_4^+
Sn/Pb alloy	H_2	Sn^+



EXTERNAL ION INJECTION INTO AN FTMS

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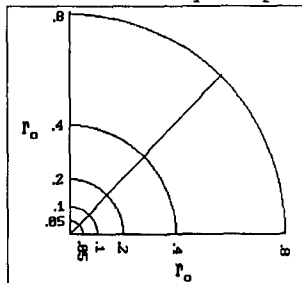
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Recently, several techniques have been proposed and tested for the injection of ions into the trapped-ion cell of the Fourier transform mass spectrometer (FTMS). The objective of the external source/FTMS is to produce ions from any of a variety of ion sources, and in particular "messy" ion sources such as FAB, API, and ion sprays, which are external to the FTMS magnetic field. The externally-produced ions are injected in the trapped-ion cell of the FTMS for mass analysis. Such "messy" ion sources are incompatible with the low-pressure optimum operation of FTMS.

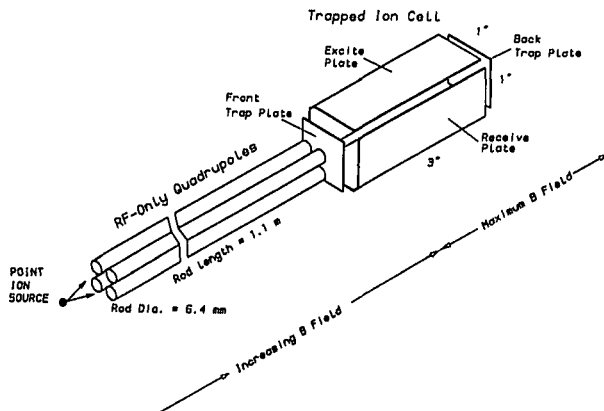
This paper investigates the properties of the RF-only quadrupole as an ion injection device for an external source/FTMS. Several RF-quadrupole/magnetic field configurations were studied by computer simulations. The models were based on the equations of motion of ions moving through an ideal quadrupole field with an externally-imposed magnetic fringe field. Some of the computer simulation results were tested by experiments on a quadrupole/FTMS system.

Several ensembles of ions were investigated in the simulations. Ions originated from a point ion source just in front of the quadrupole field. The ions were launched with initial energies of 1.0, 2.5, 5.0, or 10.0 eV. The ions were injected into the first quadrant of the RF-only quadrupole field at 15 locations as shown below. One quadrupole ion guide that was studied was 1.1-m long with 6.4-mm diameter rods and an RF frequency 870 kHz. Two RF voltages were used (500 V and 2000 V), which correspond to a low mass cutoff of m/z 460 and m/z 1860. Ions were injected into 3 T and 7 T magnetic fields with fringe fields that very closely approximated those measured. The trapped-ion cell dimensions were 2.5 cm x 2.5 cm x 7.6 cm. The front trap plate was held at 0 V until an ion entered the trap and passed the center, at which time the front trap plate voltage was raised to the same voltage as the back trap plate (3 V). Several different m/z ions were investigated including m/z 1000, 2000, 3000, 4000, and 5000.



In the simulations, an ion can be transmitted, trapped, or lost. A transmitted ion is one whose trajectory is stable through the quadrupole field; i.e., $(x^2 + y^2)^{1/2} < r_0$, where r_0 is the inscribed radius of the quadrupole structure. A trapped ion is one that is transmitted through the quadrupole field, enters the trapped ion cell, and is electrostatically reflected to the center of the cell from the back trap plate. The efficiency is defined as the percent of ions trapped relative to the number of ions that were injected.

Ions are lost by a) unstable trajectories in the quadrupoles; b) reflection because of the magnetic forces (magnetic mirror effect); and c) penetration of the back (transparent) trap plate due to high ion kinetic energy.



One important finding of these simulations is that some of the apparently desirable characteristics of the RF-only quadrupole ion guide are lost in the presence of a fringing magnetic field -- the magnetic mirror. For example, while the overall transmission through the quadrupole field appears to be mass independent, it appears to be less at higher RF-voltages (higher mass cut off). Also, the magnetic mirror affects the transmission efficiency more with low magnetic fields than with high magnetic fields. Higher RF-voltages appear to lead to an increased radial distribution about the z-axis (centerline) of the trapped-ion cell. Such an increase in the spatial distribution of injected ions will limit the coherent excitation of ions. Also, the ion injection/trapping efficiency increases with more intense magnetic fields. This is because the force exerted on the ions by the magnetic field is much stronger than the forces exerted by the quadrupole field. The strong magnetic fields tend to keep the ions bunched about the centerline. These findings are difficult to test experimentally, because at low RF voltage, the FTMS performance may be affected by the injection of large numbers of unwanted low-mass background ions that can lead to collisions and space-charge that affect the resolving power.

Those ions injected with high axial energies, for example, those at $0.4f_0$ and $0.8f_0$ are transmitted with low efficiency or not at all, and lower axial energies lead to higher efficiencies (40-50%). In practice, the ion source is tuned to minimize the angular distribution of the exiting ions. The simulations showed that ions with higher translational energies were trapped more efficiently than ions with lower translational energies. This result was confirmed by experiments with ions formed by particle-induced emission from solids and liquids.

In summary, the ion transmission properties of an RF-only quadrupole are different when it is operated in a spatially-varying magnetic field. Ion trapping is more efficient with higher magnetic fields. The higher the RF-voltage (higher low-mass cutoff), the lower the overall efficiency of injection and optimal ion trapping. A threshold ion energy is required for ions to penetrate the magnetic mirror. Further simulations and experimental studies should provide information on optimal operation of the RF-only quadrupole for ion injection into the FTMS.

**EXTERNAL ION SOURCES ON ION CYCLOTRON RESONANCE (ICR) MASS
SPECTROMETERS: ION-OPTICAL CONSIDERATIONS.**

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INTRODUCTION

An ion moving with a velocity v perpendicular to the direction of a magnetic field B experiences a force F given by:

$$F = qvB$$

This force tends to move the ion in a circular orbit perpendicular to the direction of the magnetic field. The radius of the orbit and frequency of rotation are proportional to the mass-to-charge ratio of the ion. The radius of the orbit is given by:

$$r = \frac{mv}{qB}$$

The frequency of rotation is given by:

$$f = \frac{1}{2\pi} \left(\frac{Bq}{m} \right)$$

Trajectories of ions through an electrostatic lens in a magnetic field will be different for ions of different mass-to-charge ratios due to the magnetic component.

EXPERIMENTAL

A field desorption experiment has been performed inside the magnetic field of a Bruker Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. The arrangement consisted simply of a counter-electrode and focus electrode mounted on the front of the ICR trapping cell. The trapping plate voltage was not pulsed in these experiments.

RESULTS

The compound used in the experiments was polypropylene glycol (av. mol. wt. 1000). This compound was chosen because it tends to produce strong ion currents over a broad range of masses under field desorption conditions [1]. The appearance of the polymer envelope in any mass spectrum provides an indication of the extent of any mass discrimination.

It proved very difficult in these experiments to trap ions in the cell. The three inter-dependent variables which had to be tuned were: the potential on the emitter, the emitter heating current and the potential on the focus electrode. The counter-electrode was floated at -6kV in these experiments. One particular set of conditions was found, however, under which ions were trapped in the cell. The conditions were: an emitter potential of +25V, an emitter heating current of 15mA and a focus voltage of -215. An emitter heating current of 15mA is quite high in comparison to heating currents normally used in field desorption of this compound [2]. The spectrum obtained using a short acquisition time (0.001s) is shown in Figure 1. The resolution in the spectrum is obviously very poor. It does, however, suggest extreme mass discrimination.

CALCULATED RESULTS AND DISCUSSION

Trajectories through the system were calculated for ions originating from the emitter at initial angles of 1, 2 and 3 degrees to the axis of the system in the central horizontal plane. The magnetic field strength was 4.7 tesla. Trajectories for an ion of mass-to-charge ratio of 1000 are shown in Figure 2. The emitter potential referred to in the calculations is the potential at which the ions are formed. In practice the actual voltage applied to the emitter will be greater than this by an amount equal to the voltage drop across the sample layer in the intense electric field. The trajectories have been terminated when the ions were just beginning to make their way back out of the cell (i.e. the component of velocity in the x direction became zero). Table 1 shows the results of calculations as distances (in millimeters), beyond the back face of the trapping plate, to which ions have penetrated the cell. The extent of penetration into the cell will determine how many ions are in the cell at a particular time and how long ions remain in the cell. If the ions are not excited by an RF pulse, they will remain inside the cell for only a short period of time. Under conditions where an ion of m/z 1000 penetrates to the centre of the cell the time spent inside the cell is of the order of 10^{-8} seconds. On application of the RF ions will absorb energy in the "plane of spiral", increasing the radius of the spiral. An increase in the orbital radius moves the ions out to regions of much lower electric field strength, thereby trapping the ions for a longer period of time. Negative values in Table 1 indicate distances

In front of the trapping plate i.e. these ions did not enter the cell at all. The internal length of the ICR cell is 60 mm. Values of 60 mm in Table 1 indicate, therefore, that the ion has hit the rear trapping plate. The extent to which an ion will penetrate the cell will depend upon the ions kinetic energy in the x direction (direction of cell axis). If the ion has significant angular velocity in the "plane of spiral", a lower proportion of the kinetic energy will be in the x direction, and the ion will tend not to penetrate far into the cell. This may be particularly critical under conditions of very low electric field penetration into the cell, as was the case in these experiments. Larger angles of divergence off the emitter will have greater spiralling induced by the magnetic field. For a given energy, low mass ions will tend to have a greater proportion of energy in the "plane of spiral", and are therefore discriminated against.

On studying Table 1 it would seem that masses and initial angles of divergence of ions ultimately trapped in the ICR cell will be sensitive to the actual potential at the sample/vacuum interface at the emitter. For example, consider that this potential is between +1 and +2 volts. Ions of mass around 1000 and initial divergence angle around 2 degrees will tend to be trapped. Ions of low mass will not penetrate very far, if at all, into the cell. Ions of mass around 1000 with very low initial divergence angles will tend to strike the rear trapping plate, whilst those with angles closer to 3 degrees will not penetrate the cell sufficiently. The differences in penetration distance may be sufficient to cause only a relatively small mass range to be trapped.

CONCLUSION

Mass discrimination (particularly if a broad mass range is required) is a very important consideration when designing electrostatic lenses for use in conjunction with external ion sources on ICR mass spectrometers. Under conditions of very low electric field penetration into the trapping cell and/or divergent ion beams, such as in the field desorption experiments conducted, the mass discrimination can be quite extreme. than mass 1000 under the same

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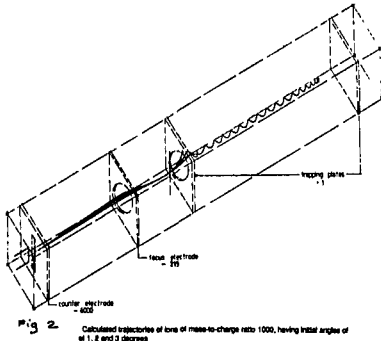
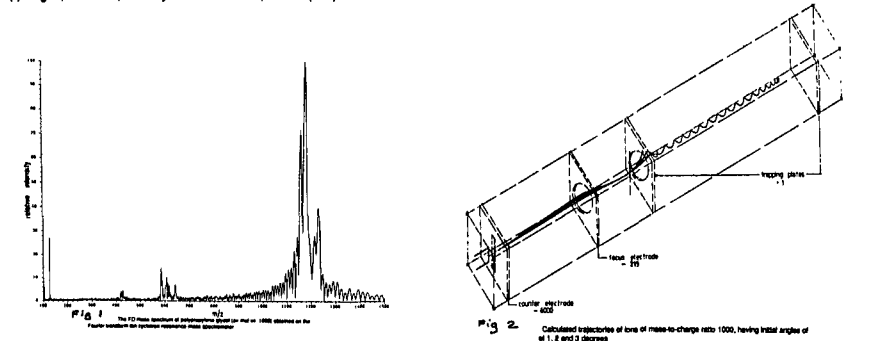


TABLE 1

m/z		emitter potential / V					
		0	1	2	3	4	5
10	1°	3.7	4.1	7.8	11.6	14.6	17.1
	2°	0.4	0.7	1.1	1.5	2.1	2.8
	3°	1.1	-1.0	0.9	0.7	-0.6	0.4
100	1°	1.8	2.5	3.8	7.8	43.4	60
	2°	-1.8	0.7	0.8	0.3	-0.1	0.1
	3°	-2.7	2.7	2.8	-2.6	2.8	2.4
500	1°	3.7	6.0	60	60	60	60
	2°	1.8	1.8	2.7	4.7	52.2	60
	3°	-1.3	-1.2	1.1	-1.0	0.9	0.7
900	1°	4.5	48.3	60	60	60	60
	2°	3.3	3.7	11.6	60	60	60
	3°	-0.4	0.0	0.9	1.2	2.3	6.1
1000	1°	4.5	49.7	60	60	60	60
	2°	3.5	4.1	48.9	60	60	60
	3°	-2.2	0.3	0.9	1.8	3.8	57.5
1100	1°	4.7	50.6	60	60	60	60
	2°	3.7	4.8	51.9	60	60	60
	3°	0.0	0.5	1.3	2.4	6.7	60
1300	1°	4.9	52.1	60	60	60	60
	2°	3.9	5.8	57.6	60	60	60
	3°	0.3	1.0	2.0	4.0	57.5	60
10000	1°	6.6	57.9	60	60	60	60
	2°	4.6	53.3	60	60	60	60
	3°	3.1	43.2	60	60	60	60

A SIMPLE DIFFERENTIALLY PUMPED
EXTERNAL SECONDARY ION SOURCE FOR FTMS

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FTMS OFFERS MANY ADVANTAGES AS A MASS ANALYZER FOR HIGH MASS IONS DUE TO ITS EXTREMELY HIGH MASS RESOLUTION, THE ABILITY TO ACCURATELY MEASURE MASSES WITH ERRORS LESS THAN 5 PPM WITHOUT CALIBRANT PRESENT, AND GOOD SENSITIVITY. HOWEVER, TO REALIZE THESE ADVANTAGES REQUIRES OPERATION AT PRESSURES BELOW 10^{-8} TORR. THIS HAS MADE THE COUPLING OF FTMS WITH LIQUID-SIMS PROBLEMATIC SINCE COMMON MATRICES HAVE VAPOR PRESSURES OF 10^{-7} TO 10^{-6} TORR. ONE SOLUTION HAS BEEN TO GENERATE SECONDARY IONS OUTSIDE THE MAGNET, IN AN EXTERNAL, DIFFERENTIALLY PUMPED SOURCE (FOR EXAMPLE THE QUADRUPOLE-FTMS OF MCIVER). WHILE CERTAINLY SUCCESSFUL, THESE HAVE NECESSITATED CONSTRUCTION OF AN EXPENSIVE INSTRUMENT DEDICATED TO THIS PURPOSE.

AN ALTERNATIVE APPROACH IS TO UTILIZE THE EXISTING DIFFERENTIAL PUMPING CAPABILITIES OF THE COMMERCIALY AVAILABLE NICOLET FTMS-2000. TO AVOID ALIGNMENT AND HIGH-VOLTAGE DIFFICULTIES THE CESIUM ION GUN IS MOUNTED TRANSVERSE TO THE MAGNETIC FIELD, WITH THE SAMPLE LOCATED OVER THE PUMP THROAT. THIS ARRANGEMENT DOES NOT INTERFERE WITH ANY OF THE OTHER EXPERIMENTS POSSIBLE ON THE INSTRUMENT (SEE FIGURE 1). THIS PRODUCES THE SECONDARY IONS ABOUT 38 CM FROM THE SOURCE TRAP PLATE, AT A MAGNETIC FIELD STRENGTH OF 0.165 TESLA, COMPARED TO 3 TESLA AT THE CELL. THE IONS ARE REPELLED BY A BIAS PLACED ON THE PROBE AND MOVE TOWARDS THE CELL WHERE THEY ARE TRAPPED BY GATING THE SOURCE TRAP PLATE TO GROUND WHILE THE ION GUN IS PULSED ON. A PULSE OF 5 MS IS LONG ENOUGH TO ESTABLISH A UNIFORM ION POPULATION FREE OF MASS DISCRIMINATION EFFECTS. BY ALSO GROUNDING THE CONDUCTANCE LIMIT THE IONS MAY BE TRANSFERRED TO THE ANALYZER CELL FOR ANALYSIS AT LOWER PRESSURE.

FIGURE 2 SHOWS A SPECTRUM OF CESIUM IODIDE CLUSTERS RECORDED IN THE SOURCE CELL. AN ION WITH m/z 8187, CORRESPONDING TO THE $Cs(CsI)_3^{+}$ CLUSTER, IS CLEARLY VISIBLE. GLYCEROL AS A MATRIX YIELDS SOURCE AND ANALYZER PRESSURES OF 4×10^{-6} AND 2×10^{-8} TORR, RESPECTIVELY. MAGIC BULLET, DITHIOHREITOL/DITHIOERYTHRITOL, YIELDS PRESSURES OF 6×10^{-7} AND 8×10^{-9} TORR, RESPECTIVELY. SPECTRA OF HISTIDINE WERE RECORDED FROM EACH MATRIX AT SIMILAR SENSITIVITY. FIGURE 3 SHOWS THE SPECTRUM WITH MAGIC BULLET AS A MATRIX, AND CONTAINS ABUNDANT $[M+H]^{+}$ IONS, WITH m/z 156. EXTRAPOLATION OF THE MEASURED SIGNAL TO NOISE DOWN TO 2:1 PREDICTS A DETECTION LIMIT UNDER THESE CONDITIONS OF TENS OF NANOMILES. THE TOTAL IONIZATION TIME TO RECORD THIS SPECTRUM WAS ONLY 50 MS. THE INSET SHOWS THE MOLECULAR ION RECORDED AT A RESOLUTION OF 160,000 FWHH, DEMONSTRATING THE RESOLUTION CAPABILITIES OF FTMS WHEN OPERATED AT SUITABLE PRESSURES.

FTMS 2000 CHAMBER

FIGURE 1

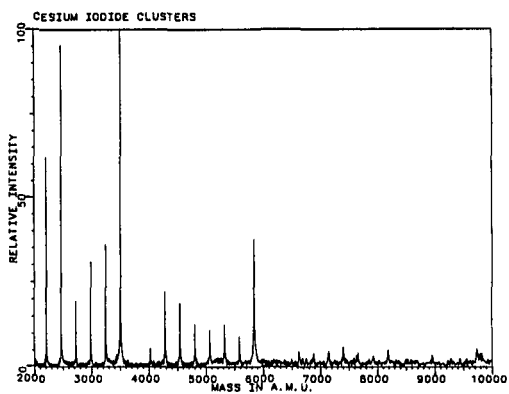
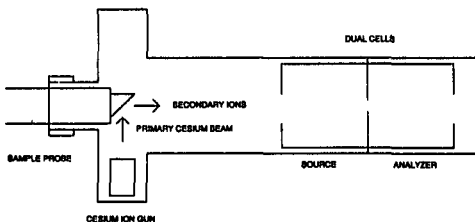
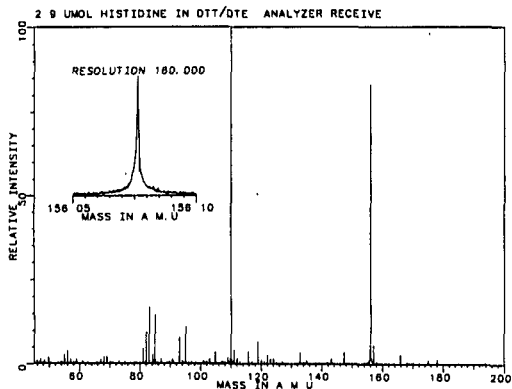


FIGURE 2

FIGURE 3



NEGATIVE IONIZATION IN QUADRUPOLE ION TRAP MASS SPECTROMETRY: EFFECTS OF APPLIED VOLTAGES:

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Successful implementation of negative ion mass spectrometry in the quadrupole ion trap mass spectrometer (QITMS) depends upon the use of various electrical fields to control the mass range of ions that have stable trajectories, as well as the kinetic energy of these ions. The electric fields may be examined by viewing the Mathieu stability envelopes for ion motion in a quadrupole ion trap (Figure 1). A detailed discussion of the Mathieu equations may be found elsewhere (1). One may consider the Mathieu parameter ' a_z ' (y-axis) as the DC voltage (U) applied to the ion trap ring electrode, divided by the m/z (m) of a given ion, multiplied by some constant (C). Similarly, the Mathieu parameter ' q_z ' (x-axis) can be considered simply as the RF voltage (V) applied to the ion trap ring electrode, divided by the m/z of a given ion, multiplied by some constant (K). Using this simplification, it becomes evident that for a given DC potential, each m/z has a unique value of ' a_z ' assigned to it. Similarly, for a given RF potential, each m/z has a unique value of ' q_z ' assigned to it.

In the RF-only mode of operation (no DC voltage), the ion trap is operated along the ' a_z ' = 0 line (point A), which allows for the storage of positive and negative ions. Since positive ions are formed more readily than negative ions (100- to 1000-fold excess), negative ion signals are quenched by positive-negative ion recombination. In order to overcome this challenge, it is necessary to examine alternative operation modes. Application of a DC voltage (negative) to the ring electrode allows operation of the ion trap at points B and C. At point B, a narrow mass range of positive ions and a wide mass range of negative ions have stable trajectories in the ion trap, while at point C, only negative ions are stored in the ion trap. Operation of the ion trap at point ($a_z > 0.15$) prevents positive-negative ion recombination and allows for observation of negative ions.

The experiments were performed with a Finnigan MAT Ion Trap Mass Spectrometer (ITMS™) equipped with mass-selective storage and a variable dynode power supply. The following operating conditions were found to be optimum: reactant gas pressures between 2 and 4×10^{-5} torr; ionization times between 40 and 100 ms; negative chemical ionization reaction times between 20 and 300 ms; multiplier setting between 400 to 500 mV greater than 10^5 gain; and the DC voltage was on during isolation of negative ions in the ion trap.

The reactant ions from water and carbon tetrachloride (produced by dissociative electron capture) and sulfur hexafluoride (produced by electron capture) were isolated with a negative DC voltage pulse applied to the ring electrode. Undesired reactant ions were ejected with a supplemental RF voltage applied across the end cap electrodes. The isolation of OH^- ion from water is shown in Figure 2. The Cl^- and SF_6^- ions did not produce negative sample ions with the compounds examined, while the OH^- ion reacted by proton abstraction with most of the compounds examined. The OH^- NCI mass spectra of nearly all of the compounds examined are characterized by an $[\text{M-H}]^-$ ion and little or no fragmentation (Figure 3).

The most important parameter for successful negative ion formation and storage in the ion trap is the applied DC voltage. This voltage must be selected such that all positive ions are ejected from the ion trap (i.e., an ' a_z ' parameter greater than 0.15). Dissociative electron capture occurs in the ion trap for a variety of reactant gases, including ammonia, water, carbon tetrachloride, and ammonia; while electron capture occurs only for compounds with extremely high electron affinities (e.g., SF_6). The OH^- NCI mass spectra of most compounds produced a mass spectrum characterized by an abundant $[\text{M-H}]^-$ ion and little or no fragmentation. This information could be particularly useful for determining molecular weights of unknown compounds.

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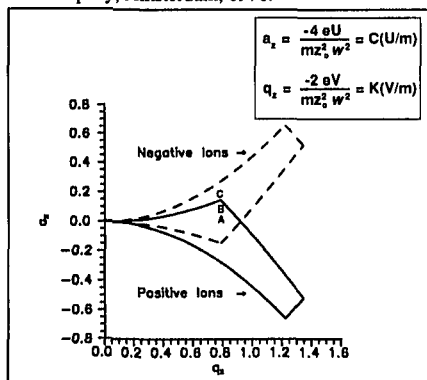


Figure 1. Mathieu stability envelopes for positive (solid line) and negative (dashed line) ions. Points 'A', 'B' and 'C' have corresponding ' a_z ' values of 0.00, 0.14 and 0.17 and ' q_z ' values of 0.76.

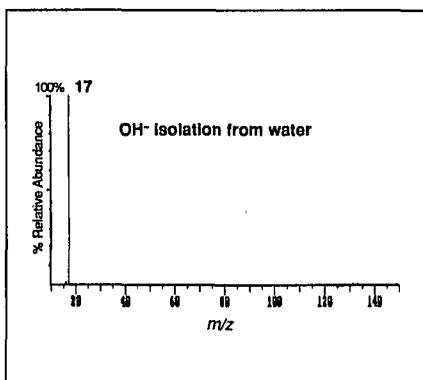


Figure 2. Isolation of OH^- from H_2O .

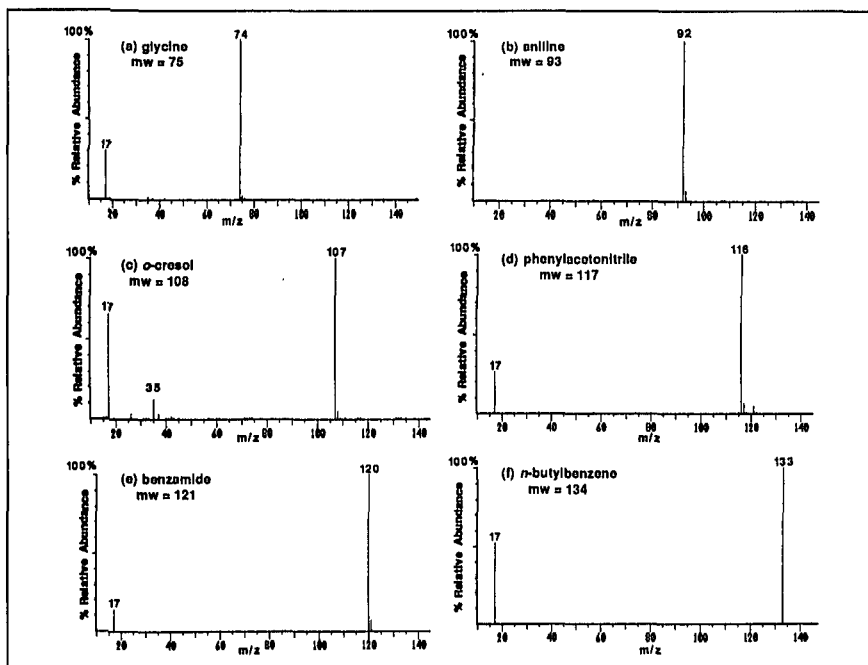


Figure 3. OH^- NCI mass spectra of glycine, aniline, *o*-cresol, phenylacetonitrile, benzamide and *n*-butyl benzene.

CALIBRATION AND ACCURATE MASS MEASUREMENT WITH GC-FTMS
USING AN EXTERNAL ION SOURCE

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Introduction

The combination of Gas Chromatography with Mass Spectrometry (GCMS) is one of the powerful methods for organic quantitative analysis. In the last few years, the combination of GC with Fourier Transform Mass Spectrometry was several times described and shown to be very successful for obtaining high resolution GC-FTMS spectra. High resolution mass spectra are an important basis for elemental composition determinations. But the determining factor in elemental composition assignments is not the high resolution but the mass accuracy, which can be obtained. Controlling the experimental parameters and calibration procedures are therefore the important factors to get accurate masses.

In this work we describe some critical parameters which influence the mass accuracy in high resolution GC-FTMS. Propositions for attaining the best mass accuracy will be drawn from the experimental data.

Experimental

A GC for capillary gas chromatography with on-column injector (HP-5790A from Hewlett Packard) was coupled to the external differentially pumped ion source of a FTMS equipped with a 4.7 Tesla superconducting magnet (CMS 47 X from Spectrospin). The GC-FTMS interface consisted of a deactivated fused silica capillary, heated to about 200 °C and inserted directly into the outlet of the GC column (25m x 0.35 mm i.d., HP-1). Helium was used as a carrier gas. The pressure in the ion source was about 4×10^{-9} mbar. Perfluorotributylamin was used as a calibration gas.

Results

In a first attempt, the mass accuracies were measured without ejecting all the ions outside the interesting high resolution mass window out of the ICR-cell (prior to the exitation pulse). This procedure showed the following results:

- Calibrating the FTMS at different GC-elution temperatures has no effect on the mass accuracy.
- The resolutions (of the mass spectra) are dependent on peak intensities: The more intense the peak, the higher the resolution.
- Measuring the accurate masses at different times during sample elution shows that the mass varies systematically with peak intensity.

The results, obtained by measuring the accurate masses with ejection of all ions out of the ICR cell (except the one which is measured are summarized in table I.

Table I

Mass Accuracy Measurements in GC-FTMS Mode with Ion Ejection

Compound	Measurend Formula	Ion Nom.Mass	Mass Accuracy
Decane (Fragment)	C ₅ H ₁₁	71	- 0.44 ppm
Decane (M) ⁺	C ₁₀ H ₂₂	142	- 2.63 ppm
2,6-Dimethyl phenol	C ₈ H ₁₀ O ₁	122	- 0.98 ppm
2,6 Dimethyl aniline	C ₈ H ₁₁ N ₁	121	- 0.62 ppm
Tridecane	C ₁₃ H ₂₈	184	- 3.52 ppm

Conclusion

The presented results indicate that highly accurate and reliable mass measurements are possible with the GC-FTMS combination, if one pays attention to the following precautions:

- The carrier gas must flow into the ion source during the calibration procedure.
- Working without ion ejection of all ions outside the interesting mass window results in short cycle times and high peak intensities. But the measured masses are dependent on the peak intensities. Mass accuracy is therefore only medium.
- Working with ion ejection needs longer cycle times but highly accurate mass measurements can be done.

RESONANT MULTIPHOTON IONIZATION ON A FOURIER TRANSFORM MASS
SPECTROMETER WITH EXTERNAL ION SOURCE

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Introduction

In the past several years, lasers have been used in mass spectrometry mainly in two ways:

Laser desorption (LD) and Multiphoton Ionization (MUPI). LD involves the interaction of a laser beam with the sample substrate in the solid phase. Ions are therefore generated by direct interaction of the laser beam with the solid sample on the target. The multiphoton technique involves the interaction of laser photons with individual molecules, atoms or ions in the gas phase (solid samples have therefore first to be desorbed by an additional laser). The technique includes MUPI of both types, the resonance enhanced (REMPI) and non-resonance enhanced variety, the resonance ionization mass spectrometry (RIMS).

It has recently been demonstrated that REMPI exhibits very attractive features: Soft ionization for polar and/or thermally labile compounds, tuneable fragmentation controlled by the laser power and selective ionization of one species in a mixture by appropriate selection of the laser wavelength.

In this work we present the combination of REMPI with Fourier Transform Mass Spectrometry (FTMS). The experimental setup is described and measurements are presented to evaluate the performances.

Experimental

The FTMS used for this work is a Spectrospin CMS 47 X with external, differentially pumped ion source, equipped with a 4.7 Tesla superconducting magnet.

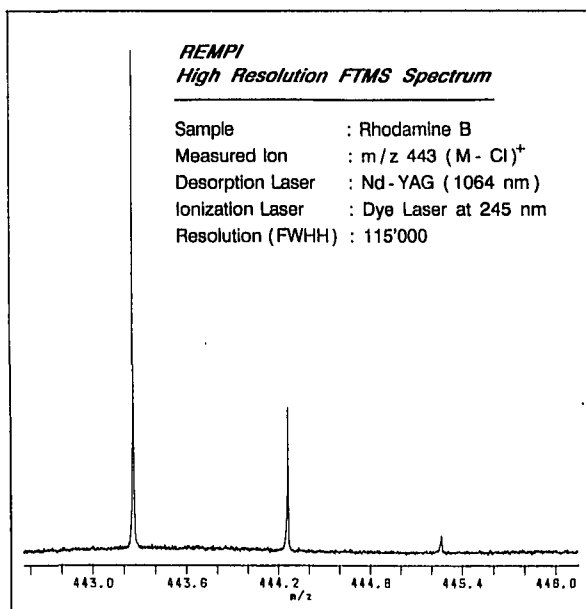
Liquid samples were introduced through the gas inlet, solid samples with the DIP or deposited on the target for LASER-desorption. Solid samples were evaporated by heating (DIP) or by laser evaporation, using a Nd-YAG laser. (wavelength 1064 nm, pulse length about 6 - 9 nsec, pulse energy was varied between 100 and 150 mJ). Ionization of the desorbed neutrals by the REMPI technique was done with a frequency doubled Eximer pumped dye laser: Eximer laser from Lambda Physik, type LPX 110 (308nm, 150 mJ). Dye-laser from Lambda Physik, type FL 3001, frequency doubled (245 - 275 nm, used laser energy about 0.04 mJ).

Results

The instrumental setup was first tested by measuring benzene. Scanning the wavelength and measuring the molecular ion and its $^{13}\text{C}_1$ isotope (m/z 78 and 79) showed that the two peaks were separated. In the broad band mode, the 70 eV EI-FTMS spectrum exhibits the well known fragmentation whereas in the REMPI spectrum only the molecular ion and no fragmentation was observed. The molecular ion could be measured with a resolution FWHH of $1'300'000$.

Coronene was introduced with the DIP and evaporated by heating or on the laser probe tip and desorbed with an additional Nd-YAG laser. Both REMPI broad band FTMS spectra showed only the molecular ion (m/z 300) and no fragmentation. By using the desorption laser (at 1064nm) and the ionization laser at 252.5 nm, the molecular ion was measured with a resolution (FWHH) of 160'000.

The REMPI FTMS spectrum of the dye Rhodamine B exhibits the quasi molecular ion ($M - Cl$)⁺ and the sodium and potassium attached ion ($M-HCl + Na$ and $M - HCl + K$). But compared to the normal LASER-desorption spectrum, less fragmentation and no signals from the target material are observed. Desorbing the Rhodamine B with a Nd-YAG laser (1064 nm, low laser energy) and ionizing the desorbed neutrals with 245 nm, one could attain a resolution (FWHH) of 115'000 of the m/z 443 ($M - Cl$)⁺ ion (see Figure).



Conclusion

The presented results demonstrate that the attractive features of REMPI (very soft and selective ionization with the possibility of tuning the fragmentation) can be combined with the salient features of FTMS: The capability for high resolution is fully preserved. Together with the inherent mass accuracy of FTMS, molecular weight determinations and elemental composition assignments of molecular ions and fragments are very much simplified.

USE OF HIGH VOLTAGE PULSES TO STUDY LASER DESORBED IONS IN A DUAL CELL FT/ICR

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In the dual cell ions produced by electron impact are transferred by temporarily grounding the conductance limit plate which separates the two cells. However in laser desorption experiments, ion transfer is often observed even when the full trapping voltages are maintained on all plates. Since ions that are "unintentionally" transferred in this way are quite often structurally important organic ions, we have decided to make a time resolved study of this process by applying short high voltage pulses to components of the icr cell. Pure metal samples were used because they yielded more reproducible spectra and exhibited this same ion transfer phenomenon.

The top portion of Scheme 1 shows the relevant features of a Nicolet FTMS-2000 dual cell for laser desorption. The pure metal sheets (Mo, Ti or Nb) were cleaned with methanol and placed ~3mm from the source cell and irradiated by a focused ir pulsed laser (Tachisto model 215G, ~10.6 μm). The laser was fired repetitively at the same spot until the signal was reproducible (within ~20%). Ions enter the source cell through a 4mm hole in the trap plate, transit a 4.7625 cm source cell and enter the analyzer cell through a 2 mm hole in the conductance limit. Ions are then detected following a 1 sec trapping period. The high voltage pulses (up to -340V, rise time ~.5 μs) were generated by a homebuilt TTL/MOSFET circuit. The high voltage pulse is either applied as a negative pulse floating on the trapping voltage or as a positive voltage up to the trapping voltage from a -340 V (Scheme 1).

Initially, a high voltage pulse was applied to the probe tip to establish a true "time zero" of the experiment relative to the laser trigger pulse. Figure 1 shows the complementary behavior of the negative quench and the positive "window" pulse. It is noted that the effect of the pulse is delayed ~6 μs relative to the laser trigger from the computer, and that since the pulses are only ~1.5 μs wide, ions are produced and/or escape from the probe tip in a relatively short time window.

A negative quench pulse was applied to the analyzer trapping plate to investigate the time of ion arrival into the analyzer cell. Figure 2 shows that a 1.5 μs pulse has a dramatic effect ~9 μs after the laser trigger but diminishes after ~30 μs . Figure 3 shows an experiment with a 10 μs pulse conducted over a longer time period. After the initial sharp effect seen in Figure 2, there is gradual and increasing ability of the pulse to quench ions in the analyzer cell beginning at 200-300 μs .

Figure 1 can be interpreted as demonstrating a ~6 μs delay between the computer trigger and the laser discharge. The laser manufacturer acknowledges possible firing delays in the μs range. Figure 2 then implies that ions have transited from the probe tip to analyzer cell in ~3 μs , which requires that the Mo (92 daltons) ion energy be ~100 eV. It is difficult to justify the production and subsequent trapping of such energetic ions in the 2V trapping well. It is also difficult to reconcile the absence of a quenching effect in the 30 to 200 μs range.

The simplest explanation for these results is that ions which are observed to be unintentionally transferred are the result of creating a dense plasma by the laser ionization process. In the probe tip region, the ions are uninfluenced by the applied voltages for several microseconds. Likewise in the analyzer cell, initially a large number of ions are trapped and not influenced by the applied voltages for several hundred microseconds, perhaps until a number are lost or diffuse in the cell. This would be consistent with the observation that unintentional transfer usually occurs at higher laser powers. Since the geometry of the icr cell is rather open with large entry holes, the electric fields which move ions are probably relatively small even with the above applied voltages. This suggests a more careful design of the icr cell should be done to produce stronger electric fields near the probe tip to draw ions into the cell.

We would like to thank Dr. Peter Todd of Oak Ridge National Laboratory for the design and construction of the high voltage pulse circuitry and the National Research Council Fellowship Program for support of JPH.

FIGURE 1

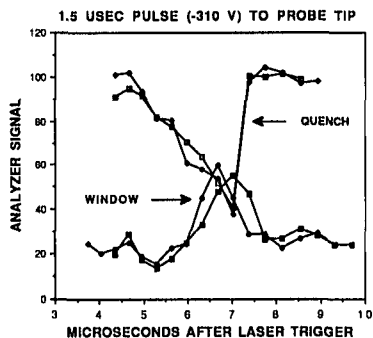


FIGURE 2

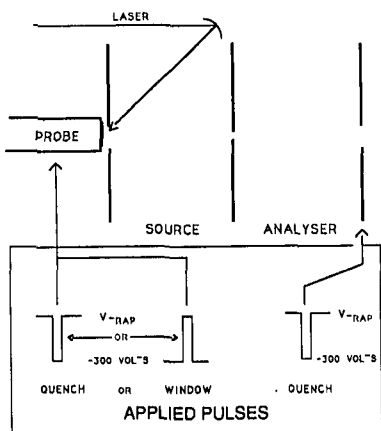
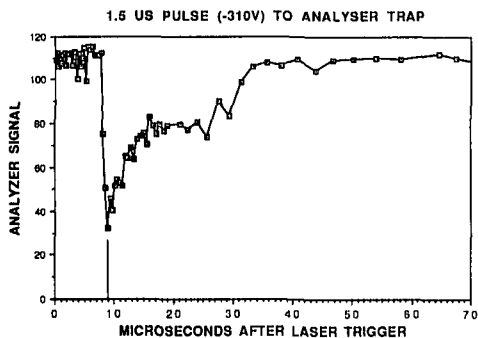
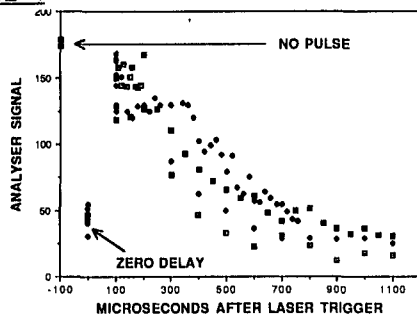


FIGURE 3

SCHEME 1 10 US PULSE (-300V) ON ANALYSER TRAP



LASER DESORPTION WITHIN THE ION TRAP CAVITY: OPTIMIZATION AND APPLICATION

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In previous work we demonstrated that a solids probe can be brought directly into the cavity of a Finnigan Ion Trap Detector (ITD) (1). A schematic diagram of this arrangement is shown in Figure 1. A non-conducting probe tip passes through a hole bored radially in the ring electrode. The probe tip sits flush with the inner wall of the ring electrode, where a CO_2 laser pulse is focussed onto it by a ZnSe lens.

This instrument was used to demonstrate the capabilities of laser desorption (LD) on the ITD. LD ions could be formed, trapped and analyzed within the ITD cavity. The ITD tuning remained the same as it was before the modifications were done, as long as the probe tip was in place. Pseudomolecular ions and fragmentation typical of CO_2 laser desorption were observed (Figure 2).

Lower mass ions did not need helium buffer gas to be trapped. For example, cluster ions K_2Cl_2^+ could be stored for 20 ms without helium, and resolution was found to increase over this long storage period. On the other hand helium was necessary to detect higher mass ions, as is the case with other ITD configurations. When using helium it was necessary to use short storage periods, 100 μs or less, because molecular ion intensity decreased over longer storage time.

The LD/ITD produced a sufficient number of ions to exceed the space charge limit, resulting in loss of resolution and mass assignment accuracy. This was controlled by minimizing the laser spot size and then adjusting the helium pressure for each sample. Unit resolution could then be obtained, with helium pressure typically 1×10^{-3} torr or higher.

Peak height was studied as a function of exclusion limit, which is the m/z value below which all ions have unstable orbits and are not trapped. In laser desorption MK^+ peak heights of sucrose and sucrose monocaprates were found to decrease dramatically as exclusion limit was raised above 40. This suggests that K^+ ions are stored before combining with desorbed neutrals, and that MK^+ complexes do not come off the probe already formed.

Because the electron filament assembly was left intact, laser-desorbed neutrals could be ionized by electron impact. This technique yields additional fragmentation due to the higher energy imparted to the molecule. Chemical ionization also took place when the LD/ITD was operated with the filament on, evidenced by appearance of MH^+ .

This work is part of our ongoing development of methods to rapidly identify marker lipids from bacteria. Molecular ions of the pigment serratamolide were observed when a lipid extract from the bacterium *Serratia marcescens* was placed on the probe. The LD/ITD was also used to produce molecular and fragment ions from lyso- (lacking one fatty acid) phosphatidylcholine, -phosphatidylethanolamine and -phosphatidylglycerol.

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FIGURE 1

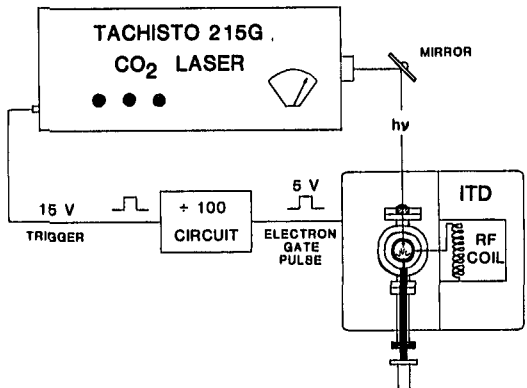
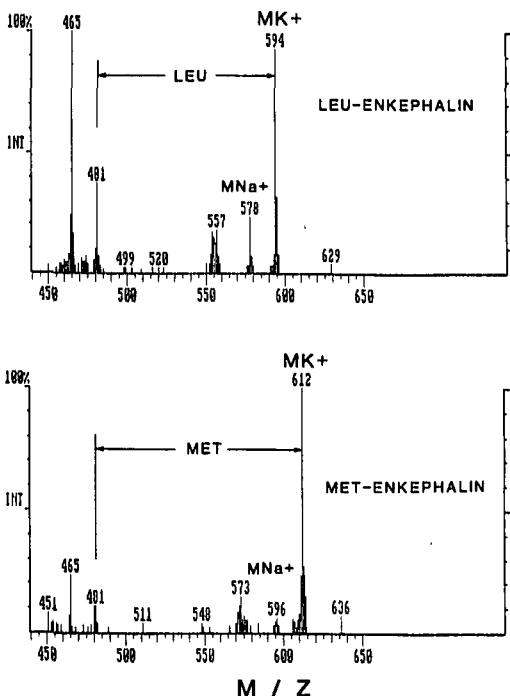


FIGURE 2



**APPLICATIONS OF LASER DESORPTION
IN AN
ION TRAP MASS SPECTROMETER**

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The three-dimensional quadrupole ion trap has recently become practical for mass spectrometry following the development of the mass selective instability mode of operation. Several features of ion trap make it attractive for combination with laser desorption: the "pulsed" nature of operation, the ability to do multiple stages of mass spectrometry, and the lack of pressure problems associated with the desorption. This paper describes the technique and results for laser desorption mass spectrometry (LDMS) experiments in the quadrupole ion trap.

The experiments were performed with the Finnigan MAT Ion Trap Mass Spectrometer (ITMSTM). The standard electrode structure was modified by drilling two 3.18 mm holes through the ring electrode, 180 degrees apart. These holes were aligned in the vacuum system such that a sample probe could be introduced into one them via the solids probe inlet. The laser beam impinged on the sample probe through an optical port and the other hole in the ring electrode. The sample was introduced on a probe constructed from a 1.25 cm diameter stainless steel rod fitted with a removable MacorTM tip. A Chromatix 1000 Nd:YAG laser was used; the 1064 nm wavelength output with a typical energy per pulse of 1.5 mJ was employed for all experiments.

The experimental scan functions were developed and data acquired using standard Finnigan software (ITMS Rev. B). A typical scan function for an LDMS experiment is shown in Figure 1. A variable delay trigger pulse from the ion trap control electronics, set via software, was amplified and used to trigger the laser. The duration of this delay was varied, depending on the length of the total scan function, to adjust the repetition rate of the laser output to about 10 Hz.

A typical ITMS laser desorption mass spectrum for trimethylphenylammonium chloride is shown in Figure 2. The intact cation is observed at m/z 136 with fragments at m/z 120 and 121. No higher mass cluster ions were observed for this compound, nor have they been seen for other compounds which have been examined. The ion trap has been demonstrated to be a very efficient instrument for performing MS/MS experiments in combination with conventional EI and CI ionization methods. This function is obtained by adding an appropriate amplitude dc voltage to the ring electrode, for parent ion isolation, followed by a "tickle voltage" to the end cap electrodes at the secular frequency of the parent ion. This technique is also possible for laser desorption ionization. The MS/MS spectrum in Figure 3 was obtained by mass selecting the laser desorbed, intact cation of trimethylphenylammonium chloride as the parent ion (m/z 136), then turning on the tickle voltage to kinetically excite the parent ion for CID.

Experiments were performed to determine the effect of ion trap operating parameters for rf trapping level (low-mass cutoff) and bath gas pressure during laser desorption. A plot of the low-mass cutoff versus ion intensity is shown in Figure 4; the trapping efficiency for laser-desorbed ions rises rapidly from the onset at an rf level corresponding to about 11 daltons, then quickly begins to level off prior to an rf level corresponding to about 20 daltons.

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The ion trap is typically operated with a He bath gas pressure of about one millitorr. The results of experiments to investigate the effect of bath gas pressure on laser desorption mass spectra in the ion trap are shown in Figure 5. The decrease in signal from run to run is due to consumption of sample during the course of the experiment. This data suggests that the bath gas pressure is important to the trapping efficiency in LDMS; without damping collisions to trap the desorbed ions in the potential well they would continue through the trap and be lost.

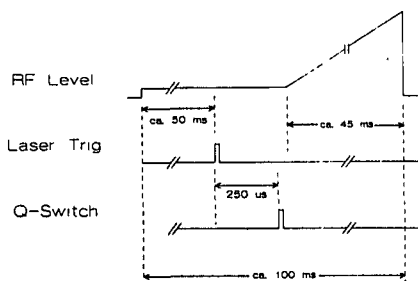


Fig. 1

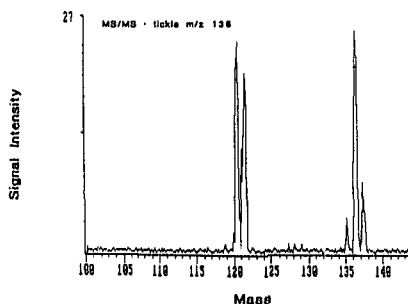


Fig. 3

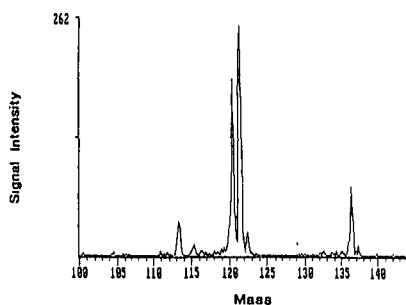


Fig. 2

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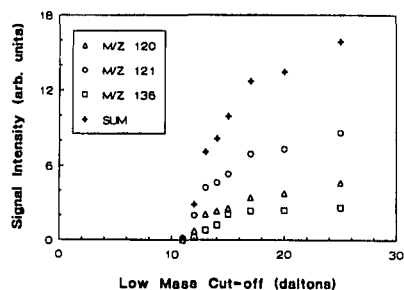


Fig. 4

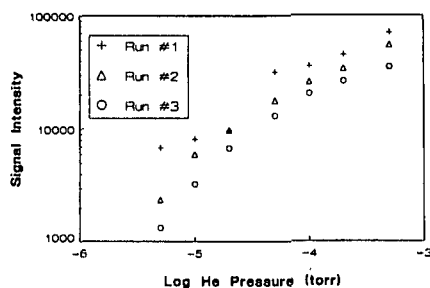


Fig. 5

AMMONIUM HYDROXIDE CHEMICAL IONIZATION MASS SPECTROMETRY

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Proton-transfer reactions are well-known in low-pressure chemical ionization mass spectrometry (LPCIMS) with ammonia reagent gas [1-3]. We have found that bimolecular ammonium attachment reactions can also occur for polar analytes [4]. Here we report a method for avoiding undesirable reactions [5] between ammonia and analyte ions. We also show that ammonium hydroxide is a convenient and inexpensive substitute for anhydrous ammonia as a reagent for LPCIMS.

Reactions can occur between ammonia and the ionized analyte [4,5] in ammonia chemical ionization. These reactions can cause a loss of sensitivity. A loss of selectivity can also occur because different reagent ions such as $[\text{NH}_3]^+$ and $[\text{NH}_4]^+$ may react with the analyte by different mechanisms. These problems can be eliminated by using a Fourier transform mass spectrometer with a dual trapped-ion cell to isolate the reagent ion from the reagent gas and from other reactive species. A schematic diagram of this experiment (which may be referred to as "isolated reagent ion chemical ionization") is shown in Figure 1. The reagent gas is introduced into one of the trapped ion cells (Cell 1) and the analyte is introduced into the other trapped-ion cell (Cell 2). Electron ionization produces ions from both the reagent gas and the analyte. The analyte ions are removed ("quenched") from Cell 2 while the ions in Cell 1 (primarily $[\text{NH}_3]^+$) react to produce $[\text{NH}_4]^+$. The $[\text{NH}_4]^+$ is then transferred to Cell 2 by grounding the common electrode (the "conductance limit") between the two cells. Ionization of the analyte occurs in Cell 2 by proton transfer from the transferred $[\text{NH}_4]^+$ or by ammonium attachment. Because the analyte is separated from the reagent gas, no undesirable reactions can occur between ammonia and the ionized analyte.

If ammonium hydroxide is used as the reagent gas, several reactive species such as $[\text{H}_2\text{O}]^+$, $[\text{H}_3\text{O}]^+$, $[\text{NH}_3]^+$, and $[\text{NH}_4]^+$ are present at short reaction times (a few ms). At longer reaction times (> 100 ms), the dominant reactive species is $[\text{NH}_4]^+$. Under typical LPCIMS conditions, ammonium hydroxide produces mass spectra that are virtually identical to those obtained with anhydrous ammonia.

Mass spectra obtained with "isolated reagent ion chemical ionization" can show striking differences from the chemical ionization mass spectra obtained with a single trapped-ion cell. This is illustrated in Figure 2 for ammonium hydroxide LPCIMS of phenetole. When the chemical ionization is carried out in a single trapped-ion cell (Figure 2a), the major analyte species is M^+ (m/z 122), formed by charge exchange from $[\text{NH}_3]^+$. If a dual trapped-ion cell are used ("isolated reagent ion chemical ionization", Figure 2b), the major analyte species are $[\text{M}+\text{H}]^+$ (m/z 123) and $[\text{M}+\text{NH}_4]^+$ (m/z 140), formed by reactions of $[\text{NH}_4]^+$.

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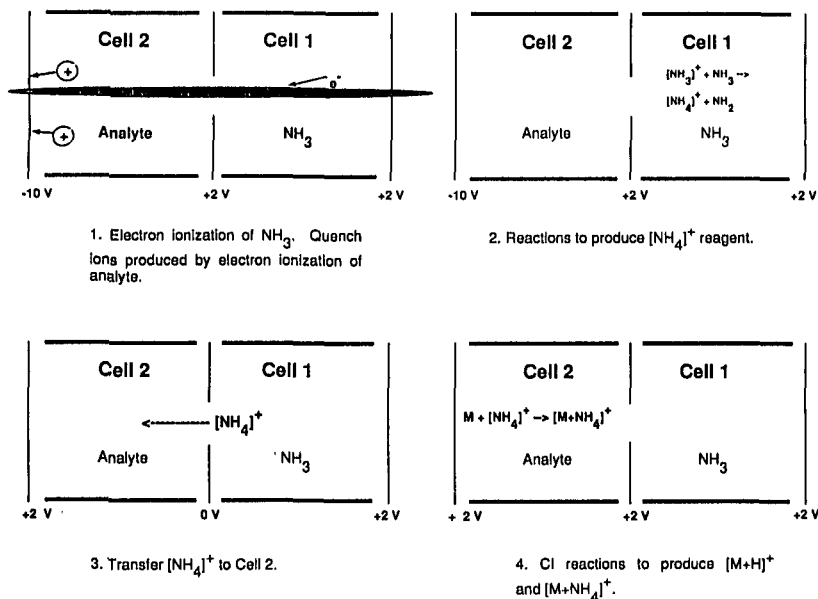


Figure 1.

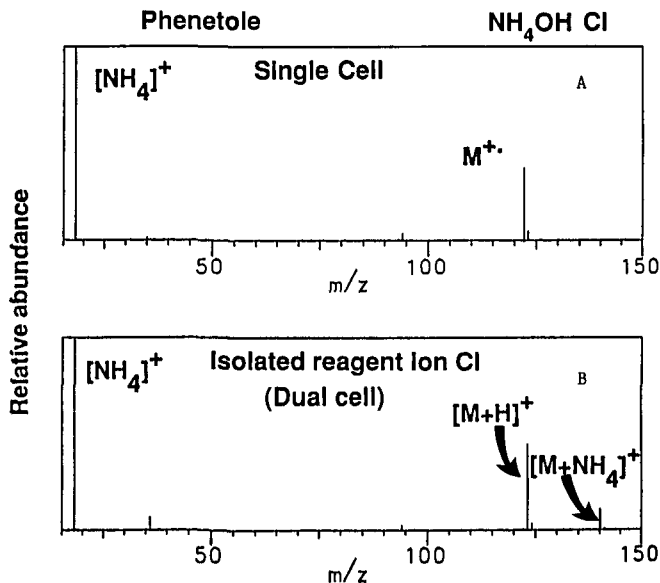


Figure 2.

ION TRAP TANDEM MASS SPECTROMETRY (MS/MS) FOR THE DETERMINATION OF EICOSANOIDS

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Introduction -- Some arachidonic acid metabolites, generally called eicosanoids, have been implicated to play a role in the inflammatory response. New drug candidates for the anti-inflammatory category often exhibit inhibition of the cyclo-oxygenase (COX) and lipoxygenase (LOX) enzymes that are involved in the production of the mediators. Simple and sensitive assays are desirable for eicosanoids to evaluate COX/LOX inhibition. Validation of radioimmuno-assay data remains important.

Our original GC/MS/MS work was sensitivity limited by the low CAD efficiency of the sector B-E instrument (1). The role triple stage quadrupoles will play is still uncertain, particularly in regard to CAD efficiencies obtained for eicosanoids. The ion trap mass spectrometer (ITMS) gives 60-75% CAD efficiency consistently for these compounds. The ITMS has been demonstrated to obtain GC/MS data on 10-50 pg of various analytes. The objective of this work is to utilize the high CAD efficiency of the ITMS and the advantage of RF/DC parent ion isolation for the determination of eicosanoids, particularly, the inflammation mediators PGE₂ and LTB₄.

ITMS Modifications -- To ensure efficient transfer of these higher boiling eicosanoid derivatives we: a) installed a thermally-stable electron multiplier for operation of the ITMS at elevated temperatures (180°C) and b) improved the GC/ITMS interface, based on use of a short TSQ-70 transfer line. While the TSQ-70 transfer line is much more convenient to work with, the elevated temperature capability is the most critical factor for the transfer of these higher boiling derivatives.

Determination of PGE₂ -- Based on other work from our laboratory (2), the structurally simplest derivative and a chemical internal standard (CIS) are used in this study. The full scan mass spectrum of PGE₂ MeMOXTMS₂ derivative is shown in Figure 1. The mass spectrum of the CIS (16,16 dimethyl PGE₂) is dominated by m/z 295 (same ionic structure as the m/z 295 in the PGE₂ derivative spectrum), probably due to the formation of the more stable neutral fragment, a tertiary radical.

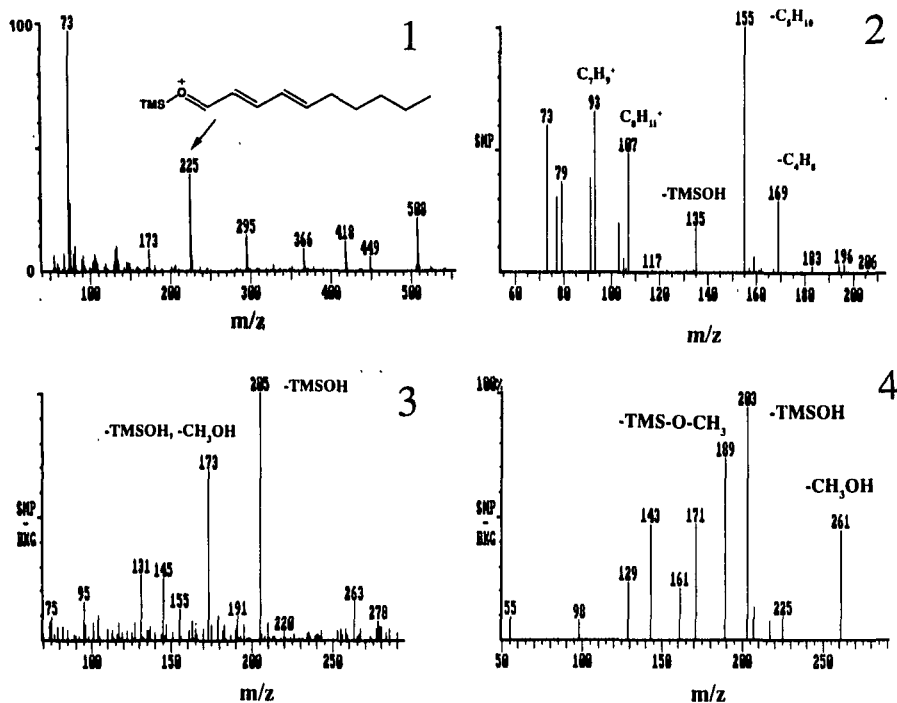
RF/DC isolation for MS/MS is employed as described in our previous work (3). This type of scan is crucial for the analysis of target compounds in complex matrices (4). It allows storage of one m/z value and ejects ions of both lower and higher masses out of the trap. In this case the parent ions isolated for MS/MS are m/z 225 and m/z 295 for PGE₂ and 16,16 dimethyl PGE₂ derivative, respectively.

Daughter ion spectra are obtainable on 100 pg. Figure 2 shows the spectrum from the GC/MS/MS analysis of the PGE₂ derivative (parent ion m/z 225). The major daughter ion is m/z 155 (-C₅H₁₀). This is a backbone specific fragmentation predicted to be highly selective for the analyte. One of the advantages of the ITMS is that all daughter ions are collected and therefore selectivity of the different types of transitions can be ascertained in a single run. The daughter ion spectra of the chemical internal standard (parent ion m/z 295) is shown in Figure 3. The major daughter ion is m/z 205 (-TMSOH), a derivative specific fragmentation. This transition is predicted to be less selective than the backbone specific fragmentation. The second largest daughter ion m/z 173 (-TMSOH, -CH₂OH) is a doubly derivative specific fragmentation and is predicted to be more selective than m/z 295⁺→205⁺.

Initial LTB₄ work -- The full scan mass spectrum of LTB₄ MeTMS₂ is similar to that obtained on other types of mass spectrometers. The most intense ion above m/z 200 is m/z 293. Qualitatively this seemed to be the best choice for the parent ion in the MS/MS experiment.

The daughter ion spectra of m/z 293 is shown in figure 4. The most intense daughter ion is m/z 203 (-TMSOH), a derivative specific fragmentation. The next most intense daughter ion is m/z 189, a rearrangement ion from loss of TMS-O-CH₃. This transition may be more selective than the loss of TMSOH due to unusual rearrangement involved to produce the specific loss of 104 u.

We are currently evaluating spiked urine and cell culture media using the above described GC/MS/MS techniques. We hope to determine the most selective detection scheme for both LTB₄ and PGE₂ in biological samples with minimal sample preparation.



ABBREVIATED REFERENCES

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ION TRAP QUANTITATIVE MS/MS ASSAYS USING RF/DC

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INTRODUCTION -- The Ion Trap Mass Spectrometer is known for its ability to analyze very small quantities of material. For instance, it is well documented that full-scan GC/MS experiments can be performed at the 10-50 pg level for a number of analytes. Our work has been aimed at exploiting the capabilities of the ion trap using combined RF/DC voltage scan functions¹.

Our principle areas of interest are: 1) high sensitivity tandem mass spectrometry (MS/MS) assays of crude biological extracts²; the trap consistently gives 60-100% observed CAD efficiencies and RF/DC is necessary to eliminate matrix ions efficiently; 2) reactive collisions of CI reagent ions stored mass-selectively by using RF/DC³.

In this paper we report on quantitation of a drug [I] with a chemical or [¹³C],[¹⁸O] stable-isotope internal standard (CIS and SIS, [II] and [III], respectively). The structures are shown in Figure 1. This particular assay is run routinely on a triple-sector-quadrupole instrument⁴.

Mass Spectrometry -- The EI mass spectrum of [I] is uncomplicated (Figure 2). McLafferty cleavage produces the odd-electron enol ion (m/z 248). Alpha cleavage of the ketone could produce the even-electron ion at m/z 233. The ion at m/z 248 was subject to RF/DC isolation and MS/MS¹. It was dissociated with 100% efficiency to only m/z 233. Loss of the methyl radical had two sources -- the t-butyl group or the methyl-phenone form of the McLafferty product (supporting isotope data not shown). This reveals other paths to m/z 233 in the EI mass spectrum. A similar spectrum is obtained for [II] and in [III] the ions are shifted by 3 u. The SIS contained less than .1% of [I].

Quantitation -- Two calibration lines were established, one using the CIS and the other using the SIS. Sample introduction was by capillary GC (Figure 3) using a high split ratio (100:1). The calibration ran from 100 pg to 10 ng (7 points) of analyte, [I], with 5 ng internal standard going to the ion trap, in both cases. Linearity over the range and the relative standard deviation (RSD) at the 100 pg level of [I] were examined (Table 1).

The calibration solutions were first examined using a typical quadrupole mass spectrometer in the selected-ion-monitoring (SIM) mode (Table 1, A,B). The regression lines were then examined in three modes on the ITMS: 1) RF-only, full scan, with automatic gain control (AGC), 2) RF/DC mass-selective storage followed by detection (analogous to SIM) and finally 3) the RF/DC isolation followed by MS/MS and detection of daughter ions.

ITMS Results -- Regression lines obtained with the co-eluting SIS gave unacceptable results while the chemical internal standard worked well in all modes tested. (Table 1, C-G). Co-elution of the internal standard is undesirable in the present configuration of the ITMS.

AGC-RF only -- Because of the relatively high concentration of the co-eluting SIS, low ionization times are selected by AGC. Therefore, for several points the number of analyte ions created is small, causing poor ion statistics and scatter in the data (Table 1, C). On the other hand, the CIS [II] is separated from the analyte on the column and AGC imparts a favorable dynamic range (Table 1, D). Long ionization times are selected for small amounts of analyte. We are examining more narrowly bracketed applications of the SIS.

RF/DC -- In this case, to avoid the problem cited in AGC control, m/z 248 and m/z 251 must be created, isolated, and analyzed in an alternate fashion, using separate RF/DC scan functions. For instance, in the case of 100 pg of analyte (m/z 248), a 10 ms ionization pulse will produce far too many ions from the 5 ng of SIS (m/z 251). However, the RF/DC sequence will reject those ions before m/z 248 is analyzed, correcting any space charge condition.

The data scatter (Table 1, E) is computer hardware related. The scan functions are continuously accessed from the hard disk of the PC and this is a slow process. Only two scans per second are acquired resulting in poor definition of the capillary peak and poor averaging of the data. The CIS works well (Table 1, F). It uses the same scan function as the analyte (m/z 248). No alternating is required. Typically, four scans are averaged every 250 ms.

RF/DC MS/MS -- The result was a linear calibration line using the CIS. The RSD was less than 4% (Table 1, G). These compounds represent a most favorable case -- only one daughter ion is formed and with 100% efficiency. We are now proceeding to examine these same calibration lines from spiked plasma samples with on-column injection of 1 μ l volumes of crude extracts.

We believe the problem with the stable-isotope internal standard will be overcome by small changes in computer hardware. RF/DC isolation will still be necessary to accommodate high dilution ratios. Finally, in drug development programs it is likely that related chemical internal standards are more readily available. The GC/MS/MS results presented here are encouraging.

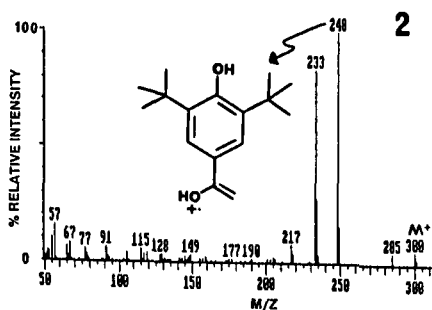
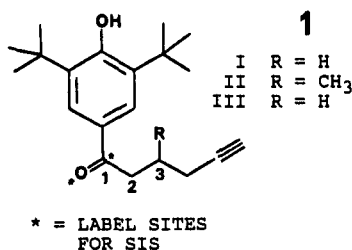
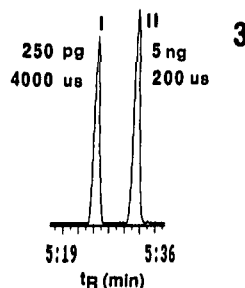


TABLE 1.

REGRESSION LINE	r ²	RSD %	INT. STD.
A) y = 1.224x - .001	.9999	1.1	SIS
B) y = 0.769x - .005	.9999	2.7	CIS
C) y = 1.151x - .051	.9927	20.0	SIS
D) y = 0.805x - .008	.9992	6.0	CIS
E) y = 1.264x - .000	.9901	50.0	SIS
F) y = 0.857x + .011	.9998	3.0	CIS
G) y = 0.854x + .011	.9997	3.7	CIS



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PHASE-SYNCHRONIZATION OF THE ION ENSEMBLE THROUGH OFF-RESONANCE EXCITATION IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE

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The FT-ICR signal is produced by accelerating an ion population into a cyclotroding packet which induces an image current in the receive plates of the ICR cell. The performance characteristics associated with FT-ICR (e.g., sensitivity, mass resolution, mass range, and Fellgett advantage) arise from the ability to produce a spatially defined ion packet from an initially random ion ensemble. Spatial definition of the ion packet is defined by a combination of the radial distribution (distribution of kinetic energy in the X-Y plane) and the radial dispersion (angular distribution). In most FT-ICR experiments (performed with electron impact ionization) the radial distribution of the initially formed ions is small and the ions have near-thermal kinetic energies. Consequently, the radial distribution of the ions does not adversely affect ion detection. It is important to note that some ICR experiments (e.g., experiments utilizing methods which involve momentum transfer, such as SIMS, collisional ionization, or ion injection from an external source) are not adequately described by models based on electron impact ionization of a static gas.

The principle factor in the production of a coherent ion packet is the ability to drive an ensemble of ions having initially random velocity vectors (e.g., random phase angles) into coherent motion (i.e., having a narrow angular distribution). We recently demonstrated that the final radial distribution of a population of ions is dependent upon how quickly the random ensemble is "driven" into coherent motion (*1*); thus, ion detection by FT-ICR is a direct result of the phase angle synchronization of the initially random ion population. Phase synchronization of ions trapped in an FT-ICR cell occurs through interaction with an applied electric field. Because no mechanism exists for moving ions out-of-phase with respect to the electric field, phase synchronization is *directional*. For example, ions which have the same relative phase angle will experience identical force vectors at any time during the oscillation of the electric field. As long as the forces acting on two ions are the same, the trajectories of the ions will be the same. Thus, two ions driven into phase coherent motion by an applied electric field, will be influenced by identical forces following phase synchronization. Because the two ions have similar trajectories, they continue to move as a phase coherent packet. Therefore, once an initially random ensemble of ions driven into phase coherent motion, it will remain coherent (in the absence of collisions or inhomogeneous fields).

The mechanism for both ion excitation and phase advancement is the application of a resonant oscillating electric field. An ion moving in-phase with the applied rf is continuously accelerated (acceleration is caused by the synchronous alignment of the ion's motion with the force vectors produced by the rf field) whereas an ion moving out-of-phase ($\neq 0$) with the applied rf field experiences a synchronous *misalignment* of the velocity and electric field force vectors. The misalignment of an out-of-phase ion with the applied rf electric field creates a net force perpendicular to the ion motion ($F_{\perp} = qE_{\perp} = qE \sin \gamma$). The frequency shift required to move an out-of-phase ion into synchronous alignment with the applied electric field is achieved by effect of $F_{\perp}$ on the ions angular frequency. The perpendicular component of the applied electric field causes a shift in the angular frequency of the ion (i.e., $\omega_{rf} \neq \omega_{ion}$) allowing phase advancement and synchronization of the ion ensemble to occur.

In a cubic FT-ICR cell, a high mass ion cannot interact with a resonant oscillating electric field as long a low mass ion because of the discrete (energy) boundaries of the cell. The impact of phase synchronization and phase advancement therefore increases with increasing mass and energy. High mass ions injected into the ICR cell (from an external ion source or from a surface bombarded by an energetic particle) with significant translational energies yield a poorly defined ion packet following excitation. The combined effects of both increasing the deviation of the different radii and relative center shifts results in an inability to produce coherent ion motion for high mass ions having

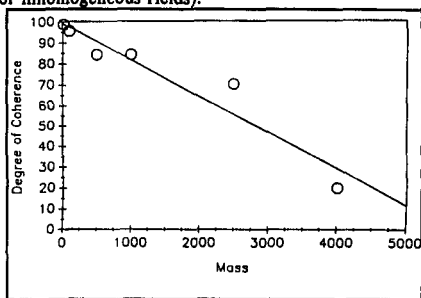


Figure 1. The plot contained in Figure 1 illustrates the decrease in the degree of coherence (defined by $(r_{ave} - r_{dev})/r_{ave}$) which is obtained by resonant acceleration as a function of mass.

initially significant (ca. 3 eV) kinetic energies and random phases. The plot contained in Figure 1 illustrates the decrease in the degree of coherence (defined by $(r_{\text{ave}} - r_{\text{dev}})/r_{\text{ave}}$) which is obtained by resonant acceleration as a function of mass. In this relationship, r_{ave} is the average radius achieved by the ion ensemble following excitation and r_{dev} is average deviation of the different ions about that radius. Such a deviation is therefore a function of both the center shift and radial deviation

In a typical broadband FT-ICR experiment, ion excitation is achieved by sweeping an rf oscillator over a range of frequencies corresponding to the mass range of interest. Individual ions experience a net gain of translational energy as their respective frequencies come into resonance. Although ions only receive a net gain in kinetic energy during resonant excitation, ions experience the electric field force during the entire excitation period. Because phase synchronization is directional, ions are driven into coherence through interaction with the electric field prior to resonant acceleration. Ions which interact with an electric field prior to a resonant excitation are phase synchronized prior to a net gain of translational energy. Contained in Figure 2 is a plot of the effect of sweep duration on the relative dimensions of the ion ensemble. The degree of coherence achieved for four ions ($\gamma=0, 90, 180, 270$ and 3 eV of kinetic energy) of m/z 500 is determined for different interaction times prior to resonant excitation. From this relationship, it is clear that longer interaction times with the rf electric field prior to resonant excitation results in a better defined ion packet for detection by FT-ICR. In order to understand, the processes of phase angle synchronization of the ion ensemble, the effect of ion interaction with a non-resonant rf electric field must be examined.

The effect of frequency swept phase synchronization on ion detection has been observed experimentally. Early Cs⁺ desorption experiments showed a dramatic loss of signal when the translationally hot ions were detected in a high resolution narrow band mode compared to a broad band detection scheme. That is, in the narrowband mode (either direct or heterodyne) off-resonant excitation does not occur. Similar observations have been made for tandem quadrupole/FT-ICR experiments. Although detection of high mass ions is possible by broadband frequency sweep excitation (chirp), the signal is greatly attenuated in the narrowband mode (i.e., near resonant excitation with reduced interaction time with the electric field). Observations such as these are difficult to quantitate, but are consistent with the problems (which we attribute to phase synchronization) presently limiting the high mass analytical utility of FT-ICR.

The process of phase synchronization on the dimensions of the ion packet following excitation is difficult to evaluate in a simple cubic ICR cell. Ions which are produced as a result of electron impact have kinetic energies corresponding to that of the neutrals prior to ionization. Thus, ions are driven into phase coherence quickly and accelerated as a well defined ion packet, thus the impact of phase synchronization is negligible. On the other hand, ions which are formed with appreciable kinetic energies are introduced into the cell with undefined velocities and spatial locations. Direct measurements of the spatial dimensions of the ion packet in a simple cubic cell is difficult due to the lack of an unambiguous diagnostic procedure for probing the effect of phase advancement and ion excitation. By utilizing energy selective partitioning in a two-section cell it is possible to evaluate the effects of ion kinetic energy and phase synchronization on the dimensions of the ion ensemble following ion excitation. Such studies of the dynamics of ion detection are further illustrated by computer simulations of ion motion. The objective of this paper is to discuss the process of phase advancement and evaluate the effects of off-resonant acceleration prior to resonant excitation on the formation of a coherent ion packet for detection by FT-ICR.

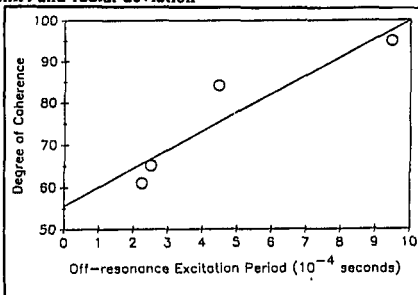


Figure 2. A plot of the effect of sweep duration on the relative dimensions of the ion ensemble.

THE EFFECT OF MAGNETIC INHOMOGENEITIES ON ION PARTITIONING IN A TWO-SECTION FT-ICR CELL

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To achieve maximum performance in Fourier transform ion cyclotron resonance (FT-ICR), ion detection must occur in the absence of ion-neutral collisions. Because the duration of observation of the time domain ICR signal directly determines sensitivity and resolution, ICR detection is most effective at pressures of 10^{-8} torr or less. The pressure limitations on FT-ICR detection can be circumvented by separating sample introduction and mass analysis. Separation of these steps can be achieved either temporally (e.g. by the use of pulsed valves^{1,2}) or spatially (e.g. by the use of external ion sources^{3,4,5,6} and the two-section cell⁷). In each of these approaches, the desired result is coupling of a high pressure (10^{-7} torr or greater) experimental step (e.g., ionization, ion-molecule reaction, or collision-induced dissociation) with ion detection at low pressure (10^{-8} torr). Remote source techniques require that ions move from the source into the detection region; however, movement of ions from one region to another requires that the ions be accelerated toward the detection cell. That is, ions which have relatively low translational energy along the axis between source and cell (Z-axis) are accelerated. Thus, the process of Z-axis excitation changes the conditions under which ion detection is performed.

Here, we describe the effects of axial magnetic inhomogeneities on ion partitioning and subsequent trapping of the ions in a two-section cell FT-ICR. Axial inhomogeneities arise because of the weakly ferromagnetic or strongly paramagnetic properties of the materials used for construction of ICR cells. The effects of axial inhomogeneities on the FT-ICR signal have been studied previously and were found to have little effect on resolution or signal broadening.⁸ Factors influencing ion partitioning in two-section cells have also been considered (e.g. pressure or ion mass) but without considering the effects of magnetic inhomogeneities.^{9,10,11} Axial magnetic inhomogeneities localized in the conductance limit aperture of a two-section cell constructed of high magnetic susceptibility material cause significant redirection and spatial distribution of low energy ions entering the ion cell. Therefore, a semi-quantitative description of trapped ion distributions due to low-energy injection through an axially inhomogeneous magnetic region is useful for considering the design requirements for external ion source FT-ICR instruments. For example, some type of ion guide (either rf only quadrupoles or electrostatic lens elements) is required for external ion source instruments. The effect of a given means of injection on ion detection must be considered. Ions which are injected into the ion cell must have relatively low Z-axis kinetic energies. Thus, at some point along the ion path (source to ion cell) low energy ions must pass through a potentially inhomogeneous magnetic field resulting in ill-defined ion trapping conditions and reduced instrument performance.

Magnetic field topologies for two two-section cell materials were calculated by using the Poisson/Superfish codes.¹² The Poisson representation of the magnetic field was solved numerically by implementing a nonuniform triangular mesh.¹³ Input for the program consists of defining the parent magnetic field strength (H), the geometry of the two-section cell, and the magnetic permeability ($1+\chi_m$) of the cell material. Magnetic susceptibilities (χ_m) were measured by the de Guoy method. Calculations were performed on a VAX 11/780 computer at TAMU. Ion trajectories were calculated and displayed using the trajectory calculation program SIMION (V4.0)¹⁴ on a math co-processor equipped PC/AT type IBM compatible computer. SIMION allows placement of electrodes in a user defined array as well as allowing magnetic fields to be defined along any axis within the array. Magnetic fields were vectorially added by coordinate as estimated from the Poisson plots. The Poisson plot for 304 SS used as cell material at TAMU ($\chi_m=0.16$) is diagrammed in Figure 1.

Using SIMION, ions were placed in a two-section FT-ICR cell such that their cyclotron orbit guiding centers coincided with the Z-axis. The ions had 0.01 eV of translational energy in the X-Y plane and various energies directed along the positive Z-axis. Figure 2 shows a trajectory plot of ten ions ranging from 0.19 to 3.48 eV of Z-axis energy as they are allowed to undergo partitioning through the axial inhomogeneity defined above. It is

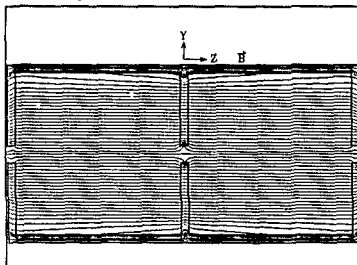


Figure 1. Poisson plot of two-section cell constructed of paramagnetic 304 SS bathed in a 3 tesla magnetic field.

apparent that the conductance limit aperture inhomogeneity dramatically affects ion partitioning trajectories. It is noteworthy that no deflection of the ion paths is observed for the same calculations performed on an oxygen free hard copper cell (not shown). Three basic trajectories (or combinations of these) are observed for the stainless steel cell; (i) the ion partitions but gains energy in the X-Y plane usually followed by collision with the conductance limit, (ii) the ion collides with the conductance limit during the first pass, or (iii) the ion undergoes reflection and subsequent collision with the conductance limit. In the first case, ions can normally be trapped in the second cell region by proper timing of the conductance limit grounding pulse. Guiding center shifts occur for all ions upon entering the axial inhomogeneity within the aperture. Projection of the data in Figure 2 onto the X-Y plane shows that the axial inhomogeneity induces significant complexity on ion partitioning trajectories at low translational energy. In addition, one observes that ions formed with little spatial distribution and equal energy in the cyclotron orbit (X-Y plane energy) are distributed spatially and can gain significant energy in the X-Y plane upon partitioning through the axial inhomogeneity. The mechanism for cyclotron orbit energy gain is deflection into the X-Y plane due to the X-Y component of the magnetic field in the conductance limit aperture.

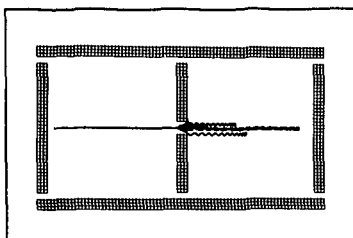


Figure 2. Y-Z plane projection of the trajectories of ten m/z 100 ions with E_z varying from 0.19 to 3.48 eV as they partition through an axial inhomogeneity.

The effect of ion mass on partitioning through an axial magnetic inhomogeneity was considered in a second study. Ions ranging from m/z 100 to 1000 were placed in the cell with 0.01 eV in the X-Y plane and 0.758 eV along the positive Z-axis. Similar results to the energy study were obtained.

Two conclusions emerge for the partitioning of ions in a two-section FT-ICR cell with an axial magnetic inhomogeneity in the region of the conductance limit aperture. First, ion deflection into the X-Y plane (measured as the X-Y plane projection of the ion's trajectory in the inhomogeneous region or the guiding center shift) decreases with increasing ion momentum. Second, the energy gain in the cyclotron coordinate during ion partitioning roughly increases with increasing momentum and is highly path sensitive. Whether energy is varied with a set m/z ratio or m/z was varied for a given injection energy, it is apparent that significant energy gain and ion deflection results for low-momentum ion injection through a magnetically inhomogeneous region. The resulting trapped ion packet has poor spatial and significant energetic distributions.

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MEASUREMENTS OF KINETIC ENERGIES OF ION MOLECULE REACTION PRODUCT IONS
BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE

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Fourier-transform ion cyclotron resonance (FT-ICR) is a versatile method for studying ion molecule reactions; however it is difficult to use FT-ICR to study dynamics of chemical reactions. For example, infrared chemiluminescence and laser induced fluorescence for determination of product ions internal energies would be extremely difficult by ICR. In addition, determination of product ion kinetic energies by ICR methods is also quite difficult. Such experiments have associated with them large errors due to undefined ion conditions (*i.e.*, ion translational energy and spatial location in the ICR cell). The inability to accurately measure either the reactant or product kinetic energies limits the utility of the instrument for chemical dynamics.

Recently, we have demonstrated that both ion location and translational energy can be mapped in a two section cell.¹ Because the radius of the cyclotron orbit depends on kinetic energy, partitioning can be used to measure radial kinetic energies. Furthermore, ion manipulation by a phase-specific excite-deexcite sequence results in defined ion location along the Z-axis.² By using the conductance limit to monitor both initial and final ion conditions, a two-section cell experiment can be designed to measure ion kinetic energies.

Kinetic energy release studies (KERD) require a uniform excitation field. The electric field lines produced in a cubic FT-ICR cell are perturbed by the detection plates which are held at a ground potential during excitation. Figure 1A contains a plot of the equipotential lines in a cell utilizing flat receive plates. It can be seen from the figure that the excitation field lines for a cubic cell contain significant radial inhomogeneities. The inhomogeneities result in electric field vectors which are not uniformly applied in the Y direction. Ions influenced by electric field vectors having components in the X direction have correspondingly different perpendicular components of the electric field during ion excitation. The difference in the angles of the applied electric field vectors produces ions having initially different spatial locations to acquire different translational energies following excitation. The resulting kinetic energy distribution limits the accuracy of detailed studies of the kinetic energies of reaction product ions. To address this issue, we have developed cell designs which significantly improve the excitation profiles such that uniform excitation can be accomplished. By replacing the detection plates with rods, the perturbations caused by the grounded detection plates are reduced resulting in an improved excitation field in the X-Y plane. Shown in Figure 1B is a plot of the equipotential lines in a cell utilizing rod receivers. The perturbations arising from the interaction of the detection plates are removed resulting in an electric field that is uniformly applied in the Y direction. The improved field lines result in a smaller kinetic energy distribution of the ion ensemble.

Although the use of receive rods improves the excitation in the X-Y plane, the interaction of the trapping field along the Z-axis causes axial inhomogeneities in the Z-direction. The resulting Z-axis excitation results in

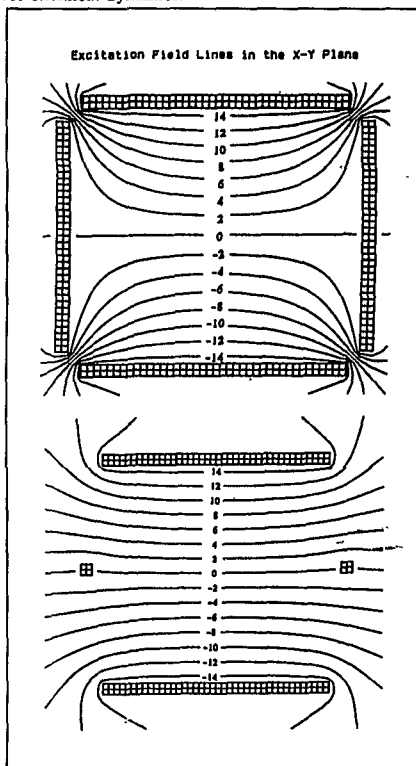


Figure 1. (A) Excitation field contours for the cubic ion cell with flat receive electrodes; (B) contours as in (A) for rod receive electrodes

non uniform excitation along that axis. To address this issue, two different cell designs have been evaluated. Shown in Figure 2 is the excitation field lines generated in a FT-ICR cell having hemispherical trap plates. It can be seen from the plot that the excitation is homogeneous throughout 80% of the cell dimensions. The ions existing at the extremes of the cell receive *no excitation* and therefore do not interact with the performed studies. The homogeneity of the excitation field can be further enhanced by incorporating electric field shimming rings. Shimming the rf excitation field was first demonstrated by Hipple and coworkers (*Phys. Rev.* 1951, 82, 697) with the introduction of the omegatron cell. The uniform excitation is achieved by linearly decreasing the magnitude of the voltages applied to the rings. This is accomplished by a voltage divider comprised of inductance-free resistors. Use of inductance-free resistors insures that there is no variation in the amplitude throughout the frequency range as well as eliminating any propagation delay across the voltage divider. By regulating the voltages applied to the rings, the field lines are redirected to produce a field which is homogeneous throughout ca. 95% of the dimensions of the ion cell. The increased homogeneity (X-Y plane) of the rf electric field enhances the uniform acceleration of the sample ions. Therefore, this type of cell design increases the ability to produce and maintain the coherent ion packet. Contained in Figure 3 is a comparison of the excitation profiles for a shimmed field corrected cell *vis-a-vis* a cubic FT-ICR cell. The excitation applied in the Y direction is dependent upon the X-Z position of the ion in the cubic trap. The amount of translational energy that an ion acquires in during a specific excitation period (dE/dT) is therefore inhomogeneous and results in a kinetic energy distribution. The excitation profile in a shimmed cell results in a small energy distribution and is therefore useful for the measurement of kinetic energies of reaction product ions.

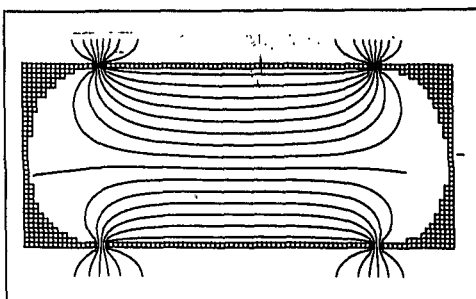


Figure 2. Excitation field contours for an ICR cell having hemispherically concave trap plates.

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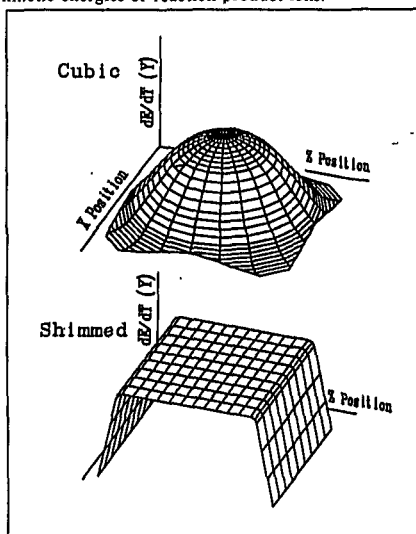


Figure 3. Comparison of the excitation profile for the cubic and the shim-corrected ICR ion cell.

A FIELD CORRECTED ION CELL FOR INCREASED PERFORMANCE IN FOURIER TRANSFORM ION CYCLOTRON RESONANCE

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The basis for mass analysis by FT-ICR is the motion of ions in a homogeneous magnetic field. Electric fields are required to modify ion motion (i.e., trapping and excitation fields). Application of inhomogeneous electric fields complicates the ion motion and leads to both loss of resolution and sensitivity. The signal is produced by observing the cyclotroding motion of a *coherent* ensemble of ions (i.e., an ion population which is both temporally and spatially coherent). Therefore, the performance characteristics associated with FT-ICR (e.g., sensitivity, mass resolution, mass range, and Fellgett advantage) arise from the ability to produce and maintain a coherent ion packet. An ion population having initially random velocities and phase angles must be driven into phase synchronous motion prior to detection.

Phase synchronization and production of the coherent ion packet requires a uniform excitation field. The electric field lines produced in a cubic FT-ICR cell are perturbed by the detection plates which are held at a ground potential during excitation. The inhomogeneities result in electric field vectors which are not uniformly applied in the Y direction. Ions influenced by electric field vectors having components in the X direction have correspondingly different perpendicular components of the electric field during ion excitation. The difference in the angles of the applied electric field vectors produces ions having initially different spatial locations and phase angles to be aligned with different temporal vectors following excitation. Such differences in the alignments of the ions following excitation corresponds to a inability to phase synchronize the ion population. The cubic ICR cell exhibits radially inhomogeneous fields which complicates both ion trapping and ion detection. Furthermore, radially inhomogeneous excitation field have been shown to cause acceleration of the ions in the Z-direction. Such coupling of the axial and radial motions of the ion population results in loss of signal and the inability to maintain a constant ion density.

We recently introduced a novel cell design which replaces the detection plates of a cubic ICR cell with rods. Utilization of receive rods significantly reduces the radial inhomogeneities in the X-Y plane created by the interaction of the rf electric field with large receive plates. Use of receive rods *vis-a-vis* receive plates results in no loss of signal sensitivity because the same number of field lines terminate on the rods as do on plate receive surfaces. The improvement of the excitation field coupled with a reduction of the capacitance of the receive surface results in an improved ability to detect ions at small radii.

Although the excitation field lines are significantly improved in the X-Y plane, interaction from the trapping plates perturbs the excitation field lines along the Z-axis. The homogeneity of the excitation field can be further enhanced by incorporating electric field shimming rings (see Figure 1). Shimming the rf excitation field was first demonstrated by Hipple and coworkers (*Phys. Rev.* 1951, 82, 697) with the introduction of the omegatron cell. The uniform excitation is achieved by linearly decreasing the magnitude of the voltages applied to the rings. This is accomplished by a voltage divider comprised of inductance-free resistors. Use of inductance-free resistors insures that there is no roll-off of the amplitude throughout the frequency range as well as eliminating any propagation delay across the voltage divider. By regulating the voltages applied to the rings, the field lines are redirected to produce a field which is homogeneous throughout ca. 95% of the dimensions of the ion cell. The increased homogeneity (X-Y plane) of the rf electric field enhances the uniform acceleration of the sample ions. Therefore, this type of cell design increases the ability to produce and maintain the coherent ion packet.

The fundamental principle of FT-ICR is the relationship between the cyclotron frequency and m/z ratio for a given ion population. It has been shown that the motion of ions in the crossed electric and magnetic

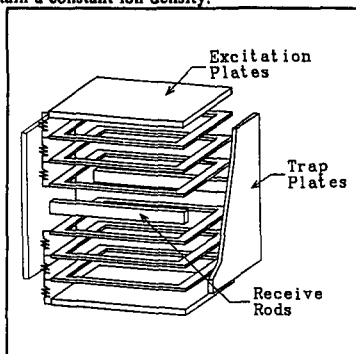


Figure 1. An FT-ICR cell is field corrected by shimming the applied electric fields. Shimming is accomplished by using a series of voltage divided rings.

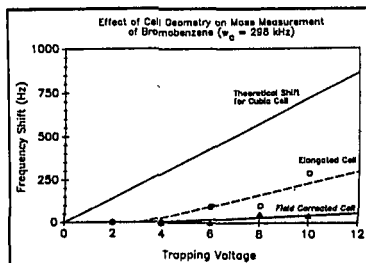


Figure 2. A plot of frequency shifts observed in a cubic, elongated and shimmed cell.

field lines arising from the trapping field cause coupling of the electric and magnetic fields (creating both ω_e and ω_d). The complicated ion motion arising from the ExB field results in loss of signal sensitivity and frequency shifts in the observe orbital frequency. The addition of the shimming rings to the ICR cell also reshapes the trapping field such that ion detection occurs in a field free region. Elimination of the ExB field effects removes the effects of ω_e and ω_d and therefore the observed frequency is equal to the ions natural cyclotron frequency. Shown in Figure 2 is the effect of the electrostatic trapping field on the observed frequency of bromobenzene (m/z 156). Frequency measurements were acquired in a two section cell comprised of an elongated FT-ICR cell ($5 \times 5 \times 7$ cm) mounted colinearly with a cubic shimmed cell ($5 \times 5 \times 5$ cm). Using combination FT allowed direct comparison of the cells under the same conditions (e.g., sample pressure, ion density, trapping voltage, etc.). It can be seen from the plot that although the elongated cell induces a smaller frequency shift than predicted for a cubic FT-ICR cell (as predicted by McIver), frequency measurements are significantly improved in a shimmed cell. Furthermore, both detected signal and peak shape are significantly enhanced in the field-corrected cell. At high trapping voltages loss of signal definition and the coherent motion of ions leads to a decrease in the signal-to-noise ratio. The degradation of peak shapes as a function of the applied electrostatic field was first proposed by Gross and co-workers. Shown in Figure 4 A&B are the narrowband mass spectra of bromobenzene achieved at a trapping voltage of 10v. Distortions of the peak shapes were not observed in the field-corrected ICR cell.

By shimming the electric fields it is possible to make frequency measurements *solely* in the presence of the magnetic field. This process eliminates both the effect of inhomogeneous excitation fields and ExB trapping effects. The result of this instrumental modification of the FT-ICR cell results in significant improvements in both ion detection and frequency measurements.

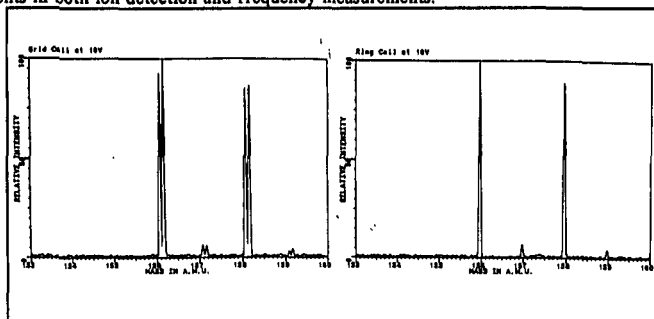


Figure 3. A comparison of narrowband spectra of bromobenzene illustrates the difference in peak shapes and signal sensitivity obtained at high trap voltages for A) a field corrected cell and B) a cubic cell.

PULSED ION INJECTION WITH AN EXTERNAL SOURCE TIME-OF-FLIGHT FTMS

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A tandem time-of-flight Fourier transform mass spectrometer (TOF/FTMS) has been constructed as a robust and versatile tandem mass spectrometer with unique capabilities for both fundamental studies of ion motion in the external source FTMS and for improved chemical analysis in realistic sampling environments. Initial experiments with the TOF-FTMS focused on development of high injection efficiency ion optics, TOF detection through the magnet, investigation of ion trapping methodologies, and procedures for front end mass selection. A diagram of the external source TOF/FTMS in its present configuration is shown in Figure 1. The system includes a 2T, 8 inch bore superconducting magnet, ultrahigh vacuum analyzer chamber, trapped ion cell and support assembly, differentially pumped pulsed electron ionization source with associated ion focusing lenses, and microchannel plate detector for TOF analysis. The field provided by this magnet is homogeneous to within two hundred parts per million over a three inch wide cubic region at the center of the magnet bore. The analyzer vacuum chamber has a total length of 1.5 meters to allow the source chamber to be mounted outside the fringing fields of the magnet. This ensures that the source electron beam is not adversely affected by the magnetic field. A conductance limit separates the ion source from the analyzer chamber and provides a pressure differential in excess of 10^3 . Electrostatic injection of ions from source to analyzer presently employs three appropriately spaced unipotential lenses to achieve near-unit injection efficiency. The first unipotential lens focuses ions out of the source and through a conductance limit aperture into the analyzer vacuum chamber. A second lens located adjacent to the analyzer side of the conductance limit focuses the ions down the length of the analyzer chamber and into a third lens located near the strong fringing field of the magnet. This third lens provides the critical focusing required to minimize ion interactions with the strong radial component of the fringing field and insure efficient injection of ions into the magnet. This series of electrostatic lenses focuses 80 to 90% of a 5uA ion beam out of the source so that it impinges on the rear trap plate of the trapped ion cell. Plots of kinetic energy distribution at the source and trap in Figure 2 are nearly identical, with the 10% reduction in longitudinal kinetic energy likely imparted to radial motion which is insufficient to reflect ions.

A fundamental requirement of the TOF/FTMS instrument as we have configured it is that acceptable TOF spectra be generated for an ion bundle which passes through the fringing field into the magnet, traverses the trapped ion cell with grounded trap plates, and then exits the magnetic field to strike a channeltron or microchannel plate detector situated outside the field. Potential disadvantages are that the ion beam will not make it through the magnet to the detector, or will generate mass spectra which are perturbed during flight by the magnetic field or electrostatic focusing. As Figure 3 demonstrates however, TOF spectra are obtained which exhibit no mass intensity discrimination and are within 5% of expected flight times based on simple TOF calculations. An additional advantage of this design is that the TOF spectrum provides an immediate indication of longitudinal kinetic energy loss or translation to radial energy. A current TOF mass resolution of about 20 will be improved in the new source operated at higher acceleration potentials.

Several ion trapping procedures have been examined in preliminary experiments including the continuous creation and injection of ions which enter the cell with trap plates grounded and are trapped when trap plate potentials above the kinetic energy of ions in the cell are established, and the pulsed introduction of ions from the source with subsequent mass selection and gated trapping. When used in conjunction with a decelerator tube positioned in front of the cell, ions injected with high efficiency at high velocities can be

trapped with small trapping voltages after deceleration. In our case, pulsed deceleration was found to easily increase sensitivity by a factor of 10 or more. A third trapping mechanism we have observed with the TOF-FTMS instrument is referred to as accumulated trapping. Even if the trap plate potentials are maintained so that a potential well exists continuously during the injection of ions with kinetic energy higher than the trapping voltages, ions are accumulated. This effect is unexpected because ions of kinetic energy greater than the trap potentials should not be retained in the cell. Because trapping potentials are maintained throughout the beam event, some longitudinal energy loss mechanism must decrease ion velocity in the well during the several microsecond flight time across the well. Possible mechanisms for this loss are: 1) ion-neutral or ion-ion collisions, 2) ion-surface collisions, and 3) translation of $V_{||}$ to $V_{\perp}$ due to electric or magnetic fields inhomogeneities in the cell. Estimates of collision rate discount ion-neutral and ion-ion interactions at the pressures and ion densities for these experiments, and our focus instead is on the latter mechanisms. Figure 4 contrasts accumulated trapping spectra of naphthalene at 8 and 18 eV accelerating potentials and suggests the possibility of a surface collision effect with increased fragmentation associated with increased internal energy deposition.

The ability to select ions in the first mass analyzer for subsequent dissociation or manipulation is essential in any tandem mass spectrometer. Hence considerable effort was devoted to developing precisely timed control of electrode potentials in the TOF tube. Although present time-of-flight mass resolution on our instrument is limited by current source design, the timing sequences are in place. This is demonstrated in Figure 3b as m/z 219 of PFTBA is selectively trapped in the cell while other ions traverse the cell and strike the TOF detector. A time-of-flight source with appropriate geometry and optics for improved mass resolution out of the source is currently being constructed.

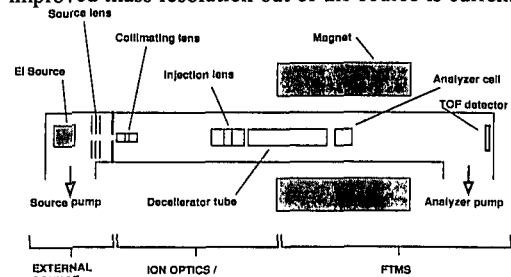


Figure 1. Diagram of TOF/FTMS in present configuration.

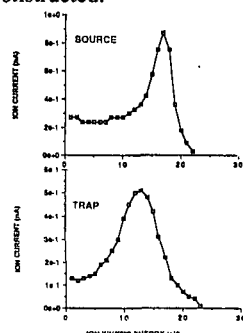


Figure 2. External source TOF/FTMS mapping of ion kinetic energy distribution.

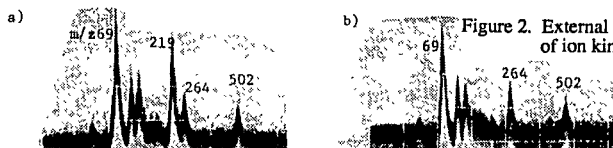


Figure 3. TOF spectrum of PFTBA acquired for an ion bundle passing through the cell and then out the magnet. a) complete PFTBA spectrum; b) PFTBA spectrum with m/z 219 ion selectively trapped in cell.

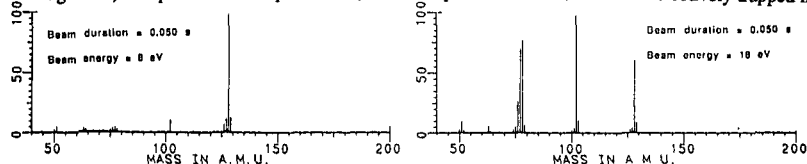


Figure 4. Accumulated trapping spectra of naphthalene at different accelerating potentials.

ANALYTICAL APPLICATIONS OF LASER DESORPTION AND PHOTODISSOCIATION FOURIER TRANSFORM MASS SPECTROMETRY

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Collaborative research efforts have led to the FTMS analysis of various samples synthesized in other laboratories. Frequently, laser desorption FTMS was able to provide analytical information where other methods had failed. For some samples FTMS provided additional data, previously unavailable, or results superior to other analytical methods.

LD/FTMS spectra of a series of methylmethacrylate/butylacrylate (MMA/BA) copolymers of different composition ratios display higher molecular weight distributions than those obtained by GPC using narrow molecular weight polystyrene standards as calibrants. As expected, as MMA to BA ratio decreases (from MMA/BA 90:10 to 70:30), the relative abundances of different composition oligomer ions shift to reflect the change in sample mix ratio.

The LD/FTMS spectrum of Zr-Pt complex was obtained to resolve whether or not the complex exists as the monomer, dimer, or a higher oligomer. The low volatility and solubility of the complex precluded characterization by thermal desorption and FAB. Abundant ions were observed which supported the proposed monomer empirical formula. Although no dimer or oligomer ions were observed, ions present at masses significantly greater than monomer ions provided evidence for the existence of some type of dimer structure or impurity. The high resolution spectrum of the monomer region is shown in Figure 1.

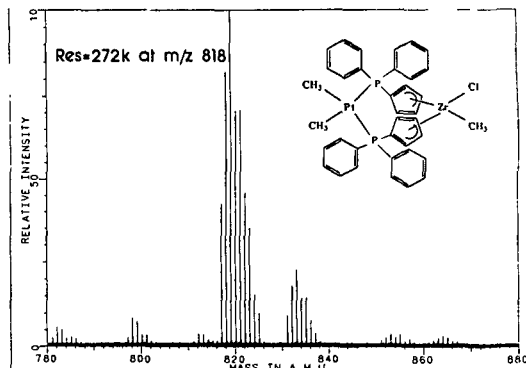


Figure 1: High Resolution LD/FTMS spectrum of $([\mu(\eta^5\text{-C}_5\text{H}_4)\text{P}(\text{C}_6\text{H}_5)_2]_2\text{ZrCl}(\text{CH}_3))\text{-Pt}(\text{CH}_3)_2$

The electrode bound polymer film formed by the reductive polymerization of an iron complex containing 4-methyl-4'-vinyl-2,2'-bipyridine (vbpy) was analyzed by LD/FTMS. Due to the steric bulk of the complex, many believed that only end-to-end dimerization of the vinyl groups could occur. Previous mass spectral analysis by thermal desorption showed the presence of monomeric vbpy and the two and three unit vbpy oligomers. In comparison the LD/FTMS spectrum clearly displays evidence for the formation of normal "polyvinyl-type" chain oligomers up to 14 vbpy units, with the most abundant oligomer in the envelope containing 7 vbpy units (Figure 2).

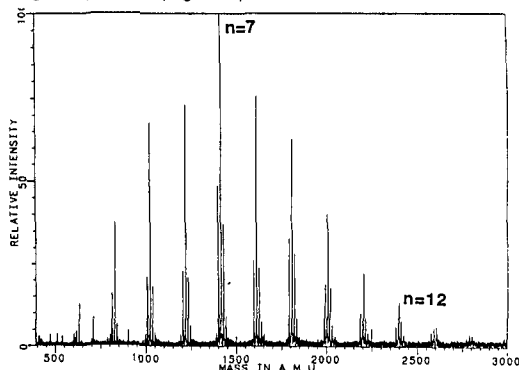


Figure 2: LD/FTMS spectrum of Poly-Methylvinylbipyridine.

LD/FTMS confirmed the synthesis of a bromine substituted tetraphenyl porphyrin iron chloride sample. The spectrum contained molecular ions and fragment ions resulting from loss of Cl and sequential loss of Br and HBr from the porphyrin. UV photodissociation of isolated $[M-Cl]^+$ ions resulted in the production of fragment ions, similar to those produced by laser desorption however the higher energetics of the photodissociation process led to an increase in the abundance of lower mass fragment ions relative to higher mass fragment ions.

CONFIRMATION OF NEWLY IDENTIFIED AMPICILLIN METABOLITES

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Ampicillin, D(-)-(α)-aminobenzylpenicillin, is a β -lactam antibiotic. Penicilloic acid and piperazine-2,5-dione have been reported as ampicillin metabolites in human urine samples. The structures of ampicillin and its known metabolites are shown in Fig. 1. The ion chromatogram was obtained from a urine sample after orally administering 500 mG of ampicillin using thermospray LC/MS. Selected ion chromatograms of this urine sample show 5 separated chromatographic peaks for ions that are characteristic of ampicillin and its known metabolites (Fig. 2). The components under chromatographic peaks 1, 2, and 5 are identified as penicilloic acid, ampicillin, and piperazine-2,5-dione by comparing the retention times and mass spectra of pure compounds. The mass spectra of chromatographic peaks 3 and 4 display ions that are characteristic of the fragment ions of ampicillin and its known metabolites. However, the identities of these compounds can not be determined from these mass spectra.

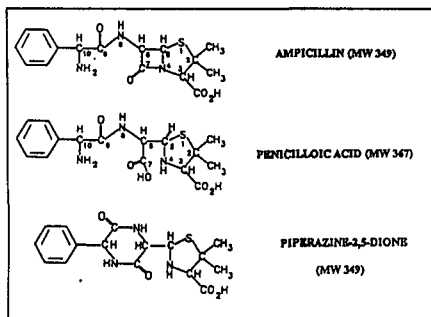


Fig. 1: Structures of ampicillin and its known metabolites

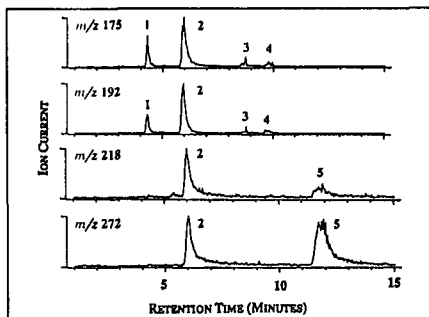


Fig. 2: The mass chromatograms of the urine sample after orally administering 500 mG of ampicillin.

The urine sample was collected as the solvent eluted from the analytical column corresponding to cut that contained peaks 3 and 4. The samples were evaporated to dryness under vacuum at room temperature. The FAB mass spectrum of this urine extract displays many ions that represent the unknown compounds and other unknown endogenous components in the urine. Several fragment ion spectra were obtained for the selected ion to determine the molecular ion of the unknown ampicillin metabolites. Only the ions at m/z 324 and m/z 350 exhibited fragment ion characteristic of a β -lactam ring (the thiazolidine ring at m/z 160). The FAB MS/MS spectrum of the ion at m/z 350 shows fragment ions corresponding to a combination of ampicillin and ampicillin piperazine-2,5-dione.

The FAB MS/MS spectrum of ion at m/z 324 shows fragment ions of the thiazolidine ring and the benzylamine group at m/z 160 and m/z 106, respectively (Fig. 3). Scheme 1 shows the structure of the ion at m/z 324 as penicilloic acid. The structure was deduced using the assumption that these unknown metabolites should have a similar structure as ampicillin and must fragment to give rise to a thiazolidine ring and a benzylamine group.

Ampicillin penicilloic acid was synthesized and the product contains two major components which have the same retention time as components 3 and 4 using LC/MS under the same conditions. These two compounds were separated using a semi-preparative column. The FAB mass spectra of penicilloic acid 3 and penicilloic acid 4 show the protonated molecule at m/z 324 and proton bound dimer at m/z 647. The ion at m/z 336 was not observed when *m*-nitrobenzyl alcohol was used as a matrix. The FAB MS/MS spectra of ion at m/z 324 from penicilloic acid 3 and penicilloic acid 4 are similar to each other and also comparable to the FAB MS/MS spectrum in Fig. 3. The FAB mass spectrum of penicilloic acid 3 and FAB MS/MS spectrum of protonated penicilloic acid 3 (at m/z 324) are shown in Figures 4 and 5. Components 3 and 4 in urine samples were identified as stereoisomers of penicilloic acid. The structure of these two compounds are (3S,5R) penicilloic acid and its epimer, (3S,5S) penicilloic acid.

The epimerization of penicilloic acids are demonstrated by incubation of an aqueous solution containing penicilloic acid 3. An increase in the amount of penicilloic acid 4 and a decrease in the amount of penicilloic acid 3 was observed using HPLC with UV detection. The conversion of penicilloic acid 3 to penicilloic acid 4 was observed by incubation of an aqueous solution of penicilloic acid 4.

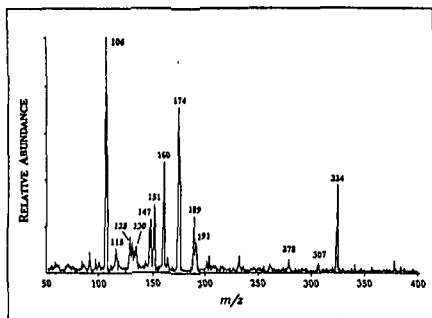
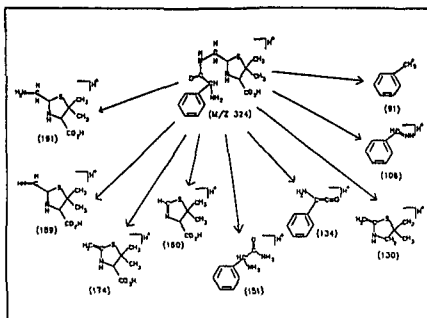


Fig. 3: FAB MS/MS spectrum of ion at m/z 324 from urine extract containing peaks 3 and 4.



Scheme 1: Postulated structure of fragment ions in Fig. 3.

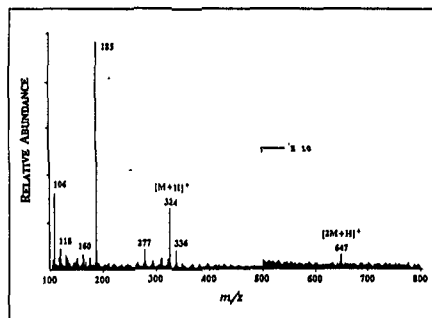


Fig. 4: FAB mass spectrum of penilloic acid 3.

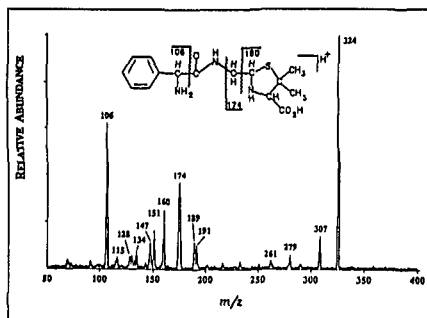


Fig. 5: FAB MS/MS spectrum of protonated penilloic acid 3.

Penilloic acid can arise from the decarboxylation of penicilloic acid. It is known to be a degradation product of penicillins. Experiments were performed to determine whether the penilloic acid in urine sample is a metabolite of ampicillin or a degradation product of ampicillin and its known metabolites. A urine sample was collected from a human subject 2 hours after orally administering ampicillin. The urine sample was immediately frozen at -78°C and analyzed the same day using LC/MS. The selected ion chromatograms of this urine sample display chromatographic peaks 1, 2, and 3 which are identified as penicilloic acid, ampicillin, and penilloic acid, respectively. The blank urine sample spiked with ampicillin was incubated at 37°C for 2 hours. Then, the urine sample was treated and analyzed using the same procedure as previously described. No detectable amount of penicilloic acid was observed. The similar results were observed in a blank urine sample spiked with penicilloic acid or piperazine-2,5-dione. However, penilloic acid 3 was detected in a urine sample that was spiked with ampicillin and was kept at -5°C for 72 hours.

CONCLUSION: Mass spectrometry was used in the structural elucidation and identification of ampicillin and two known metabolites, ampicillin penicilloic acid and ampicillin piperazine-2,5-dione. Two additional unreported ampicillin metabolites were detected in urine samples using thermospray LC/MS. These ampicillin metabolites were identified as (3S,5R) penilloic acid and its epimer, (3S,5S) penilloic acid using MS/MS. The identifications were based on the fact that metabolites products usually contain a substructure of the parent drug.

ACKNOWLEDGEMENTS: We thank Dr. Marvin L. Vestal and Dr. Donald A. Flory for their valuable assistance and the Texas Advanced Research Program for partial support of this work.

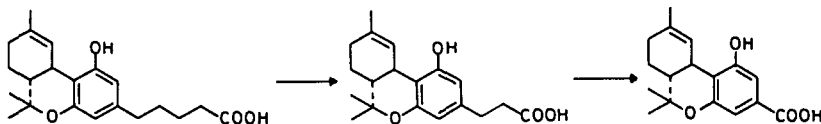
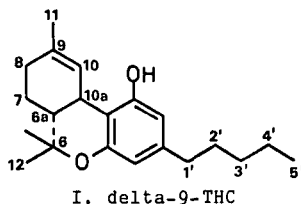
NOVEL PATHWAYS FOR THE OXIDATIVE DEGRADATION OF ALIPHATIC CHAINS IN CANNABINOID METABOLISM.

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Introduction

Many cannabinoids, such as delta-9-tetrahydrocannabinol (THC, I), the active constituent of marihuana, are metabolized by beta-oxidation of the pentyl chain to acids with C₃ and C₁ chains (Scheme 1). However, metabolites also occur with C₄ and C₂ chains; these are formed by undescribed mechanisms which appear not to involve intermediates that are common to the pathways leading to beta-oxidation. The mechanisms leading to formation of these metabolites are investigated in this paper.



Scheme 1. Metabolism of delta-9-THC by two stages of beta-oxidation.

Methods

Mice (Charles River, CD-1, male) were treated (IP) with one of the primary THC metabolites: 2'-, 3'-, 4'- or 5'-hydroxy-delta-9-THC, killed after 1 hr, and the hepatic metabolites were extracted. After concentration by chromatography on Sephadex LH-20, they were converted into TMS, Me/TMS and [²H₉]TMS derivatives and examined by GC/MS using a VG 70/70F mass spectrometer (70 eV) interfaced to a Varian 2440 gas chromatograph containing a 25 m x 0.2 mm OV-1 fused-silica capillary column.

Results

Metabolites were identified by mass spectral comparison with published data. 5'-Hydroxy-delta-9-THC, as reported previously (1), gave hydroxylated derivatives of the beta-oxidation product, 4',5'-bis,nor-delta-9-THC-3'-oic acid as the major metabolites (Scheme 2); no metabolites containing C₄ or C₂ side-chains were formed. 4'- (Fig. 1) and 3'-hydroxy-delta-9-THC (Fig. 2), unexpectedly, also gave these products by undescribed biochemical pathways. 4'-Hydroxy-delta-9-THC additionally gave the acids containing four carbon atoms in the side-chain (Scheme 2), thus identifying their source. 3'-Hydroxy-delta-9-THC gave metabolites with a hydroxy-ethyl side-chain (two carbon chain, Scheme 2), again by an undescribed mechanism; compounds of this type are known to be major metabolites of cannabidiol in humans but their mechanism of formation has not been determined. 2'-Hydroxy-delta-9-THC was not metabolized by oxidative mechanisms.

These results show that the 3'-oic acids are not only products of classical beta-oxidation, but can be formed by other mechanisms. These may, however involve a common delta-3' intermediate by elimination of water from either 3'- or 4'-hydroxy-5'-oic acid. The mechanism involving loss of three carbon atoms from 3'-hydroxy-delta-9-THC, on the other hand, is not related as the product is an alcohol rather than an acid.

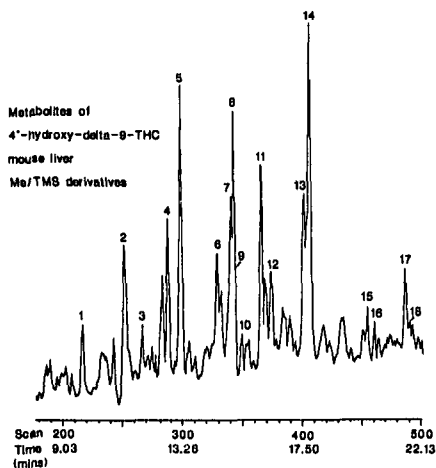


Fig. 1. Limited ion chromatogram (m/z 300-700) of metabolites of 4'-OH-delta-9-THC. Major metabolites: peak 4, 6-OH,3'-oic acid; 5, 7-OH-3'-oic acid; 7, 6-OH,4'-oic acid; 14, 4'-OH,11-oic acid.

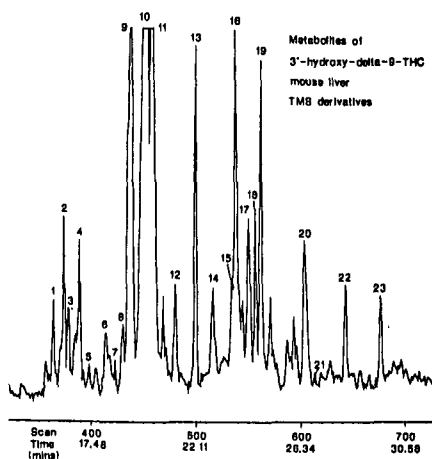
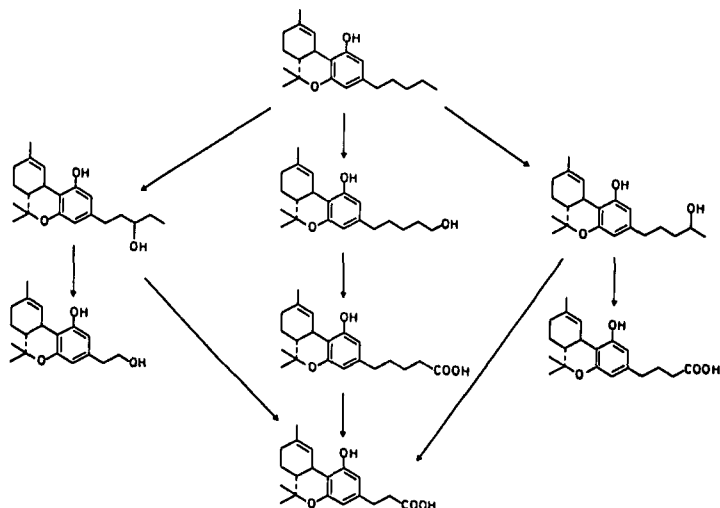


Fig. 2. Limited ion chromatogram (m/z 300-700) of metabolites of 3'-OH-delta-9-THC. Major metabolites: peak 8, 2',7-di-OH-3',4',5'-tris,nor-THC; 16, 3',6-di-OH-THC; 17, 11-OH-THC,3'-oic acid; 19, 3',7-di-OH-THC.



Scheme 2. Metabolism of 3'-, 4'-, and 5'-hydroxy-delta-9-THC

References

1. D.J. Harvey and J.T.A. Leuschner, *Drug Metab. Dispos.*, 1985, **13**, 215.

Acknowledgements

This work was funded by NIDA and the Wellcome Trust.

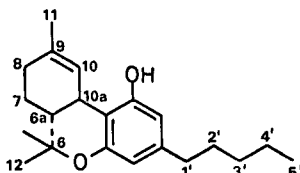
A METHOD FOR THE IDENTIFICATION OF TETRAHYDROCANNABINOL METABOLITES BY GC/MS
USING CATALYTIC HYDROGENATION.

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Introduction

Tetrahydrocannabinols (THC), of which the delta-9-isomer (I) is the major psychoactive constituent of marihuana, are extensively metabolized by hydroxylation. The TMS derivatives of these compounds frequently give similar and undiagnostic mass spectra and the quantities produced in the usually complex mixtures are frequently too low to permit isolation. This paper describes a method to facilitate identification by converting the metabolites into hydroxy-hexahydrocannabinols (hydroxy-HHCs) by catalytic hydrogenation. Most of the reference compounds for these products can readily be synthesised from available isomeric hydroxy-THCs.



I, delta-9-THC

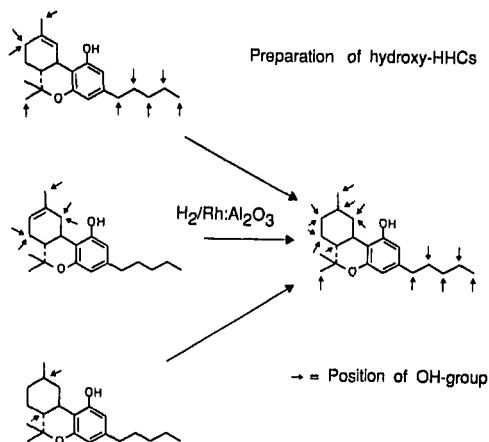
Methods

Reference samples of the following compounds were available: 1'-, 2'-, 3'-, 4'-, 5'-, 8-alpha-, 8-beta- and 11-hydroxy-delta-9-THC, and 7-alpha-, 7-beta-, 10-alpha-, 10-beta- and 11-hydroxy-delta-8-THC. 12-Hydroxy-delta-9-THC was synthesized. These compounds were hydrogenated for 5 min. over a rhodium/alumina catalyst to the 9-(R)- and 9-(S)- isomers of the appropriately hydroxylated HHC. 6a-, 9-alpha- and 9-beta-hydroxy-HHC were synthesized from THC epoxides by reduction with LiAlH_4 . GC/MS data of the TMS derivatives of these compounds were recorded with a VG 70/70F mass spectrometer (70 eV) interfaced to a Varian 2440 gas chromatograph containing a 25 m x 0.2 mm OV-1 fused-silica capillary column. Metabolism of delta-11-THC was performed with mouse liver microsomes, metabolites were hydrogenated as above except that the reaction was allowed to proceed overnight.

Results

Scheme 1 shows the structures of the available hydroxy-THCs and hydroxy-HHCs and their conversion by catalytic hydrogenation to hydroxy-HHCs. All reactions proceeded quantitatively and the TMS derivatives of the hydroxy-HHCs gave informative spectra with, in most cases, a very abundant and unique diagnostic ion. These are listed in Table 1. Two compounds, isomeric about C-9 were produced, but, except for the compounds hydroxylated at C-9 or C-11, the spectra of these isomers were almost identical. All compounds except the TMS derivatives of 10-alpha- and 10-beta-hydroxy-HHC gave unique spectra. In many cases they also gave unique base peaks, but for those compounds that did not, eg, 8-beta- and 9-beta-hydroxy-HHC, other major peaks in the spectrum were diagnostic as listed in Table 1. In general, HHCs containing an equatorial hydroxy group in the terpene ring, eg, 8-beta- and 9-beta-hydroxy-HHC, gave abundant molecular ions, whereas those compounds with an axial hydroxy-group tended to show a more favourable loss of TMSOH . HHCs hydroxylated at the 7- and 10-positions usually gave spectra with ions (eg m/z 143, 157) initiated by alpha-cleavage at the adjacent tertiary carbon atom. Diagnostic ions in the spectra of the side-chain hydroxylated HHCs were the same as those reported earlier for other cannabinoids (1).

This method for structural determination of hydroxy-THCs was evaluated for metabolites of delta-11-THC from rat liver microsomes where the same metabolites were found as reported earlier (2) with the major metabolite being 6-beta-hydroxy-delta-11-THC.



Scheme 1. Hydrogenation of hydroxy-THCs to hydroxy-HHCs

TABLE 1

Ions diagnostic of hydroxy substitution in various positions of HHC			
Compound	Base peak	Ion	
		Other ions [m/z and (%)]	
6a	433	a	
7-alpha	371	b	157 (7), 143 (15)
7-beta	319	c	157 (84), 143 (93), 476 (80)
8-alpha	371	b	
8-beta	476	M^+	343 (75), 420 (30)
9-alpha	386	d	371 (71)
9-beta	476	M^+	343 (47), 371 (42), (no 420)
10-alpha	461	e	143 (7)
10-beta	461	e	143 (8)
11-alpha	371	b	476 (90), 317 (79), 265 (84)
11-beta	476	M^+	265 (90)
12	373	f	
1'	419	g	
2'	145	h	
3'	332	i	
4'	476	M^+	
5'	476	M^+	332 (84)

(a) $[\text{M} - \text{C}_2\text{H}_7]^+$, (b) $[\text{M} - \text{TMSOH} - \text{CH}_2]^+$, (c) $[\text{M} - 157]^+$, (d) $[\text{M} - \text{TMSOH}]^+$,
(e) $[\text{M} - \text{CH}_3]^+$, (f) $[\text{M} - \text{CH}_2\text{OTMS}]^+$, (g) $[\text{M} - \text{C}_4\text{H}_9]^+$, (h) $[\text{C}_3\text{H}_7\text{CHOTMS}]^+$,
(i) $[\text{M} - \text{C}_4\text{H}_7\text{OTMS}]^+$

References

1. M. Binder, S. Agurell, K. Leander and J.-E. Lindgren, *Helv. Chim. Acta*, 1974, **57**, 1626.
2. M. Binder and U. Barlage, *Helv. Chim. Acta*, 1980, **63**, 255.

Acknowledgements

This work was funded by NIDA and the Wellcome Trust.

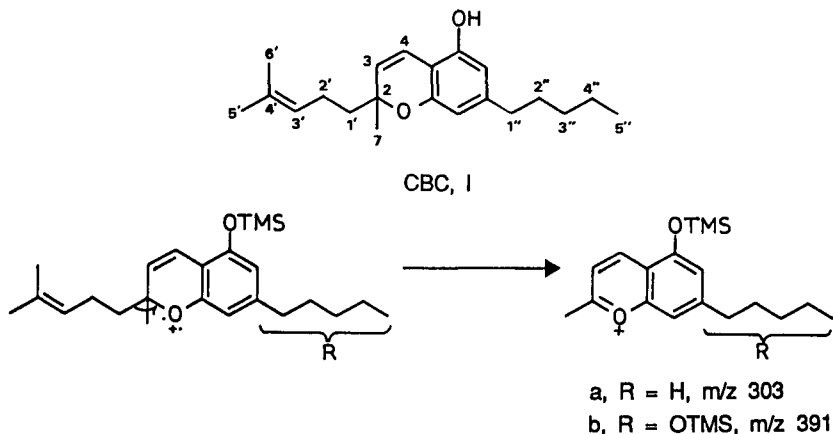
CANNABICHROMENE METABOLISM IN THE RABBIT: A NEW METHOD FOR METABOLITE IDENTIFICATION AND THE CHARACTERIZATION OF EIGHT NEW DIHYDROXY METABOLITES.

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Introduction

Cannabichromene (CBC, I), although a relatively minor cannabinoid from *Cannabis sativa* L., the source of the drug marihuana, can be the second most abundant cannabinoid in certain strains of the plant grown in the USA. A major problem encountered in the identification of its metabolites by mass spectrometry is the dominance of the chromenyl ion (a,b, Scheme 1); this suppresses, often to zero, the abundance of structurally informative fragment ions. This problem has now been overcome by catalytic hydrogenation of the cyclic double-bond (concomitantly with the acyclic double-bond) to redirect the fragmentation to the site of metabolic attack.



Scheme 1. Formation of the chromenyl ion from CBC metabolites

Methods

CBC metabolites were extracted from rabbit hepatic microsomal preparations, concentrated by chromatography on Sephadex LH-20 and converted into TMS and [$^2\text{H}_9$]TMS derivatives both before and after hydrogenation over a rhodium/alumina catalyst. Metabolites were examined by GC/MS using a VG 70/70F mass spectrometer (70 eV) interfaced to a Varian 2440 gas chromatograph containing a 25 m x 0.2 mm OV-1 fused-silica capillary column.

Results

Fig. 1 shows a limited ion (m/z 300-700) profile of the metabolites of CBC from the rabbit. The mass spectrum of the TMS derivative of a representative metabolite, 3"-hydroxy-CBC, is shown in Fig. 2. The abundant chromenyl ion at m/z 391 clearly indicates that the metabolite is hydroxylated in the pentyl chain, but the major ion diagnostic of the position of hydroxy substitution, m/z 330, is not significantly larger than the background ions. Spectra of other hydroxy metabolites were even less diagnostic. After catalytic hydrogenation, the formation of the chromenyl ion was considerably suppressed and fragmentation was initiated by charge localisation on the derivatized hydroxy function, giving the mass spectrum shown in Fig. 3. The ion diagnostic of the position of hydroxylation (m/z 334) is now the base peak. Other metabolites behaved similarly allowing the structural assignments shown in the legend to Fig. 1 to be made. 12 Monohydroxy and 8 new dihydroxy metabolites, an epoxide and its derived dihydrodiol were identified.

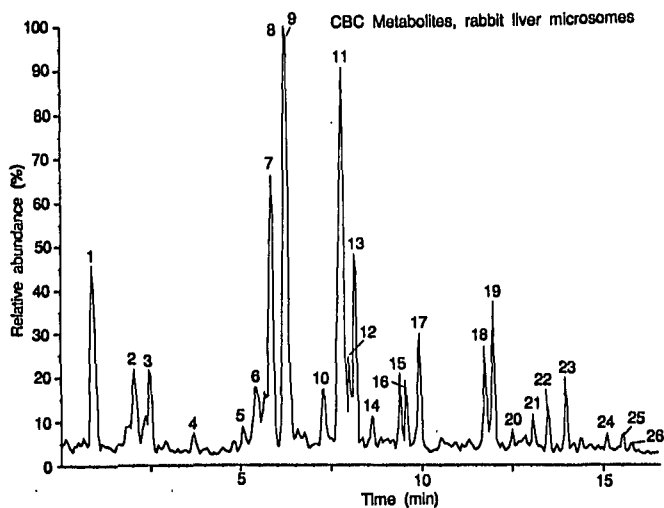


Fig. 1. CBC metabolites from the rabbit. Major peaks are: 7, 3"-OH; 8, 4"-OH; 9, 6'-OH; 11, 5'-OH; 13, 5"-OH; 18, 3",5'-di-OH; 19, 4",5'-di-OH.

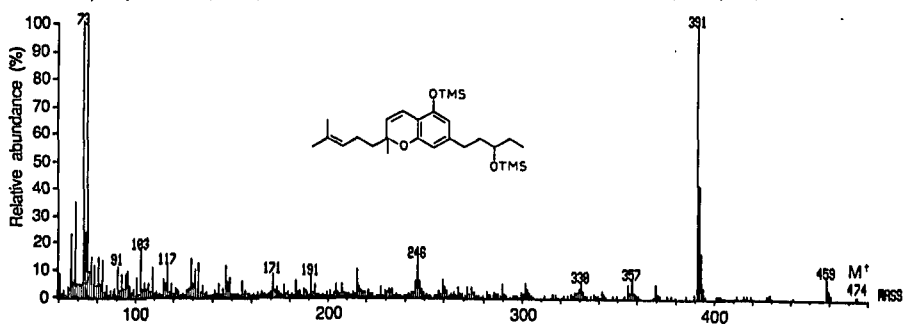


Fig. 2. 70 eV Mass spectrum of the TMS derivative of 3''-hydroxy-CBC

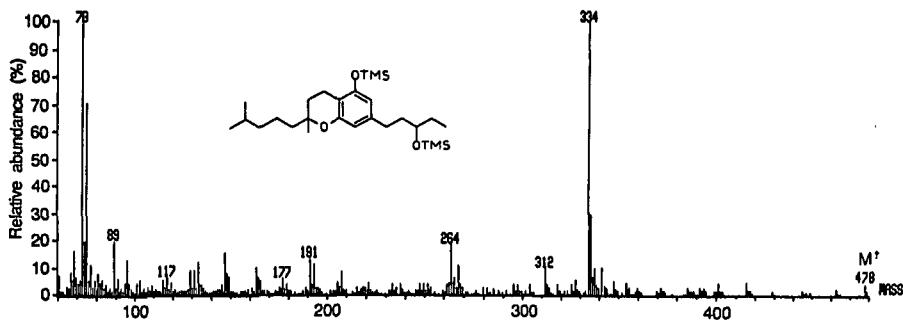


Fig. 3. 70 eV Mass spectrum of the TMS derivative of 3''-OH-tetrahydro-CBC

Acknowledgements. This work was funded by NIDA and the Wellcome Trust.

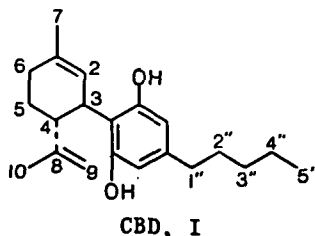
METABOLISM OF CANNABIDIOL BY THE DOG: IDENTIFICATION OF GLUCOSE CONJUGATES
AS THE MAJOR URINARY METABOLITES AT EARLY TIMES AFTER DRUG ADMINISTRATION.

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Introduction

Cannabidiol (CBD, I) is a major non-psychoactive cannabinoid from *Cannabis sativa*; it has clinically useful anticonvulsant properties. Its urinary metabolites are being studied in man, dog and rat. This paper describes the identification, by GC/MS, of unconjugated metabolites of which 37 were found, and a series of glucose conjugates which were excreted as the major metabolites at early times after drug administration. This is only the second report of such *O*-glucosides as drug metabolites in mammals.

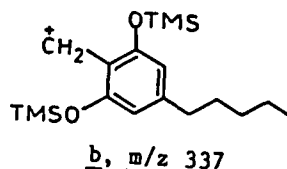
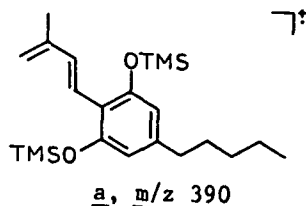


Methods

Three dogs were treated (IV) with CBD and urine samples were collected at intervals for 25 hours. Metabolites were hydrolysed (beta-glucuronidase, type VII), extracted with ethyl acetate, concentrated by Sephadex LH-20 column chromatography, and converted into TMS, [²H₉]TMS, Me/TMS and Me-Oxime/TMS derivatives for examination by EI GC/MS. The mass spectrometer was a VG 70/70F interfaced to a Varian 2440 gas chromatograph and the GLC column was a 25 m x 0.2 mm OV-1 fused-silica capillary terminating inside the ion source. Spectra were acquired with a VG 11-250 data system at 70 eV.

Results

Fig. 1 shows a limited ion chromatogram of the metabolites of CBD in a pooled sample of urine collected over 25 hours after drug administration. Unconjugated metabolites were identified from their EI spectra which contained diagnostic retro-Diels-Alder (a) and tropylium ions (b). The shifts in these ions, coupled with the appearance of abundant additional diagnostic ions were sufficient to enable most metabolites to be identified.



Metabolites were mainly products of beta-oxidation with additional hydroxylation at C-6 rather than at C-7 which is the major position hydroxylated by most other species. Major metabolites are identified in the Figure legend. At early times, glucose conjugates of 4'-, 5'-hydroxy- and 6-oxo-CBD were the major metabolites (Fig. 2). EI spectra of the TMS derivatives contained ions diagnostic of the sugar [e.g. m/z 450, 361 (base peak), 217, 147, 129, 103] and the aglycone. The latter ions were formed by

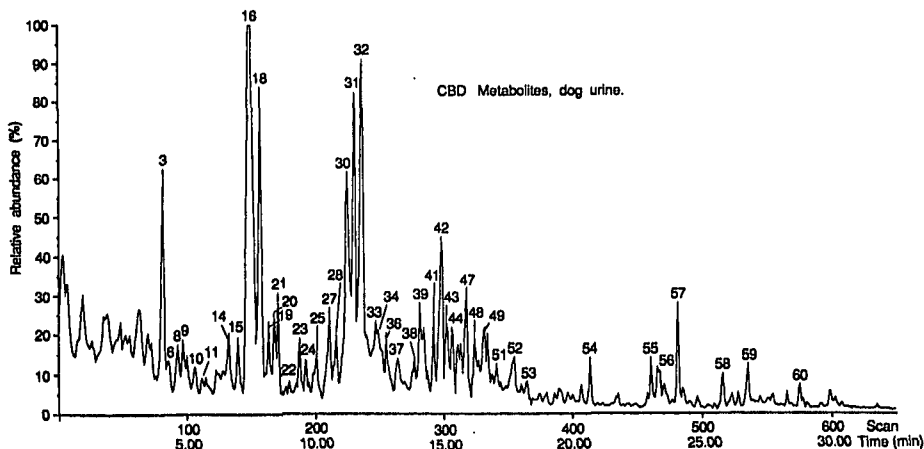


Fig. 1. Metabolites of CBD extracted from the 0-25 hour dog urine sample. Major metabolites are: peak 3, 6-hydroxy-2",3",4",5"-tetrakis,nor-CBD-1"-oic acid; peak 16, 6-hydroxy-4",5"-bis,nor-CBD-3"-oic acid; peak 18, 6-oxo-4",5"-bis,nor-CBD-3"-oic acid; peak 30, 4"-hydroxy-CBD-7-oic acid; peak 31, 4",6,7-trihydroxy-CBD, peak 32, 6-hydroxy-CBD-5"-oic acid; peak 42, CBD-5",7-dioic acid.

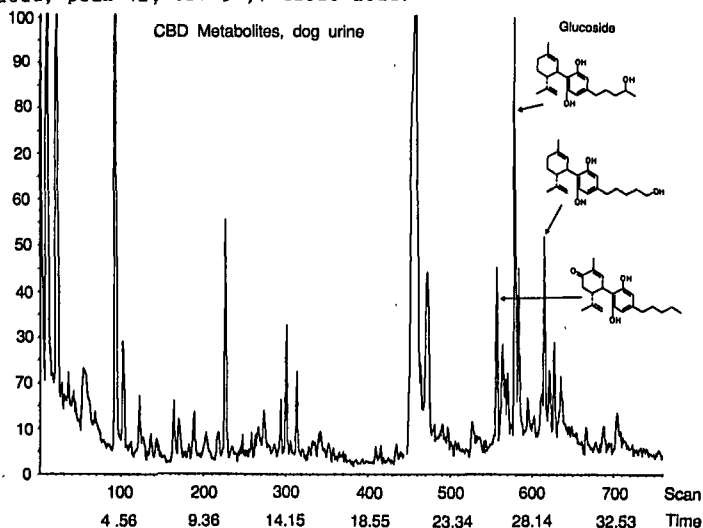


Fig. 2. Metabolites of CBD extracted from the 0-3 hour urine sample. The metabolites in scans 100-400 are the same as those shown in Fig. 1

TMS migration to the aglycone thus characterising the conjugates as phenol glucosides. Hydrolysis gave the free metabolites which were also identified by GC/MS.

Acknowledgements

This work was funded by NIDA and the Wellcome Trust.

TANDEM (MS/MS) MASS SPECTROMETRY UTILIZING ELECTRON IMPACT IONIZATION AND MULTIPLE REACTION MONITORING FOR THE RAPID, SENSITIVE, AND SPECIFIC IDENTIFICATION AND QUANTITATION OF MORPHINE IN WHOLE BLOOD WITH 5 METER CAPILLARY COLUMN CHROMATOGRAPHY. W.H. PHILLIPS, JR., N. Wade, K. Ota, R. Kitagawa, D. Warden, S. Zehnder, T. Appel, and G. Davis. California Department of Justice, Toxicology Unit, Sacramento, California 95820

ABSTRACT: The power of tandem (MS/MS) mass spectrometry virtually eliminates background interferences and increases sensitivity, thus allowing for a minimum of sample cleanup. A single step liquid-liquid extraction procedure with pentafluoropropionic anhydride derivatization of the resultant extract and 5 meter column chromatography provides a 3 minutes analysis of free morphine in blood samples. The specificity of the method is further enhanced by multiple reaction monitoring which is essentially selective ion recording of two quadrupoles. The first quadrupole is set to transmit only the molecular ion, 577, which then undergoes collision induced dissociation with argon. The last quadrupole is set to transmit only the daughter ion, 414. In the next scan, the ion, 414, is then analyzed for its daughter ion, 266. Deuterated morphine was included as an internal standard and analyzed similarly. Linear and reproducible calibration curves have been obtained for morphine at concentrations from 1.0 to 500 ng/ml achieving correlation coefficients of greater than 0.994. A signal to noise ratio of approximately 7:1 was observed for the 1.0 ng/ml samples.

INSTRUMENTS: A model TRIO-3 Triple Quadrupole Mass Spectrometer (VG MassLab) with a Model 5890A GC (Hewlett-Packard) controlled by a micro PDP 11/73 computer (Digital Equipment Corp.) was employed when operated in the electron impact (EI) mode. The ion source temperature was maintained at 200°C and electron energy of 70eV was routinely used. Sample automation was accomplished by a Model 7673A autosampler (Hewlett-Packard). The instrument's quadrupole sets (Q_1 and Q_3) were both calibrated prior to each use with perfluorotributylamine (FC-43). Argon was used as the collision gas.

A fused silica capillary column, DB-5 (J&W Scientific, Inc.) 30-M x 0.25MM i.d., was cut into six, 6 meter lengths. One five meter length was installed. The helium head pressure was maintained at less than 1.0 PSI with an apparent linear velocity of 68 cm/sec at a GC temperature of 300°C. A split ratio of 10:1 was employed. From an initial temperature of 100°C, the oven was programmed at 70°C/min to 300°C, and held for 2 minutes. The injector and transfer line were maintained at 250°C and 200°C, respectively.

EXTRACTIONS: Disposable syringized glass vials and teflon lined screw caps were used in all analyses. Blood obtained from a local blood bank and screened by radioimmunoassay for opiates and free morphine (Diagnostic Products Corp. Coat-A-Count), was determined to be negative for opiates. Morphine standards were prepared by spiking with appropriate volumes of 100 ng/ml stock morphine in methanol. For each set of unknowns, at least 3 blood standards of known concentration, one quality control specimen and one blank blood were analyzed. 100 μ l of 1.0 μ g/ml morphine-d₃ internal standard was added to each 2 ml aliquot of specimen. The pH of the specimen was adjusted to approximately 9.0 with 1 ml of carbonate/bicarbonate buffer. The mixture was extracted with 8 ml of ethyl acetate for 10 minutes on a rotator at 12 rpm. Extraction tubes were centrifuged at 3,000 rpm for 10 minutes and the organic layer transferred to clean disposable 15 ml centrifuge tubes and evaporated to dryness, using a vortex-evaporator heated to 70°C.

DERIVATIZATION: Pentafluoropropionic anhydride (PFP)(100 μ l) was added to the dry extract, which was then stoppered and heated for 15 minutes at 70°C in a heating block. The derivatized extracts were allowed to cool at room temperature for 5 minutes and again evaporated to dryness in a vortex-evaporator heated to 70°C and reconstituted with heptane (60 μ l). 1.0 μ l was injected into the GC MS/MS by autosampler.

RESULTS AND DISCUSSION: Figure 1 is the EI spectra of 3,6 di-PFP-morphine molecular weight 577, as seen at the intermediate detector (refer to ion optics diagram Fig. 11) prior to MS/MS analysis. The most abundant fragment occurs at 414 and corresponds to the loss of a pentafluoropropionate group from the morphine derivative. Figure 3 shows the daughter ion spectrum of the 577 molecular ion, which thus supports the hypothesis that the 414 is a daughter ion of 577. The reverse of a daughter ion scan is a parent ion scan and should and indeed does verify that 577 is the parent of 414 (fig 4). The neutral loss scan (fig 5) verifies the results of these experiments. This development was done utilizing the direct probe. As a further verification of the first parent to daughter transition, 577 to 414, of the 3,6 di-PFP-morphine, the parent ion 414 was transmitted with an expected loss of 163 from the 6 position corresponding to the remaining PFP group. What was observed was a loss of 148 and a corresponding daughter of 266 (fig 6). It is assumed that the oxygen of the 6 position remains, due to the aromatic charge distribution of the phenolic ring of the morphine, and some sort of a hydrogen rearrangement occurs. Without proper deuteration studies, exact details of this rearrangement are unknown. The daughter transition from 414 to 266 AMU is confirmed by its corresponding parent scan and is also verified by the neutral loss scan of 148.

The collision induced dissociation (CID) of a parent ion to its corresponding daughters has two components that are associated, the collision energy and collision gas pressure. Small changes in either, but particularly collision energy can have dramatic effects in parent to daughter production. Figure 7 depicts the ratio of the intensity of the M/Z 266 to 414 transition as the collision energy and collision gas pressure are varied. The best transmission of both 414 and 266, and 577 to 414 occurred with the collision energy set at 20 volts and argon collision gas pressure of 2.8 millitorr. This would likely vary from instrument to instrument.

To compare the selectivity of the MS/MS multiple reaction monitoring (MRM) to the GC/MS selective ion recording (SIR), drug free blood and a 1 ng/ml morphine blood standard were extracted, derivatized and analyzed by each technique. Ions monitored for SIR included M/Z 414, 417, 577, and 580. Ion transitions monitored for MRM included M/Z 414 to 266, 417 to 269, 577 to 414, and 580 to 417. A signal to noise ratio of approximately 7:1 was consistently observed for the 1 ng/ml (1 part-per-billion) whole blood samples analyzed by MS/MS MRM using a simple single step extraction and derivatization (fig 9,10). Using SIR, it was difficult to detect the internal standard which was spiked at 50 ng/ml, and no discernable peak was seen for the 1.0 ng/ml morphine.

As of this time, 14 consecutive standard curves ranging in concentration from 1 ng/ml to 500 ng/ml and with correlation coefficients of greater than 0.994 have been obtained. Figure (8) shows a representative calibration graph for morphine.

Tandem mass spectrometry (MS/MS) in combination with short capillary chromatography has been found to be a powerful tool in the analysis of a difficult compound. The results of the application demonstrate that GC/MS/MS for the analysis of morphine can be selective, and sensitivity is enhanced by way of the virtual elimination of background interference even with a minimum of sample cleanup. The use of a short, 5 meter capillary column to introduce the sample, enables rapid autosampler analysis. Approximately 60 samples can be extracted and analyzed in an 8 hour day. The gas chromatographic oven program used is not optimized for morphine analysis, but had been developed for general use in baseline separation of several drugs, including methamphetamine, meperidine, phenylephrine, codeine and diazepam. A high isothermal temperature could, in fact, shorten analysis time even further. A trade off in shorter column life maybe realized, but with 5.0 meters of column at risk, the savings in decreased analyst time and increased case throughput is well worth the cost.

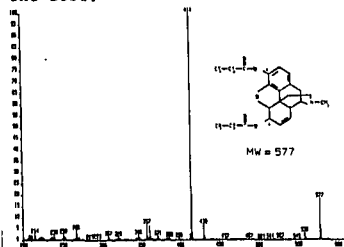


FIGURE 1.
First Quadrupole EI Scan Spectra of 3-6-di-PPP-Morphine

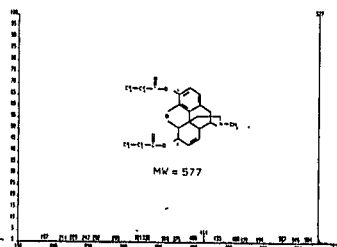


FIGURE 4.
Parent Ion MS/MS Spectra of the Daughter Ion at m/z 414

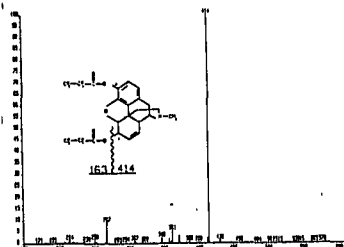


FIGURE 3.
Daughter Ion MS/MS Spectra of m/z 577 Parent Ion

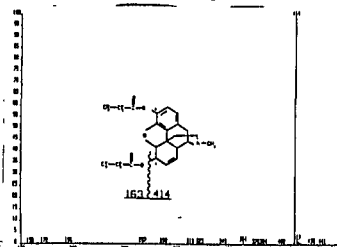


FIGURE 5.
Neutral Loss MS/MS Spectra of m/z 148

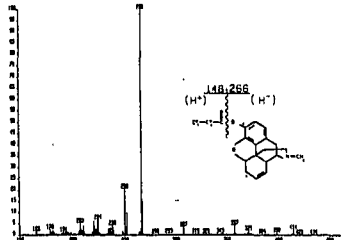


FIGURE 6.
Daughter Ion Spectra of 414 Parent Ion

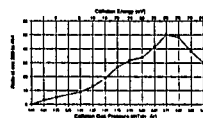


FIGURE 7.
The Ratio of the m/z 386 to 414 as the Collision Energy and Collision Gas Pressure are Varied

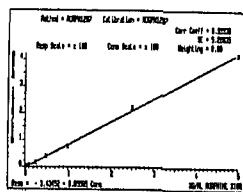


FIGURE 8.
Calibration Graph for Free Morphine Determining Linearity from 1 to 500 ng/ml

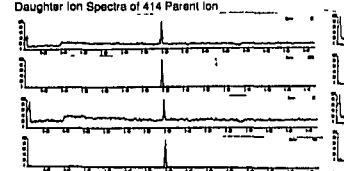


FIGURE 9.
Immune Reaction Monitoring of the 1.0 ng/ml Whole Blood Standard and Blank Blood with Morphine-d3 as the Internal Standard

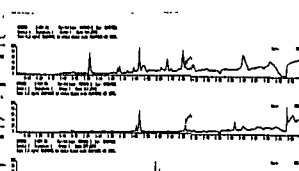
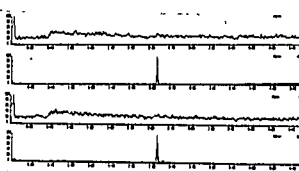


FIGURE 11.
Immune Reaction Monitoring of the 1.0 ng/ml Whole Blood Standard and Blank Blood with Morphine-d3 as the Internal Standard

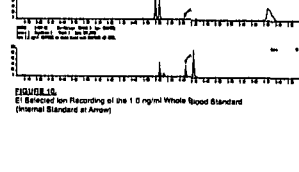


FIGURE 13.
Immune Reaction Monitoring of the 1.0 ng/ml Whole Blood Standard and Blank Blood with Morphine-d3 as the Internal Standard

AN IMPROVED METHOD FOR EXTRACTING OPIATES FROM URINE
SAMPLES PRIOR TO FULL SCAN GC/MS ANALYSIS

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Toxicology Testing Service Inc., Miami, Florida

Drug testing to monitor employees is increasing dramatically. Most of these analyses are performed with the ultimate identification of the substance resting on mass spectrometry using selected ion monitoring (SIM) of as few as three ions. As has been pointed out by de Jong, et al. (1988) when recommending the use of MS/MS, this may not be sufficient. The argument that some other compound does not yield these same few ions, in the same relative ratios, may not be pertinent. In a urine or blood extract the GC does not resolve all compounds from each other. If a mixture of compounds co-chromatographs and yields the correct ion ratios within 20%, for the ions being monitored, an incorrect identification may occur.

With the implementation of Federal regulations requiring quantitation of drugs including codeine and morphine, a simple method which would produce clean extracts suitable for GC/MS is required. A method which produces full scan spectra and enables quantitation begins with hydrolysis. After hydrolysis at 15 psig of 2 mL of urine to which 600 ng of each deuterated analog and ethyl morphine have been added as internal standards the pH is adjusted to approximately 8.5 with KOH. Samples are then transferred to extraction tubes containing 0.26 grams of sodium sesquicarbonate, 1.5 grams sodium chloride and 1.5 mL solvent (100 mL 1,2 dichloroethane + 63 mL isopropanol + 100 mL dichloromethane + 140 mL iso-octane). The top organic layer is transferred to a 10 X 75 mm culture tube and evaporated to dryness at 90 degrees C in an aluminum dry block under a gentle stream of nitrogen. After drying the nitrogen flow is stopped and 100 uL of BSTFA is added to each tube and is allowed to reflux for 15 minutes. Evaporate off the BSTFA with nitrogen in about 20 seconds and immediately remove from the heat. Dissolve the residue in DMSO and extract with iso-octane by vortexing for 30 seconds. Transfer the top iso-octane layer to a new test tube and evaporate just to dryness. Cover tube and store under nitrogen until ready for GC/MS analysis. A J&W on-column injector and a 12 meter 0.32 mm DB-5 column with a 1 micron film thickness is used. The extract is reconstituted with iso-octane just prior to injection.

Analysis of control samples during a 6 month period for codeine and morphine at 50, 100, 300, 450, 600, 900 and 1000 ng/mL prepared in negative urine produced excellent results. A sample chromatogram for codeine and morphine at 300 ng/mL, the Federally mandated action level, is given in Figure 1. It shows well resolved peaks for trimethylsilyl (TMS) derivatives of codeine (406), ethyl morphine (423) and morphine (434). Mass spectra for TMS derivatives of codeine and morphine are shown in Figures 2 and 3. These spectra are obtained by subtraction of spectra due to their deuterated analogs. Derivatives of D-3 codeine and D-3 morphine chromatograph approximately 1 to 2 seconds earlier than their non-deuterated analogs. This permits subtraction of the internal standard if it is present at the same or less concentration than the non-deuterated analog. Automatic quantitation of codeine and morphine is accomplished by integration of the TMS derivatives' molecular ions at 371 and 429 respectively (Figure 4). Data for linear calibration curves from 50 to 900 ng/mL is displayed in Table 1.

TABLE 1

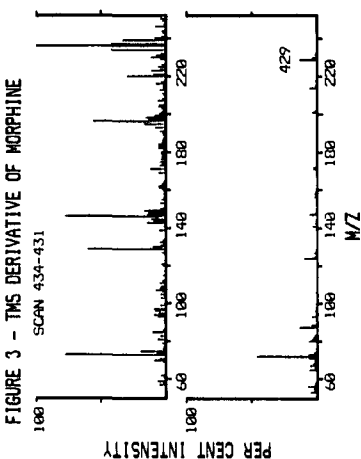
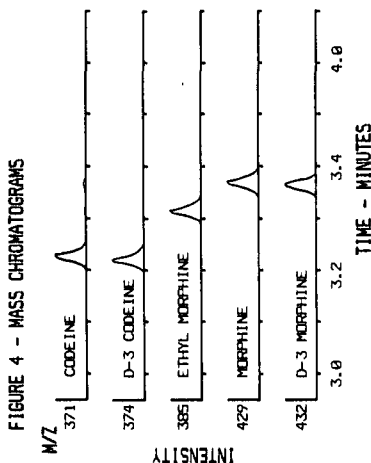
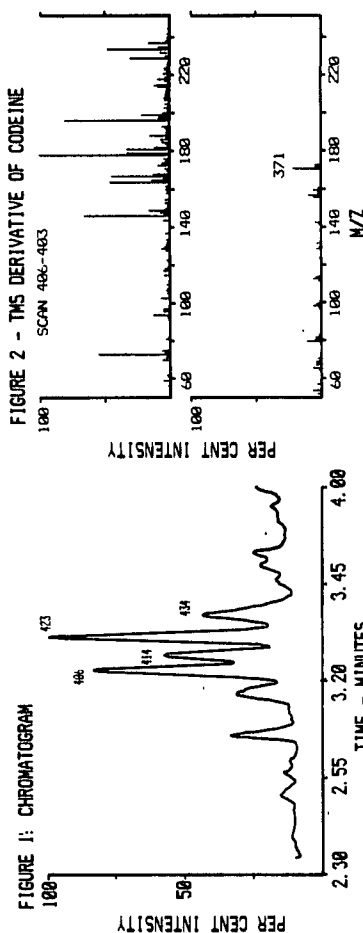
ION RATIO	INTERNAL STANDARD	EQUATION	CORRELATION COEFFICIENT
371/374	D-3 codeine	$C = 245x + 34$	0.996
371/385	ethyl morphine	$C = 261x + 24$	0.995
429/432	D-3 morphine	$C = 190x + 49$	0.997
429/385	ethyl morphine	$C = 940x + 76$	0.946

C = concentration

x = ion ratio

In conclusion, this method produces extracts which are clean enough for reconstructed ion chromatograms of urine extract analyses to show resolved peaks for codeine and morphine at the Federally mandated action level of 300 ng/mL. Full scan mass spectra can be obtained at this level by subtracting the spectrum due to the deuterated analog. Codeine may be quantitated using calibration curves obtained with both codeine D-3 and ethyl morphine as internal standards. Morphine is best quantitated using morphine D-3 as an internal standard. Ethyl morphine was successfully used for many analyses but it is apparently not a sufficiently good surrogate for morphine and any variation in experimental conditions greatly decreased precision in morphine quantitation. Quantitation within 10% of the level expected has been obtained for a control urine sample purchased from Hycor Biomedical, Inc. containing 400 ng/mL of both codeine and morphine as morphine glucuronide.

de Jong, E.G.; Maes, R.A.A.; van Rossum, J.M. Biomed. Environ. Mass. Spectrom. 1988 16, 75-80.



Triple Quadrupole and Quadrupole Ion Trap Tandem Mass Spectrometry (MS/MS) for the Determination of Morphine Alkaloids

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Introduction. The task of determining morphine alkaloids in physiological fluids has been and continues to be a major analytical challenge due to the effective low dosage of the morphine alkaloids. Over the past decade, triple quadrupole (TQMS) tandem mass spectrometry (MS/MS) has been successfully used for the determination of a variety of trace analytes in complex matrices with little or no sample cleanup or separation. Recently, a quadrupole ion trap (ITMS) has been developed which has the capability of performing MS/MS. The objectives of this research were a) to characterize and optimize the MS and MS/MS behavior of the morphine alkaloids, b) to compare the MS and MS/MS capabilities of the TQMS and ITMS, and c) to evaluate TQMS and ITMS MS/MS for the determination of trace levels of morphine alkaloids.

Experimental. All data were acquired on Finnigan MAT TSQ70 and ITMS tandem mass spectrometers. The ITMS was operated in the RF-only mode for the results presented here. Samples were introduced into the TSQ70 via a short (2.5 m) capillary GC column for optimization studies and via solids probe (SP) for quantitation. With the ITMS, all samples were introduced via a solids probe. Electron energies of 70 and 100 eV were used for EI and PCI, respectively, on the TSQ70. The RF level of the ITMS was maintained at 10 amu during the ionization and CI reaction time periods. The TSQ70 ion source was maintained at 150°C while the ITMS was kept at 100°C. After optimization of their respective CAD parameters, the sensitivities of the two tandem mass spectrometers were compared for the SP/EI-CAD of the M^+ ion determination of morphine standards in methanol.

Characterization of Normal MS. The EI normal mass spectra on the TQMS and ITMS are characterized by relatively strong M^+ ions at m/z 285 (100 and 20 %RA on the TQMS and ITMS, respectively) and extensive fragmentation (Figure 1). The higher relative abundances of low m/z ions to high m/z ions in the ITMS spectrum is most likely due to poor trapping of the higher m/z ions at an ionization RF level of 10 amu. Due to the short ion residence times (μs) in the ion source, the TQMS required 1-2 torr of CH_4 to achieve PCI. In contrast, with the capability to trap ions and, thus, have long CI reaction times (ms), the ITMS required only 2×10^{-5} torr CH_4 . The PCI normal mass spectra on both instruments are characterized by a large $(M+H-H_2O)^+$ ion at m/z 268 (100 %RA) and the $(M+H)^+$ ion at m/z 286 (30-40 %RA). The TQMS and ITMS sensitivities for the $(M+H)^+$ ions were ca. 30 and 2 times, respectively, those for the M^+ ions. Thus, it was anticipated that PCI would be the preferred ionization technique.

Characterization of Daughter MS. The EI-CAD daughter spectra of the M^+ ions (m/z 285) are characterized by efficient fragmentation of the parent molecular ion to produce only a relatively few, characteristic daughter ions, with the m/z 162 daughter ion being the most abundant and characteristic of the morphines (Figure 2). The PCI-CAD daughter spectrum of the $(M+H)^+$ ion obtained on the TQMS is characterized by inefficient fragmentation to a large number of low intensity daughter ions. With the ITMS, it has not been possible to fragment the $(M+H)^+$ ion of morphine under several different sets of CAD conditions. Although on the TQMS PCI was 30 times more sensitive than EI, PCI-CAD was only 5 times more sensitive than EI-CAD due to the inefficient CAD of the $(M+H)^+$ ion. Due to the inability to fragment the $(M+H)^+$ efficiently on both instruments, the EI-CAD of the M^+ ion was only chosen for further study.

Optimization of EI-CAD Parameters. The CAD fragmentation of the M^+ ion on the TQMS is largely controlled by the ion's axial energy (collision energy) and collision gas pressure. The maximum yield of the m/z 162 was found to occur with a collision energy of approximately 20 eV and a collision gas pressure of approximately 3 mtorr nitrogen. With 2.0 mtorr nitrogen collision gas, a maximum overall CAD efficiency of 65% was obtained with 20 eV collision energy.

The CAD fragmentation of the M^+ ion on the ITMS is affected by the frequency, magnitude, and length of time for which the auxiliary AC voltage (tickle voltage) is applied. The maximum CAD is achieved at the secular frequency of the parent ion while the width of the effective tickle frequency band increases (decreases

the mass resolution of parent ion resonance) with increasing tickle voltage and tickle time. The resolution of parent ion resonance increases with an increase in the RF voltage applied during the CAD period. With judicious selection of conditions, it is possible to excite only the parent ion of interest. The extent of CAD increases with increasing tickle voltage and tickle time. With variation of the tickle voltage, a maximum overall CAD efficiency of 95% was obtained with 500 mV tickle voltage and 10 ms tickle time.

Quantitative Comparison of the TQMS and ITMS. With the optimized EI and CAD conditions, calibration curves were obtained for the SP/EI-CAD of the M^+ ion of morphine in methanol. With selected reaction monitoring (SRM) of $285^+ \rightarrow 162^+$, the TQMS had a limit of detection (LOD) of approximately 10 ng of morphine applied to the probe. With a wide mass range scanning (m/z 160 to 330, 0.3 s), identifiable daughter spectra could be obtained on the TQMS on 50 ng of morphine (Figure 3). In contrast, the ITMS required about 75 ng of morphine to obtain comparable spectra (Figure 4). This was attributed to the Rulon fitting on the ion trap which mates with the end of the solids probe. As this Rulon fitting was at 100 C and morphine was not vaporized until above a SP temperature of 250 C, considerable broadening and tailing of the morphine peak occurred.

Conclusions. Although PCI was a more sensitive than EI, the EI M^+ ion was more efficiently fragmented than the PCI ($M+H$) $^+$ ion. Thus, EI-CAD was the preferred method for the determination of morphine in these studies. Although the ITMS yields a higher overall CAD efficiency than the TQMS, the lack of a heated solids probe interface to the ion trap results in the ITMS having a higher LOD than the TQMS. Finally, LODs of 10 and 30 ng of morphine on the solids probe were obtained for EI-selected reaction monitoring and EI-CAD daughter scanning, respectively, with the TQMS.

Acknowledgment. This research was funded by the Division of Pari-Mutuel Wagering, Florida Department of Business Regulation.

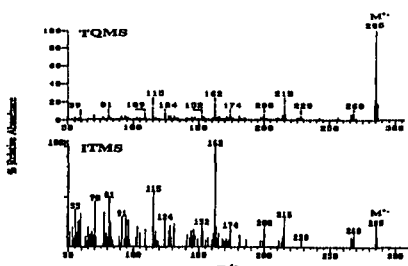


Figure 1. EI-MS of Morphine

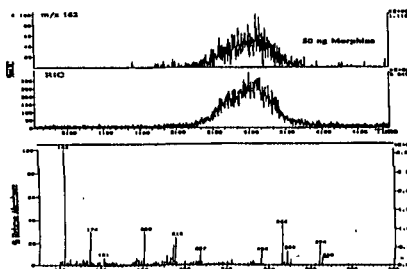


Figure 3. TQMS: SP/EI-CAD daughter scan determination of 50 ng morphine

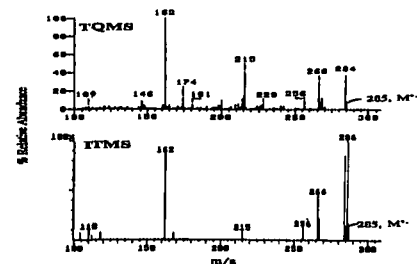


Figure 2. EI-CAD daughter spectra of M^+ of Morphine

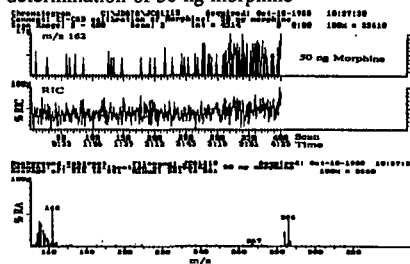


Figure 4. ITMS: SP/EI-CAD daughter scan determination of 50 ng of morphine

IDENTIFICATION OF FOUR PREVIOUSLY UNREPORTED METABOLITES OF COCAINE
IN HUMAN URINE SPECIMENS

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Cocaine remains one of the most widely abused illicit drugs in the United States. Although the major metabolites of cocaine have been extensively studied, some of the minor metabolites have not been previously reported. In order to further elucidate the metabolic pathways of cocaine in man, we undertook a search for additional cocaine metabolites in urine specimens previously found to contain high concentrations of benzoylecgonine.

Two ml of urine was hydrolyzed with 20,000 units of β -glucuronidase in 4 ml of sodium acetate buffer (pH=5) overnight at 37°C. After the incubation, the solution was adjusted to pH 8.5 with 1 N NH₄OH and extracted twice with 5 ml of chloroform:isopropanol (3:1). Then 5 ml of 0.1 N HCL was added and the solution was shaken for 15 min. The organic layer was discarded, the aqueous phase was adjusted to pH 8.5 and extracted twice with 5 ml of chloroform:isopropanol (3:1). The organic layer was evaporated to dryness and derivatized with 70 μ l of pentafluoropropionic anhydride (PFPA) and 30 μ l 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) by heating at 70°C for 10 min. After the derivative was evaporated to dryness, 20 μ l of ethyl acetate was added, and a 1 μ l aliquot of the solution was injected into the GC-MS.

The GC/MS used was a Finnigan-MAT model 800 Ion Trap Detector (ITD) combined with a Hewlett-Packard 5890 gas chromatograph. The chromatographic column was a 20 m x 0.2 mm DB5 fused-silica capillary column (J&W Scientific, Rancho Cordova, CA). The GC oven was initially held at 100°C for 0.5 min, then temperature programmed at 15°C/min to a final temperature of 300°C.

The total ion current chromatogram of a derivatized urine extract is shown in Figure 1. Cocaine and 11 of its metabolites are detectable. Four previously unreported metabolites were identified: ecgonidine (1), methyl-norecgonidine (5), norecgonine methyl ester (6) and m-hydroxybenzoylecgonine (8). Their structures were confirmed by comparison of their mass spectra (Figures 2a-2d) and their retention times with those obtained for synthetic standards. Other cocaine metabolites identified in this urine specimen were benzoylecgonine (7), ecgonine methyl ester (4), ecgonine (2), methylecgonidine (3), norcocaine (11), m-hydroxycocaine (10) and p-hydroxycocaine (12). Also, cinnamoylcocaine (14), which is often present in coca leaves and in illicit preparations of cocaine, was detected in this urine specimen along with its metabolite, cinnamoylcocaine (13).

The known metabolites of cocaine are shown in Figure 3. The arrows indicate likely metabolic pathways. The main biotransformations include: deesterification, N-demethylation, and arylhydroxylation.

Acknowledgement: This work was supported by a grant from the Finnigan Foundation.

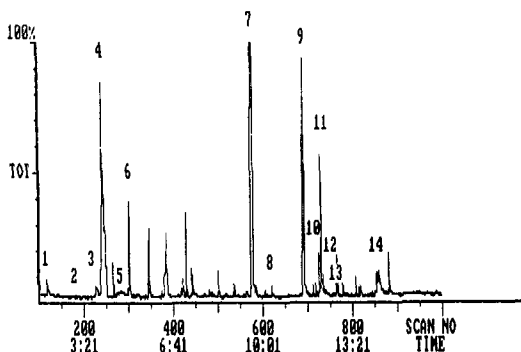


Fig. 1 Total ion chromatogram of derivative of urinary metabolites of Cocaine in human urine

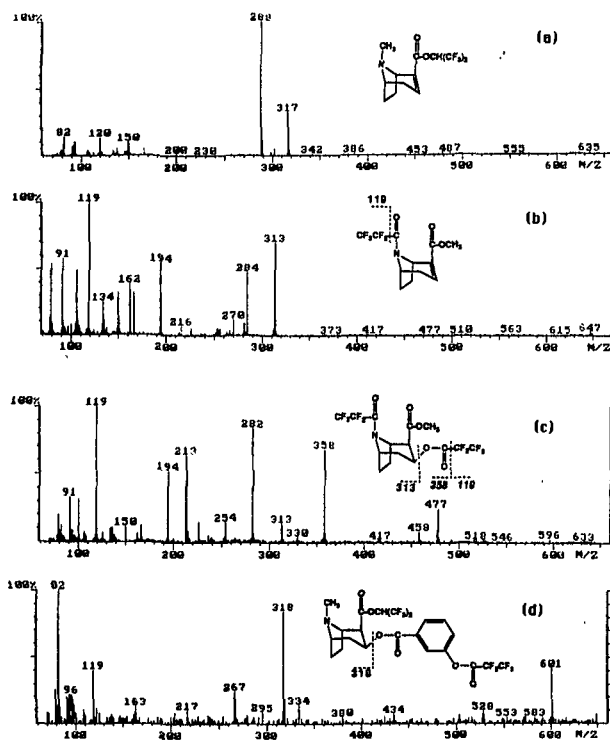


Fig. 2 EI mass spectra of four new metabolites of Cocaine

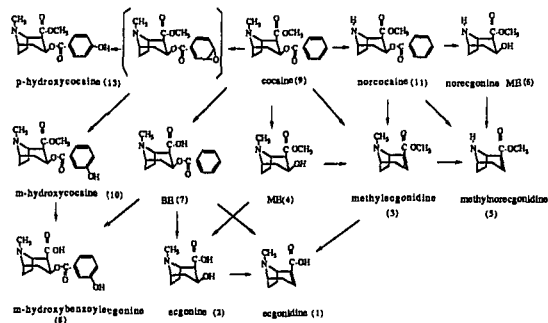


Fig. 3 Proposed biotransformation routes of Cocaine in human urine

CHARACTERIZATION OF URINARY METABOLITES OF
NAPAMEZOLE HCL IN RATS, DOGS, MONKEYS AND HUMANS.

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Napamezole HCL is an orally active naphthalenyl imidazole anti-depressant agent. It is structurally novel in that it lacks the fused three-ring system of the tricyclic antidepressives and physiologically novel in that it has both alpha-2 adrenergic antagonist activity and serotonin selective uptake inhibitory activity.

Following oral administration, napamezole was well absorbed in all species and excreted primarily via the kidneys. Dogs and monkeys excreted 80% of the dose in urine in 72 hr while rats excreted slightly less (50-60%). Intact napamezole represented less than 2% of the dose in rat urine but accounted for approximately 16% and 11% of the dose in dog and monkey urine, respectively. In humans, there was an apparent dichotomy of subject populations based upon the extent of drug metabolism. Poor metabolizers excreted up to 80% of the dose into the urine as intact drug while extensive metabolizers had less than 20% intact drug excreted into the urine.

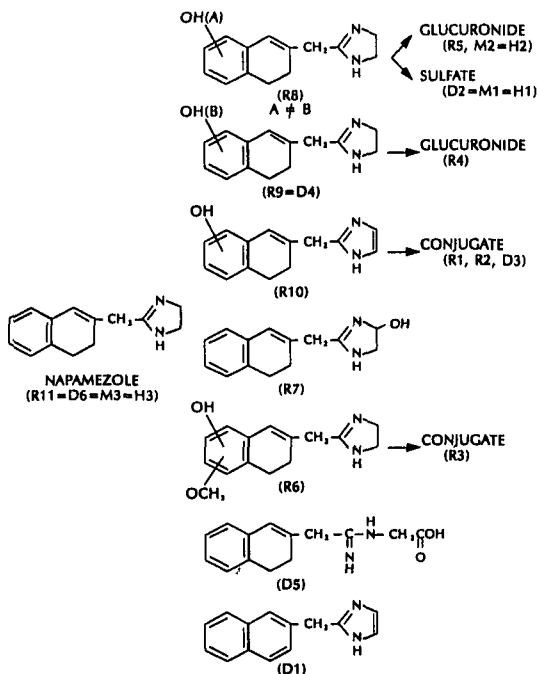
Twelve different metabolites plus the parent compound were isolated and characterized. Structural analysis by mass spectrometry revealed alterations of the aromatic and imidazole moieties of napamezole by oxidation, hydroxylation, cleavage and conjugation. A considerable interspecies divergency was observed. The biotransformation pattern was most complex in the rat (10 metabolites) compared to the dog (5 metabolites), monkey (2 metabolites) and human (2 metabolites).

The biotransformation of napamezole in rats, dogs, monkeys and humans may be summarized as follows. Hydroxylation and conjugation were the major routes of metabolism. Two different metabolites OH(A) and OH(B), each with an intact dihydroimidazole ring and hydroxylation on the aromatic ring, were isolated based on differences in retention time by HPLC. Free OH(A) was isolated in rat urine while free OH(B) was found in both rat and dog urine. Two different glucuronide conjugates of OH(A) were observed in rat, monkey and human urine while a sulfate conjugate of OH(A) was observed in dog, monkey and human urine. A glucuronide conjugate of OH(B) was isolated in rat urine. A "catechol" like compound, both free and conjugated, was identified in rat urine. Although the possibility exists that the O-methyl may be an artifact of purification, the dihydroxy configuration is of metabolic origin.

A metabolite hydroxylated in the alpha position on the dihydroimidazole ring was isolated in rat urine. The formation of carbinolamines from substituted amines has been proposed to take place during the intermediary oxidative metabolism of a number of drugs. In the biotransformation of napamezole, the existence of a carbinolamine intermediate represents a potential route for the conversion of the drug to the ring-opened carboxylic acid metabolite observed in dog urine. Oxidative ring opening is a significant metabolic route for several tertiary N-heterocycles.

Imidazole metabolites with hydroxyl substitutions on the aromatic ring were observed in both free and conjugated forms. One cannot determine unequivocally whether the imidazole moiety in these metabolites arose from alpha-C-hydroxylation and dehydration via the mechanism proposed for metabolite R7. However, since there was no mass spectral evidence to support an acyclic linear aldehyde intermediate with a hydroxyl substitution on the aromatic ring, it is probable that some other mechanism is involved.

SUMMARY OF METABOLITES OF NAPAMEZOLE



IDENTIFICATION OF PHASE II METABOLITES OF PSYCHOTROPIC
DRUGS USING HPLC COMBINED WITH FAB-MS

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Psychotropic drugs like fluphenazine, doxepin and haloperidol have been shown to undergo a wide variety of metabolic transformations *in vivo*. When plasma samples obtained from patients and healthy volunteers given such medication are examined indirect evidence indicates that various phase II conjugated metabolites may be present. The indirect evidence has been obtained after enzymatic or non enzymatic hydrolysis followed by analysis for the parent drug or functionalized metabolite(s). When such experiments are performed there is an apparent and significant increase in the measured levels of parent drug or metabolite. The conclusion reached from such studies is that conjugated metabolites are present in significant concentrations. In order to obtain direct evidence for the identification of phase II metabolites of psychotropic drugs a series of preliminary experiments were designed using rats.

EXPERIMENTAL

Animal Studies:

The phase II metabolism of doxepin, fluphenazine and haloperidol was studied on separate occasions in female Lewis rats (220-350 g) which were anesthetized with aqueous urethane solution (2 ml, 66 mg/ml, i.p.). The common bile duct was cannulated with polyethylene tubing (PE-10, 1.0 mm O.D.) and blank bile was collected for the first hour. An aqueous solution of the drug in question (25 mg/kg) was injected into the peritoneal cavity, after which the incision was closed with surgery clips. Bile was collected over a period of 4-8 h.

Instrumentation:

1) HPLC Conditions - Two Waters M-45 solvent delivery pumps were used controlled by a Waters 720 gradient system controller. A Waters Model 441 ultraviolet detector (254 nm) and Rheodyne valve loop injector were employed. Data was recorded on a Hewlett Packard Model 3390A integrator. The column was packed in-house with Spherisorb nitrile (3 μ m, 0.46 x 15 cm) and operated at 50°C (Sys-Tec, CH 1445) under gradient conditions. During the entire period the flow rate was maintained at 1.5 ml/min.

2) Mass Spectrometry - Fast atom bombardment mass spectrometry was performed on the various HPLC eluted and collected peaks. Two instruments were employed - a VG 70SQ and VG 7070 HE. Both positive and negative ion spectra were recorded depending on the suspected identity of the peaks. In all cases the FAB gas was argon; gun emission 6KV and sample matrix glycerol and/or thioglycerol. Data was collected using a Dec II/250J data system, spectra were averaged and background subtracted to remove matrix ions.

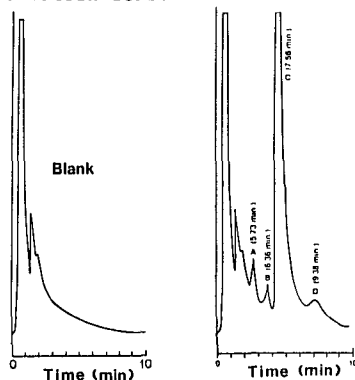
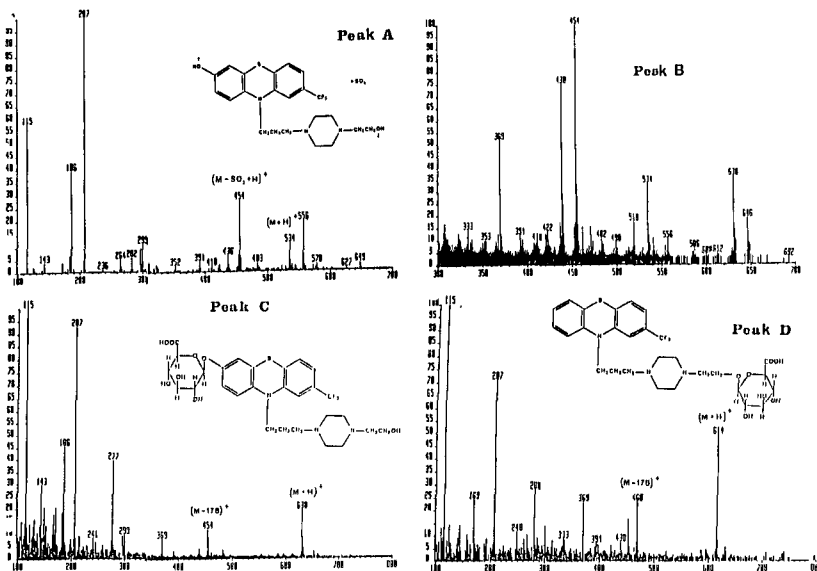


Figure 1 - HPLC chromatograms of blank bile and bile obtained from the same rat previously administered fluphenazine (25 mg/kg, i.p.). Peaks A, B, C and D were collected and analyzed by FAB-MS.



RESULTS AND DISCUSSION

Administration of fluphenazine resulted in the production of at least four peaks (A, B, C and D, Figure 1) which when individually analyzed by FAB-MS were tentatively identified as 7-hydroxyfluphenazine plus sulfate, fluphenazine sulfate, 7-O-glucuronide fluphenazine and fluphenazine glucuronide respectively. The FAB-MS of all these metabolites yielded quasi-molecular ion information which was reinforced in some cases by the presence of natiurated adduct ions. In addition the aglycone or de-sulfated fragment ions could also be readily identified.

In the case of peak A, Figure 1, the resultant FAB-MS with a quasi-molecular ion at m/z 534 demonstrated a very low abundance of a fragment ion at m/z 518. This would tend to indicate the additional oxygen in this species is in the form of a hydroxyl group rather than a sulfoxide which readily lose 16 amu under FAB conditions. We can not say however whether the sulfation of this hydroxylated metabolite is on the phenol or alcohol hydroxyl moiety. In addition the assignment of the hydroxyl group to the 7-position of the phenothiazine ring is based on the previously reported location of hydroxylation during phase I metabolism. Peak B in this chromatogram appears to be a mixture of peaks A and C and a previously unreported poly oxygenated metabolite giving rise to m/z 646.

Haloperidol and doxepin phase II metabolites were more simple in that unambiguous assignments of haloperidol glucuronide and two isomeric glucuronides of hydroxylated doxepin could be made (data not shown). In the case of doxepin, sufficient quantities of these metabolites were collected to obtain NMR data in order to confidently assign the metabolites as (E)-3 and (E)-2-O-glucuronide doxepin respectively.

The comparison of the spectra obtained to authentic reference spectra in most cases facilitated the assignment of metabolite structures.

These results are for the most part the first direct evidence for the presence of these metabolites in rat bile. Their importance in the human disposition of these drugs still remains to be clarified.

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UTILITY OF LC-MS AND TANDEM MS FOR THE ANALYSIS OF THE RAT URINARY
METABOLITES OF PYRILAMINE

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INTRODUCTION

Pyrilamine maleate is a member of a class of antihistamines that is used in cold and allergy formulations. At least one member of this class of antihistamines has been shown to be carcinogenic in laboratory animals (1). Therefore, there is a continued interest in the metabolic products of these compounds. Thermospray (TS) is a mass spectrometric (MS) ionization technique that is ideal for direct high performance liquid chromatography-mass spectrometric (HPLC-MS) analysis of samples (2,3). We have recently shown that TSMS is a useful technique for the analysis of several antihistamines and their metabolites (4-7). Recently, Lee and Yost (8) described the utility of tandem mass spectrometry for the identification of drug metabolites. In this study, we have used high performance liquid chromatography-thermospray mass spectrometry (HPLC-TSMS) combined with tandem mass spectrometry for the direct analysis of urinary extracts from rats which have been dosed with pyrilamine.

EXPERIMENTAL

A gradient HPLC system was connected to one inlet of a mixing T; the other inlet was connected to a syringe pump. The gradient HPLC was programmed from 100% 0.5 M ammonium acetate in water to 100% methanol over a 10 minute period at a constant flow rate of 1 ml/min through the HPLC column. The HPLC column was a 25 cm x 4.6 mm Supelco 5 μ m LC-18 column. The syringe pump was filled with a solution of 0.3 M ammonium acetate in water and was operated at a flow rate of 0.4 ml/min. The total effluent from the two pumps was mixed in the T and flowed into the mass spectrometer. The mass spectrometer (MS) was a Finnigan-MAT TSQ 70 triple quadrupole MS instrument equipped with a Finnigan-MAT thermospray probe and controller. When operated in the tandem MS mode, the collision gas was argon set to about 0.5 mtorr and collision energy of 25 EV. Both parent ion spectra and daughter ion spectra were obtained.

RESULTS AND DISCUSSION

Based on our previous studies of the TSMS/MS characteristics of pyrilamine and related metabolites, it was known that all of these compounds produced an m/z 121 ion in the daughter ion spectra produced from the collision-activated dissociation (CAD) of their $[MH]^+$ ions (4). In addition, it was observed that the m/z 107 ion in the daughter ion spectra of these metabolites was indicative of an O-dealkylated pyrilamine metabolite (4). Therefore, as shown in Figure 1, the rat urinary extract sample was analyzed via HPLC-TSMS/MS in the parent ion mode set to obtain parent ion spectra for the m/z 121 ion. In addition, a parent ion analysis for the m/z 107 ion was also performed. These parent ion analyses provided several new potential metabolites for further analysis. Daughter ion spectra for these potential metabolites were then obtained. For example, Fig. 2 shows the m/z 272 daughter ion analysis of the sample; m/z 272 corresponds to the $[MH]^+$ ion for O-dealkylated pyrilamine. By this method, several unexpected metabolites were detected and preliminary structures have been assigned to them. The success of this experiment was due to a combination of the separation of these metabolites in time via the gradient HPLC system and the ability to search for potential metabolites using the parent ion mode of the tandem MS system followed by the use of daughter ion spectra to give a preliminary identification of these metabolites.

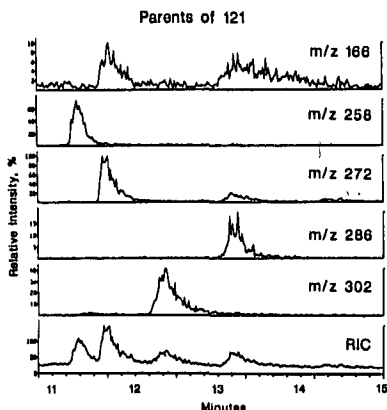


Figure 1 Mass chromatograms for the m/z 121 parent ion analysis of the rat urine extract sample.

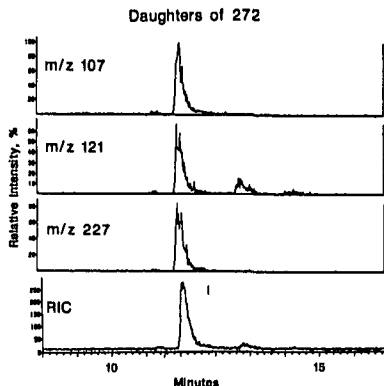


Figure 2 Mass chromatograms from the m/z 272 daughter ion analysis of the rat urine extract sample. Peak I is the O-dealkylated pyrilamine metabolite.

CONCLUSIONS

HPLC-TSMS and HPLC-TSMS/MS were successfully utilized for the direct analysis of a rat urinary extract which contained several metabolites of pyrilamine. By using the parent ion MS mode, potential pyrilamine metabolites were located in the sample. By successive use of the daughter ion MS mode, individual pyrilamine metabolites could then be characterized.

ACKNOWLEDGEMENT

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CHARACTERIZATION OF THE METABOLITES OF TIBENELAST FROM URINE IN MALE HUMANS USING LIQUID SECONDARY ION (LSIMS), DIRECT PROBE CHEMICAL IONIZATION (DCI) AND THERMOSPRAY (TSP) MASS SPECTROMETRY

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INTRODUCTION Tibenelast, (figure 1, I), has been identified as a potential antiasthma compound. After pharmacokinetic steady state was achieved with tibenelast, each volunteer received approximately 50 mg (100 μ Ci) of radiolabeled tibenelast. The major urinary metabolite was identified as the glucuronide conjugate of tibenelast by LSIMS (figure 1, IV). Minor metabolites of tibenelast, the conjugates of the O-desethylated metabolites, (figure 1, II+III) were identified by thermospray LC/MS.

METHODS AND PROCEDURES **Direct Chemical Ionization (DCI) Analyses** DCI analyses were performed on a Nermag R 30-10 triple stage. The samples, dissolved in methanol, were applied onto the direct probe filament tip that was initially at ground then increased to 600 mA at 30 mA/min. The ion source was maintained at 120 °C with an ammonia gas pressure of 7×10^{-5} torr. A mass range of 200-700 amu was scanned every 0.5 seconds.

Liquid Secondary Ion Mass Spectrometry (LSIMS) LSIMS analyses were performed on a Nermag R 10-10 single stage quadrupole mass spectrometer equipped with a cesium ion gun. The sample, dissolved in methanol, was applied to the probe tip that was coated with glycerol. The cesium gun operated at a heater current of 250-300 mA to produce a cesium ion beam of 0.8 mA. The mass range of 200-1200 amu was scanned every 10 seconds.

LC/MS All TSP spectra were obtained using a model TS101 Vestec TSP interface installed on a Finnigan 4500 MS/DS. The TSP source block temperature was 250 °C. TSP probe tip temperature was 230 °C. All analyses were performed in the discharge-on mode at a flow of 1 ml/min. Most acquisitions scanned across a 30-50 amu mass range in 2 seconds. Samples were introduced via manual injection using a 50 μ l fixed loop. The mobile phase with eluting peaks passed from the column to a U.V. detector set at 313 nm and then to the TSP probe.

RESULTS and DISCUSSION The DCI analysis of a standard of O-desethyl tibenelast (figure 1, II+III) produced a prominent pseudomolecular ion and an intense ammonium adduct ion. Treatment of the standard with ethereal diazomethane produced the methyl ester with the pseudomolecular ion shifted 14 mass units. DCI analysis of isolated O-desethyl tibenelast did not produce the prominent ions observed with the standards. Selected ion display of the pseudomolecular ion revealed a major peak at scan #21 indicating that a pure component at this mass was possibly in the sample (figure 2). Treatment of the component with diazomethane did not affect the major ions present.

The LSIMS spectrum of the putative glucuronide had significant ions (figure 3) that corresponded to the mono and di-natriated adducts of a postulated glucuronide (figure 1, IV). The assignment of these peaks as natriated adducts was supported by the intense natriation of the glycerol matrix ions. The ion at $m/z=556$ was the glycerol adduct of the mono-natriated glucuronide. LSIMS was not successful for the analysis of other putative glucuronides. These samples were successfully analyzed by Thermospray LC/MS.

The TSP spectrum of other isolated glucuronides produced prominent pseudomolecular ions at $m/z=239$ indicative of the aglycone of O-desethylated tibenelast glucuronide. Thermospray LC/MS did not yield molecular ion information of the intact glucuronides, only the aglycones.

The LSIMS experiments were limited by the presence of sodium in the matrix, a common problem in FAB and LSIMS mass spectrometry. The DCI analyses produced excellent results with standards but interferences dominated the sample spectra. Thermospray LC/MS was able to overcome the matrix problems associated with other techniques.

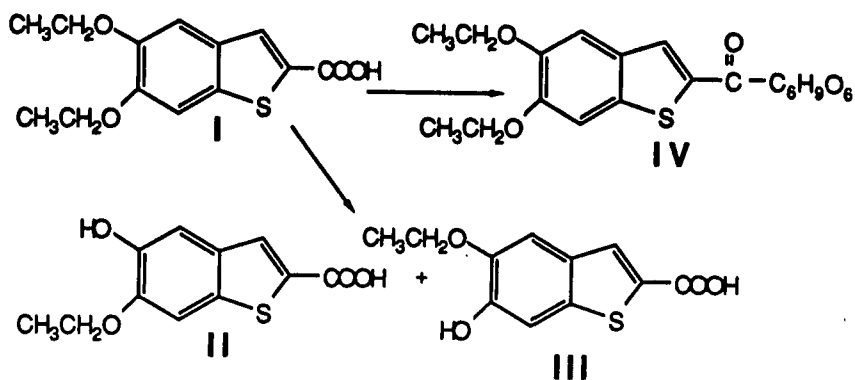


FIGURE 1

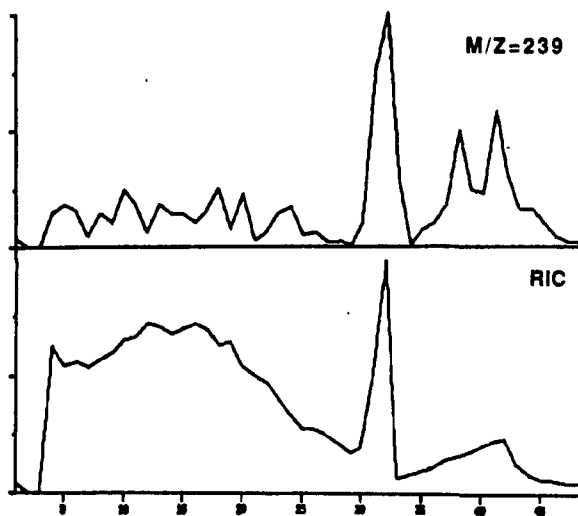


FIGURE 2

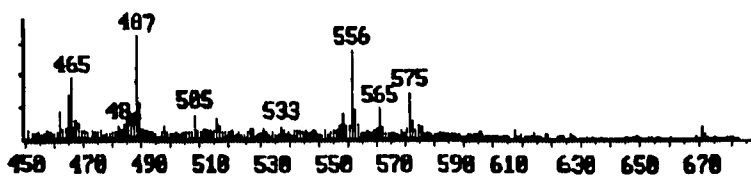


FIGURE 3

**APPLICATION OF THERMOSPRAY LC-MS FOR THE ANALYSIS OF THENYLDIAMINE AND
TRIPLENNAMINE AND THEIR MICROBIAL METABOLITES**

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INTRODUCTION

Thenyldiamine and tripeleennamine are ethylenediamine-type antihistamines. Antihistamines in this class have been used in various over-the-counter cold remedies. The determination of the metabolism and toxicity of these compounds is of interest because similar antihistaminic drugs have been found to be carcinogenic in laboratory animals (1). The use of microbial systems to model phase I mammalian drug metabolism has been reported (2,3). Research in our laboratories has also shown the applicability of microbial models to complement mammalian metabolism studies of various classes of antihistamines (4,5).

Thermospray (TS) is a recently developed mass spectrometric (MS) ionization technique that is ideal for direct high performance liquid chromatography-mass spectrometric (HPLC-MS) analysis of samples (6,7). In this report, we describe the direct HPLC-TSMS analysis of methylene chloride extracts containing microbial metabolites of these two antihistamines.

EXPERIMENTAL

Biotransformation Conditions. Typically, 5 mg of the antihistamine was dissolved in 0.5 ml sterile physiological saline and then incubated with cultures of the filamentous fungus Cunninghamella elegans ATCC 9245. Methylene chloride extracts of these fungal cultures were evaporated to a dry residue and then redissolved into 5 ml of the mobile phase prior to injection into the HPLC-TSMS system.

HPLC-TSMS System. An Isco (Lincoln, NE) model LC-5000 syringe pump was connected to a Rheodyne (Cotati, CA) 7125 injector that included a 20- μ l sample loop. The mobile phase was pumped through a 25 cm x 4.6 mm Altex (Berkeley, CA) Ultrasphere 5 μ m Cyano-phase HPLC column at a flow rate of 1.0 ml/min. The mobile phase consisted of 0.1 M ammonium acetate in water adjusted to pH 5 with acetic acid/acetonitrile (75/25, v/v). The HPLC eluant went directly into the TSMS interface. TSMS analysis was performed using a Delsi-Nermag (Houston, TX) R1010C quadrupole mass spectrometer equipped with a Delsi-Nermag TS source and a Vestec (Houston, TX) TS interface. The TS source was operated in the positive ion, "filament off", and "discharge off" mode. Typically, the TS source block was set to 160 °C, and the control temperature was set to 132 °C.

RESULTS AND DISCUSSION

The parent compounds and the N-demethylated metabolites provided [MH]⁺ ions as the base peak in their TS mass spectra. The N-oxide metabolites provided [MH]⁺ ions plus several fragment ions in their TS mass spectra. Figures 1 and 2 show the LC mass chromatograms for the thenyldiamine extract and the tripeleennamine extract, respectively.

CONCLUSIONS

By this method, TS mass spectra of the N-oxide and N-demethylated metabolites of these antihistamines were obtained directly from the on-line HPLC-TSMS analyses. These results demonstrate the utility of HPLC-TSMS for the direct analysis of thenyldiamine and tripeleennamine and their methylene chloride-extractable microbial metabolites.

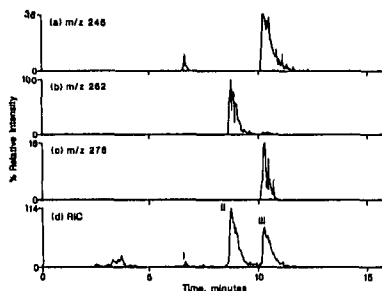


Figure 1. LC mass chromatograms from the HPLC-TSMS analysis of an extract from the fungal metabolism of thenyldiamine, displaying (a) m/z 246, (b) m/z 262, (c) m/z 278, and (d) the RIC. Peak I is N-desmethylthenyldiamine; peak II is thenyldiamine; peak III is thenyldiamine N-oxide.

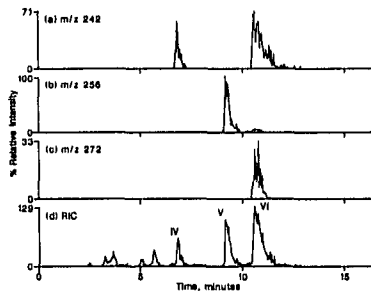


Figure 2. LC mass chromatograms from the HPLC-TSMS analysis of an extract from the fungal metabolism of tripeleonnamine, displaying (a) m/z 242, (b) m/z 256, (c) m/z 272, and (d) the RIC. Peak IV is N-desmethyltripeleonnamine; peak V is tripeleonnamine; peak VI is tripeleonnamine N-oxide.

ACKNOWLEDGEMENT

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THE DETERMINATION OF SERUM URIC ACID BY ISOTOPE DILUTION LC/MS AND LC/MS/MS

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INTRODUCTION

Quantitation using LC/MS is often difficult to perform with satisfactory precision because of rapid fluctuations and drift in the signal. One approach to overcoming these problems is to use an isotope labeled internal standard, which coelutes with the analyte.

Provided the peak is sufficiently wide, enough alternating measurements of the intensities of the unlabeled and labeled ions can be made to average out the rapid fluctuations. Drift problems are partially overcome by using the coeluting internal standard. Even then, absolute ratios measured for a given sample can vary considerably with time. Performing measurements of standard mixtures (that are close in ratio to the samples) between measurements of samples can largely overcome this problem.

We chose to test how well quantitation could be performed using isotope dilution/LC/MS by measuring uric acid in SRM 909, Lyophilized Human Serum. The concentration of uric acid in this SRM was determined by an isotope dilution/GC/MS candidate definitive method. Results from this GC/MS method are very precise and are free of significant bias. The method is however, very time-consuming. The serum is spiked with a known amount of uric acid- $^{15}\text{N}_2$, the uric acid is isolated from most of the serum matrix with use of an anion exchange column, the sample is freeze-dried, the uric acid is converted to a tetra(t-butyl-dimethylsilyl) derivative, and the derivatized mixture is injected onto a nonpolar capillary column interfaced directly to a magnetic sector mass spectrometer extensively modified for high precision isotope dilution measurements.

An LC/MS method would require less sample preparation and no derivatization. If results proved satisfactory, LC/MS methods could be rapidly developed and instituted for determinations not requiring the precision and accuracy of a definitive method.

In contrast to the GC/MS method, the LC method we developed is very simple. After spiking the serum with a labeled internal standard, the serum is treated to precipitate protein. The supernatant is then ready, without further treatment, for injection onto the HPLC column.

Because LC does not have the resolving power of GC, separation from all interferences is less likely with LC. The addition of a collision induced dissociation (CID) step should add sufficient specificity for accurate measurements of analytes in complex matrices. We wanted to test whether this additional step was necessary and whether it would have adverse effects on the precision.

EXPERIMENTAL

Reconstituted serum (SRM 909) was spiked with a known amount of uric acid- $^{15}\text{N}_2$ and treated with trichloroacetic acid to precipitate protein. Fifty microliters of the supernatant was then injected directly onto a C-18 HPLC column which was interfaced to a triple quadrupole mass spectrometer via a thermospray device. Best results were obtained when the thermospray ion source was tuned for best signal stability, rather than for maximum sensitivity. For tuning we bypassed the HPLC column with a constant flow

of a solution of our eluting solvent containing uric acid at about the concentration of our samples. The eluting solvent was an aqueous solution of 0.075 M ammonium acetate and 0.003 M tetrabutylammonium hydroxide adjusted to pH 5.2. For the LC/MS measurements, (M-H)⁻ ions for the unlabeled and labeled forms of uric acid were measured and their ratio calculated. To determine the quantity of uric acid in the serum, such ratios were compared with ratios from uric acid standard solutions spiked with the same concentration of labeled uric acid as were the samples. Injections of samples and standards were alternated to provide frequent checks on the instrument stability.

For the LC/MS/MS measurements, we selected the (M-H)⁻ ions generated by thermospray, performed CID on them, and measured the intensity of specific fragment ions. The instrument was set in the neutral loss mode for monitoring loss of 43 from m/z 167 and loss of 44 from 169. This loss of 44 suggests that one of the labeled nitrogens is lost in the fragmentation.

RESULTS

The results for three samples of SRM 909 measured by LC/MS are shown in Table 1. The precision was very good for these three samples. However, our other experiences with quantitation by LC/MS suggest that cv's of 1 - 1.5 % are more typical. The results are slightly higher (3%) than the certified value, suggesting the possibility of a small interference in the measurements. Nevertheless, the ease of the method and the satisfactory results, in terms of both accuracy and precision, demonstrates that isotope dilution LC/MS can be a viable technique for quantitation.

The results from the LC/MS/MS measurements of these same samples are also shown in Table 1. They agreed better with the certified value than did the LC/MS results, suggesting that the MS/MS process may have eliminated the interference that affected the LC/MS results. Precision of the measurements was very good and again, somewhat better than we would expect for this type of measurement.

These evaluations demonstrate that the combination of isotope dilution with LC/MS can provide accurate and precise results. This technique should be very useful for determining analytes that are difficult to measure by GC/MS, provided an isotope labeled internal standard is available. The use of a CID step provides additional specificity, with little loss of precision and little increase in effort.

Table 1
URIC ACID IN SRM 909 USING LC/MS AND LC/MS/MS
mmoles/L/g

Sample	LC/MS	LC/MS/MS	
	Run 1	Run 1	Run 2
A	0.583	0.580	0.557
B	0.583	0.573	0.566
C	0.586	0.574	0.570
Mean	0.584	0.576	0.564
CV %	0.30	0.60	1.13

Certified value by ID/GC/MS Definitive Method = 0.567
Difference of LC/MS mean = +3.00%
Difference of LC/MS/MS mean = +0.53%

QUANTITATIVE DETERMINATION OF LEUCINE TRANSAMINATION IN THE FETUS
FOLLOWING TRACER INFUSION OF ^{15}N - ^{13}C - LEUCINE

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The use of ^{15}N - ^{13}C -leucine as tracer combined with MS analysis provides a method by which the reversible transamination of leucine in the fetus can be dissected from irreversible degradative processes involving the carbon skeleton. Transamination of the stable isotope of leucine to the ketoacid produces a subsequent tracer enrichment of alpha-ketoisocaproic acid (KIC). Quantitative GC-MS-SIM allows for accurate and repeated assessment of the rates for both leucine transamination to KIC and irreversible degradation of the carbon skeleton to isovaleryl-CoA.

METHODS AND RESULTS

Whole blood and plasma amino acid concentrations are determined by standard ion exchange chromatography. Branched chain alpha keto acids are determined by a modification of the HPLC procedure described by Koike et.al. (1984). Alpha-ketoglutarate is used as the internal standard.

Isotopic enriched leucine is determined as the N-propyl, N-acetyl ester by the method of Matthews (1979). A Finnigan 4500 with a 15 meter, 0.25mm I.D., DB-17 Column with a flow rate of 1.5 ml/min of helium was used for the analysis. The oven temperature profile was 80° for 1 minute to 260° at 10°/min. Injector temperature was set at 250° and transfer line at 280°. Injections were made under splitless conditions. Chemical ionization was used and the molecular ions at m/e 216, 217 and 218 were monitored by selective ion monitoring to determine the isotopic ratio of the non-labelled (m/z 216) singly labelled (1- ^{13}C ; m/z 217) and doubly labelled (1- ^{13}C , ^{15}N ; m/z 218) species of leucine respectively (Fig. 1).

Isotopic enrichment of keto-isocaproate was determined by modifications of procedures described by Wolfe (1984). In brief, samples were deproteinized with 50% SSA, centrifuged at 10,000g, 0° C for 30 minutes, and transferred to reaction vessels. The samples were then derivatized with OPDA at 100° for 30 minutes. Following drying with anhydrous sodium sulfate, samples were extracted with methylene chloride and evaporated to dryness. BSTFA (50 ul) and pyridine (25 ul) were then added to the extraction vial, heated to 70° for 20 minutes, and sealed. A Hewlett Packard 5970 MSD outfitted with a 15 meter, 0.25mm I.D., DB-5 column with a flow rate of 0.85 ml/min of helium was used for analysis. The oven

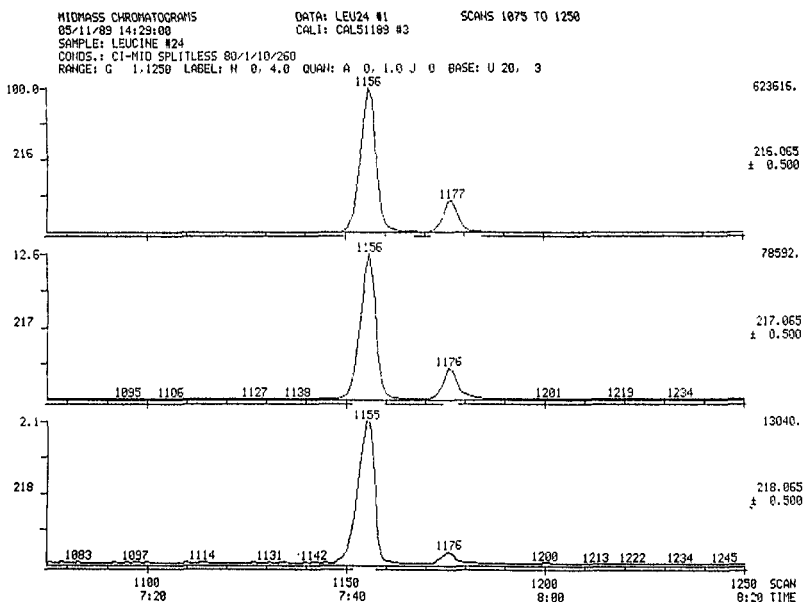
temperature profile was 130° for 1 minute to 260° at 10°/min. Injector temperature was set at 250° while the transfer line was at 280°. A 10:1 split injection was used. Ionization was by electron impact and the ion ratio at 232/233 determined by SIM for non-labelled and singly labelled (1-¹³C) KIC was used to calculate ¹³C isotopic enrichment.

Combined with the quantitative HPLC analyses for leucine and KIC, these GC/MS procedures provided a sensitive and repeatable method for monitoring of leucine metabolism as a marker for protein synthesis and amino acid oxidation on a repeated basis in the intact fetus.

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FIGURE 1



**ISOTOPE DILUTION METHOD FOR QUANTITATIVE GAS CHROMATOGRAPHY -
MASS SPECTROMETRY OF HYDROXY AND KETO ACIDS IN URINE.**

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Introduction.

The work described here involves primary hyperoxaluria which is a general term for two rare genetic disorders characterized biochemically by the continuous synthesis and excretion of excessive oxalic acid.

In type I (glycolic aciduria), the oxalic acid is excreted together with large quantities of glyoxylic and glycolic acids. Alpha-ketoglutarate:glyoxylate carbonylase activity has been implicated in this disorder¹.

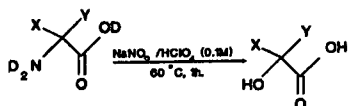
In type II (glyceric aciduria), large amounts of glyceric acid, which is formed from the reduction of hydroxypyruvate, is excreted. The hydroxypyruvate is thought to accumulate because of reduced leukocyte D-glyceric dehydrogenase activity¹.

The existing methods for the analysis of these hydroxy and keto acids involve enzyme assay for each compound; in the case of hydroxypyruvate, one does not exist.

We are working on a method, part of which is described here, which will analyse for all five organic acids in a single GC-MS run.

Methods.

Synthesis of the Internal Standards. 2,2-²H₂-glycolic and 3,3-²H₂-glyceric acids were synthesized by diazotizing perdeuterated glycine and 3,3-dideuteroserine, respectively.



X = Y = D, Glycine

X = H, Y = CD₂OD,
Sérine

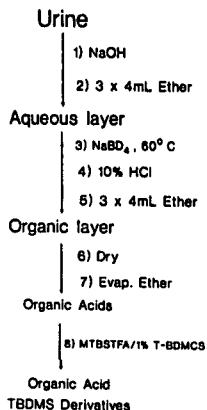
X = Y = D, Glycolic Acid

X = H, Y = CD₂OD,
Glyceric Acid

Extraction and Silylation of Organic Acids. Aqueous solution of, or urine containing the five hydroxy and keto acids was spiked with deuterated internal standards. The sample was then treated as shown in the opposite scheme. The TBDMS derivatives are then analysed by GC-MS.

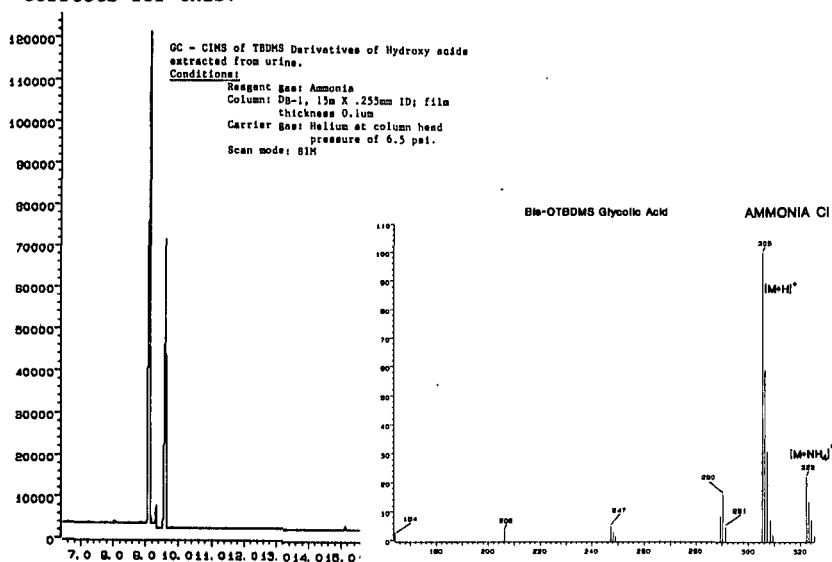
Results and Discussion.

The sodium borodeuteride reduction afforded the monodeuterated glycolic and glyceric acids from glyoxylic and hydroxypyruvic acids, respectively. EI spectra



show intense $[M-t-Bu]^+$ ion for the TBDMS derivatives of all the hydroxy acids. The ammonia CI give very interesting results; glycolic acid shows the $[M+H]^+$ ion as the most abundant. On the other hand oxalic acid gives the $[M+NH_4]^+$ as the base peak. This indicates that oxalic acid-TBDMS is a poorer proton acceptor than ammonia. The mass spectrum below is that of glycolic acid consisting of $NaBD_4$ -reduced glyoxylic, glycolic- d_0 , and glycolic- d_2 .

Selected ion monitoring was performed on a urine extract of the hydroxy acids after silylation. The ions at m/z 305, 306, 307 for glycolic acid, 336 and 338 for oxalic acid (^{12}C and $^{13}C_2$), and 449 and 450 for glyceric acid were monitored. The SIM TIC below shows the three compound at 9.05 min. for glycolic acid, 9.60 min. for oxalic acid, and 15.2 min. for glyceric acid. The glyceric acid is known to extract poorly. The isotope dilution technique, however, corrects for this.



Reference.

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Acknowledgement.

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DETERMINATION OF ^{13}C -, ^{15}N -, AND ^2H -ENRICHMENT USING CAPILLARY GAS CHROMATOGRAPHY - REACTION INTERFACE / MASS SPECTROMETRY (GC-RIMS)

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We have expanded the ability of a capillary gas chromatographic-reaction interface mass spectrometer technique (GC-RIMS) from the selective detection¹ of ^{13}C , ^{15}N , and ^2H (D) to quantify their enrichments. The microwaved-powered reaction interface converts materials from their original forms into small molecules whose mass spectra serve to identify and quantify the nuclides which make up each analyte¹. The presence of carbon, nitrogen, and hydrogen is followed by monitoring the isotopic variants of CO_2 , NO , and H_2 . Chromatograms showing only enriched ^{13}C and ^{15}N are produced by subtracting the abundance of naturally occurring isotopes from the observed $M + 1$ signal. A selective chromatogram of HD is obtained by measuring HD at m/z 3,0219 with a resolution of 2000. Mole enrichment of ^{13}C or ^{15}N is quantified by measuring the ratio of enriched $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ or enriched ^{15}NO to ^{14}NO .

When isotope dilution studies are done in biological experiments, one usually has a fixed amount of an unlabeled, endogenous compound and a variable, small amount of its labeled analog. Phenytoin was the test molecule for these experiments because: isotope ratio measurements in RIMS do not depend on structure; phenytoin is commercially available in a variety of isotopic forms; and our familiarity with its chemical properties. Prior to an isotope dilution study, an analyst must decide how much labeled compound is to be administered so that it can be detected and quantified in the presence of its unlabeled analog. The quantity of label administered is determined in part by the method and its detection limits for enrichment. To reduce any alteration in pool sizes, the concentration of a labeled compound is kept low. Therefore, a method with the ability to detect low levels of enrichment is preferred. We have carried out a series of experiments to determine the characteristics of GC-RIMS in measuring ^{13}C - and ^{15}N - enrichment as well as the detection limits of deuterium.

In separate experiments, either [2- ^{13}C -, 1,3- $^{15}\text{N}_2$]- (99 atom %, Cambridge Isotopes), [2,4,5- $^{13}\text{C}_3$]-, (90 atom %, Cambridge Isotopes), or [ring-D- 10], (99 atom %, MSD Isotopes)- phenytoin was added to 0.5 μg , 5 μg , and 50 μg of phenytoin to produce mole ratios ranging from 0.00059 - 0.494. For deuterium experiments, 2.5 μg of [ethyl D₅]-primidone (98 atom %, KOR Isotopes) was also added to each sample as an internal standard. Samples were derivatized by extractive ethylation using tert-butylammonium hydroxide and ethyl iodide in methylene chloride. The derivatives were reconstituted in toluene to produce a final volume of 100 μL . One μL of this derivatized extract was analyzed by GC-RIMS thus yielding 5, 50, or 500 ng of unlabeled phenytoin on column.

A Varian Model 3300 gas chromatograph equipped with a Varian Model 1075 split/splitless injector was used with a 30 meter DB-5, 0.25 mm i.d., 0.25 μm film thickness, capillary column (J & W). Ultra pure carrier grade helium fitted with high purity stainless steel regulators was operated at 38 - 40 psi to produce a helium flow of 1 or 2 mL/min. The injector temperature was 265°C and the initial column temperature was 80°C. Following injection of a 1 μL sample, the chromatograph was programmed at multiple rates up to 250 °C. All experiments were carried out on a Dupont 21-492 mass spectrometer which was equipped with a Trek Model 667 power supply. This power supply was controlled by a Teknivent data acquisition interface, software, and an IBM XT Model 286 computer.

Selective detection of ^{13}C was achieved using SO_2 as the reactant gas and monitoring $^{12}\text{CO}_2$ at m/z 44 and $^{13}\text{CO}_2$ at m/z 45. The isotopic contribution of $^{13}\text{C}^{16}\text{O}_2$ and $^{12}\text{C}^{17}\text{O}^{16}\text{O}$ at m/z 45 from unenriched material was 1.19% which is equivalent to its natural abundance. A chromatogram showing only enriched ^{13}C can be generated using Equation 1.

$$\text{Eq.1} \quad \text{Enriched } m/z \text{ 45} = \text{Measured } m/z \text{ 45} - [0.0119 * \text{Measured } m/z \text{ 44}]$$

Selective detection of ^{15}N was achieved using SO_2 as the reactant gas and monitoring ^{14}NO at m/z 30 and ^{15}NO at m/z 31. The isotopic contribution of $^{15}\text{N}^{16}\text{O}$ and $^{14}\text{N}^{17}\text{O}$ at m/z 31 from unenriched material was determined experimentally to be 0.440%, slightly greater than the theoretical 0.403%. We used this factor and equation 2 to generate a RIMS capillary chromatogram showing only ^{15}N -enriched substances.

$$\text{Eq.2} \quad \text{Enriched } m/z \text{ 31} = \text{Measured } m/z \text{ 31} - [0.00440 * \text{Measured } m/z \text{ 30}]$$

Selective detection of deuterium was achieved using H_2 as the reactant gas and monitoring HD at m/z 3,0219 with a resolution of 2000. A D- enriched chromatogram could not be generated because H_2 , the reactant gas, was greatly off scale. However, the natural abundance of deuterium is low and this means a calculation similar to equations 1 and 2 is not needed.

For each sample derivatized and analyzed, the mole enrichment was obtained by calculating the ratio of moles of ^{13}C , ^{15}N , or D present in labeled diethylphenytoin in excess of natural abundance to moles of ^{12}C , ^{14}N , or H present in the mixture including both unlabeled and labeled diethylphenytoin.

Enrichment of ^{13}C - or ^{15}N - labeled phenytoin was determined using GC-RIMS by measuring the ratio of the area under the peak at the retention time (r.t.) of diethylphenytoin (14.6 min.) in the enriched isotope chromatogram (generated using equation 1 or 2) to the area under the same peak in $^{12}\text{CO}_2$ or ^{14}NO chromatogram (representing total $^{12}\text{CO}_2$ or ^{14}NO). The observed mole ratio of D-labeled phenytoin to D-labeled primidone was also calculated in a similar manner. These enrichment data can also be manipulated using conventional mathematical expressions such as the atom % excess (A.P.E.) and the $\delta/\text{‰}$ (delta per mil).

The enrichments found at 3 concentrations of phenytoin were plotted against the calculated enrichments for ^{15}N and ^{13}C . A regression analysis was performed using a weighting of $1/y^2$ on the data set for each isotope. The reproducibility of RIMS was determined by analyzing 6 replicates each of samples with calculated mole enrichments of 0.008, 0.00198, and 0.0095 for ^{13}C , ^{15}N , and D, respectively, in the presence of 500 ng of phenytoin on column.

The experimentally determined ^{13}C enrichment for 500, 50, and 5 ng of unlabeled phenytoin on column was linear with a correlation coefficient of the entire data set of 0.96. The slope of this regression was 1.29 indicating that the determined ^{13}C enrichment corresponds closely to the calculated enrichment. The coefficient of

variation for the analysis of a sample at a calculated mole enrichment of 0.008 was 3%. As little as 10, 3, and 2.5 ng of ^{13}C -labeled phenytoin were detected in the presence of 500, 50 and 5 ng of unlabeled phenytoin, respectively. The lowest detectable mole enrichment for ^{13}C labeled phenytoin was 0.0028. This is equivalent to a calculated atom % excess (A.P.E.) 0.28 or a δ ‰ of 234.

As with ^{13}C , the experimentally determined ^{15}N -enrichment for 500, 50, and 5 ng of unlabeled phenytoin was found to be linear with a correlation coefficient of the entire data set was 0.98. The slope of the regression was 0.95 indicating that the determined ^{15}N enrichment corresponds closely to the calculated enrichment. The coefficient of variation for a sample representing an calculated mole enrichment of 0.002 was 6%. As little as 0.3 ng of ^{15}N -labeled phenytoin was detected in the presence of 500, 50, or 5 ng of unlabeled phenytoin. The lowest mole enrichment detected was 0.00059 which is equivalent to a calculated A.P.E. of 0.059 or a δ ‰ of 148.

A segment of a RIMS chromatogram showing ^{14}NO at m/z 30 and ^{15}NO at m/z 31 for 0.3 ng of ^{15}N -labeled phenytoin in the presence of 50 ng of unlabeled phenytoin is shown in Figures 1a and 1b. An enriched ^{15}N chromatogram computed using equation 2 is shown in Figure 1c.

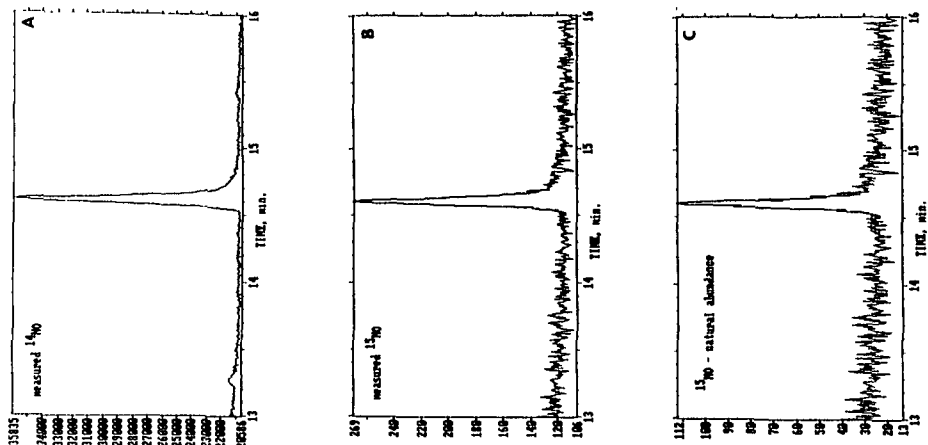
To evaluate the linearity of the deuterium analysis, the experimentally determined ratio of D-labeled phenytoin to D-labeled primidone was calculated and plotted against the calculated mole ratio. The correlation coefficient of the entire data set was 0.92 with a slope of the regression of 0.6. These results indicate that the quantity of ^2H -labeled phenytoin detected corresponds linearly with the quantity of D-labeled phenytoin added. Because the internal standard is not an isotopic variant of the analyte, the slope is not expected to be unity. The coefficient of variation of a sample representing a mole enrichment of 0.009 was 4%. The detection limits were 1 ng of D-labeled phenytoin in the presence of 500 ng of unlabeled phenytoin. The enrichment could not be experimentally determined although a theoretical mole enrichment of 0.00096 for this mixture was calculated. In practice, D-enrichment can be evaluated from the ratio of deuterium to carbon in each peak.

RIMS offers several advantages over conventional GC/MS methods in that it is a structure-independent technique for determining isotope ratios. Not only can RIMS quantify enrichment of a preselected set of compounds of interest but also any other compound present in a chromatogram which had become enriched through metabolism. This makes RIMS versatile. GC-RIMS also has the ability to quantify enrichment of more than one isotope simultaneously, e.g. ^{13}C , ^{15}N . Another characteristic of GC-RIMS is that the mass spectra enriched compounds can be obtained by turning off the reaction interface power, repeating the chromatographic analysis and analyzing the mass spectra at the retention time of the enriched compound found using RIMS. Multiple collector isotope ratio instruments lack this capability. Reproducibility is 3-6%. The limits of detection are 100 fold poorer than that available from on-line combustion GC-multiple collector isotope ratio mass spectrometers, however we show good quantitation for mole enrichments low enough to enable many biological experiments.

¹Chace, D.H. and Abramson, F.P., "Metabolism Studies using Stable Isotopes: Application of a Capillary Gas Chromatograph - Reaction Interface / Mass Spectrometer Technique (GC-RIMS) to the Selective Detection of ^{13}C , ^{15}N , AND ^2H , submitted for publication to Anal. Chem.

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Figure 1. Sections of a RIMS capillary gas chromatogram demonstrating the selective detection of ^{15}N -phenytoin (0.3 ng) in the presence of 50 ng of unlabeled phenytoin. Panel a: Detection of ^{14}NO at m/z 30. Panel b: Detection of ^{15}NO at m/z 31. Panel c: Detection of enriched ^{15}N using equation 2.



QUANTIFICATION OF THIODIGLYCOL IN URINE AFTER MUSTARD GAS EXPOSURE.
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A new assay has been proposed to quantify thiodiglycol, a major breakdown product of mustard gas, in urine by gas chromatography-mass spectrometry (GC-MS) using heptafluorobutyric anhydride (HFBA) derivatization. Mustard gas was allegedly used during the Iran-Iraq conflict in 1984. Conclusive chemical verification was not possible at that time due to the presence of high levels of thiodiglycol in control urines.¹ Subsequent reports have indicated thiodiglycol levels in control urines may be low enough to be useful in verifying mustard gas exposure.^{2,3} Our objective was to develop a reliable, portable assay for thiodiglycol that would verify mustard gas exposure. Reliability was increased by maximizing analyte recovery during sample preparation, stabilizing the thiodiglycol against oxidation, and preventing against contamination. The assay was made more portable by using electron ionization on a benchtop instrument. Verification of mustard exposure using thiodiglycol would be possible only if control levels of thiodiglycol are shown to be very low. Therefore, a data bank of human urine control levels was started.

In the first phase of the study, spiked human, rat, or guinea pig urine (1 to 500 ng/mL) served as samples. Sample preparation consisted of incubation with glucuronidase, acidification to pH 2, evaporation with N₂ at 90°C, derivatization with an excess of HFBA, and then extraction into ethyl acetate. A Hewlett-Packard GC-MSD with a DB5 (30m) capillary column was used for separation and detection in the selected ion monitoring mode. Derivatization of thiodiglycol with HFBA produced the di-heptafluorobutyric acid ester (di-HFBA) in 95% yield. The molecular ion at m/z 514 of the thiodiglycol derivative was not used because of low abundance. Instead, the analyte ions at m/z 300 and 301 were used for quantification along with m/z 309 of the deuterated internal standard. These ions represented loss during fragmentation of one of the two HFBA substituents present on the molecule. The di-HFBA thiodipropanol standard was monitored using ions m/z 328 and 542. Standard curves (1, 5, 10, 50, 100, and 500 ng/mL thiodiglycol) were prepared using an internal standard approach. Both thiodipropanol and dg-thiodiglycol were used as internal standards.

The second phase of this study consisted of animal exposure to mustard gas by both subcutaneous injections and by vapor exposure. Rats were exposure subcutaneously at two doses. Each group of twelve rats received either 450 ug/kg or 750 ug/kg mustard with collection and analysis of the urine for thiodiglycol. A separate group of twelve rats served as a control. Urine was collected in 4 and 8 hour intervals from the rats for two weeks post-exposure. Several chemical and physical methods of stabilizing the urine were attempted during the rat study. Guinea pigs were exposed to a mustard saturated vapor over a small area of their back (4-6 cm²) for two to ten minutes. A single urine specimen was collected from the guinea pigs at 24 hr post-exposure.

Both internal standards yielded linear relationships over the entire concentration range. Precision using the deuterated internal standard ranged from 15% RSD (percent relative standard deviation) at 1 ng/mL to 2% RSD at 500 ng/mL of thiodiglycol in rat and human urine. Thiodipropanol (1 ug/mL sample) was found to prevent degradation of the analyte. Analyte recovery was better than 90% which included the 95% conversion of thiodiglycol to the di-HFBA derivative.

During the development of the assay, several other derivatives of

thiodiglycol were made such as the trifluoroacetic acetate derivative. In general, the molecular ions of thiodiglycol and its derivatized forms had very low relative abundances. Conversely, thiodipropanol and its derivatized forms yielded relatively stable molecular ions. For example, the M⁺ of underivatized thiodipropanol (m/z 150) represented 80% of the total ion abundance versus 4.5% in the case of thiodiglycol (m/z 122). One reaction that contributed to the lower abundance of molecular ion in the case of thiodiglycol was the elimination of water or its equivalent. Loss of water could have produced either 1,4-thioxane or 2-hydroxyethyl vinyl sulfide. If 1,4-thioxane was formed then the d₈-thiodiglycol under the same mass spectrometric conditions should retain all eight deuteriums. However, loss of one deuterium was noted which supported the formation of the vinyl analog of thiodiglycol. Furthermore, the relative abundance of the M⁺ for the deuterated thiodiglycol was 6.5 times that of the undeuterated species. This indicated a primary kinetic isotope effect which supported the breaking of a carbon hydrogen bond in the rate determining step. An E_{1cb} type of mechanism described the dehydration of underivatized thiodiglycol. A similar elimination reaction was observed when thiodiglycol was derivatized. However, the isotope effect dropped to 2.5 in the case of HFBA derivatives. The heptafluorobutyrate ion was a better leaving group and made a larger contribution to the elimination reaction than did the hydroxide ion.

Animal exposure to mustard has typically resulted in the production of many metabolites.^{4,5} Thiodiglycol has been one of the most accessible metabolites of mustard to quantify. Mustard injections in the rat were approximately 1/4 and 1/3 of an LD₅₀. In general, mustard at this level of exposure had no effect on the hematology or health of the animals. Determinations of thiodiglycol levels were possible for approximately a week after the subcutaneous injections. Collecting the samples over ice or the use of anti-oxidants such as nordihydroxyguairitic acid proved to be no better at stabilizing urine samples than the use of thiodipropanol alone. Thiodiglycol levels in rat urine post mustard exposure were 70.5±19.5 ng/mL (24hr) and 60.9±21.4 ng/mL (48hr) for the low dose. The higher dose of mustard produced levels of 196±109 ng/mL (24hr) and 113±58.1 ng/mL (48hr). Control levels of thiodiglycol were below detectable limits. Thiodiglycol levels in guinea pig urine after vapor exposure ranged from 34 to 185 ng/mL. Studies were also undertaken to develop a data base on human background levels of thiodiglycol in control groups. At present, background levels of thiodiglycol in urine collected from human volunteers (15 samples) have been 2 ng/mL or less. These results have indicated that thiodiglycol would be a suitable metabolite to verify and monitor mustard exposure. In man mustard metabolites are eliminated only half as fast as in rats.⁴ Therefore, detection and verification of mustard contamination should be possible for up to two weeks after exposure depending on degree of exposure.

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Application of FAB and Thermospray Mass Spectrometry to the Identification of Two Glucuronide Metabolites of Methapyrilene

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INTRODUCTION

Ethylenediamine antihistamines although once widely used, may have undesirable toxicological effects. Methapyrilene is reported to be a rat hepatocarcinogen (1). Pyrilamine induces unscheduled DNA synthesis (2). For this reason, we have developed mass spectral methods for the identification of metabolites of ethylenediamine antihistamines as well as metabolites from other less problematic compound classes (3-5).

We report the characterization of two glucuronide metabolites of methapyrilene, (from mouse-hepatocytes) using TS and FAB/MS. One metabolite was incompletely separated (HPLC) from more abundant components presumed to be from the biological matrix. This problem is typically encountered in the identification of radiolabeled metabolites in biological systems. Because of the complementary nature of the TS and FAB mass spectra it was possible to ascertain that the metabolites had the same molecular weight as likely glucuronide metabolites, that a glucuronide substituent was present, that the glucuronidation occurred remote from the dimethylamine moiety, and that the metabolites differed by the metabolic loss of a single methyl group.

EXPERIMENTAL

FAB mass spectra were obtained with a Kratos MS-50 equipped with an M-Scan fast atom source and gun. FAB ionization employed 10-keV xenon atoms. Approximately 0.5 μ g of sample was loaded on a copper target after 2-3 μ l of thioglycerol matrix liquid.

Thermospray mass spectra were obtained using a Delsi Ribermag RC10-10C equipped with a Vestec TS controller and a Delsi TS source. A liquid carrier composed of 80/20 methanol/0.5M aqueous ammonium acetate was used at a flow rate of 1.5 ml/min. The TS conditions were control temperature 118C, tip 207C, and block 237C. Approximately 5 μ g of sample was injected in 10 μ l.

Metabolites were generated from suspension-cultures of isolated mouse-hepatocytes dosed with methapyrilene hydrochloride (specific activity 50 mCi/mmol). The metabolites were isolated, purified and quantified according to previously published HPLC (6) and scintillation methods.

RESULTS AND DISCUSSION

Two methapyrilene metabolites had HPLC retention characteristics similar to those of other glucuronide metabolites. Prior to mass spectral analysis these HPLC fractions were further purified (C-18 SEP-PAK) to remove salts from the HPLC system. Signal levels in the FAB spectra of similar metabolites have been reduced when this step was omitted (3).

Methapyrilene glucuronide

Initial FAB/MS analysis of the major metabolite HPLC fraction, showed a series of cation adduct molecule ions at m/z 454, 476, 492, 498, 514, and 530 corresponding to $[MH]^+$, $[MNa]^+$, $[MK]^+$, $[MNa_2-H]^+$, $[MNaK-H]^+$, and $[MK_2-H]^+$. Major ions were also observed at m/z 409, and 278 and 233. The ion at 409 daltons corresponds to loss of dimethylamine, and has been shown to be a characteristic cleavage for ethylenediamine antihistamines (4). Based on this loss, glucuronidation of the antihistamine occurred remote from the dimethylamine moiety. The ion, at m/z 278 corresponds to loss of the glucuronide sugar (176 daltons), and is reported to be a primary and initial loss from the FAB produced protonated molecules of many glucuronide metabolites (7). The other ion, m/z 233, corresponds to loss of both the glucuronide sugar and dimethylamine moiety.

The TS mass spectrum also showed a protonated molecule, albeit much

weaker relative to m/z 278 (sugar loss). Loss of a dimethylamine moiety (45 daltons) was not observed using TS ionization. A weak ion at m/z 233 was also observed via TS/MS, suggesting sequential loss of the sugar and dimethylamine.

Desmethylmethapyrilene glucuronide

FAB and TS/MS analysis of the other HPLC fraction initially showed major ions at m/z 371 and 391 (FAB) or 388 (TS), not corresponding to a glucuronide or any other known metabolite. Closer examination of the TS spectra revealed weak ions at m/z 440 and 264 corresponding to the protonated molecule, and subsequent sugar loss from a glucuronide of desmethylmethapyrilene. Reexamination of the FAB spectrum also revealed an ion at m/z 440 whose time dependence in mass/time plots differed significantly from the more abundant ions at m/z 371 and 391. By selecting a scan so as to minimize the unwanted signals (m/z 371 and 391) with respect to the suspect protonated molecule (m/z 440), it was possible to produce a FAB spectrum with minimal contribution from the biological matrix. The protonated molecule, and a signal corresponding to the loss of methylamine (m/z 409) were then observed. A difference of 14 daltons relative to the major metabolite, as well as the loss of 45 daltons (methylamine) are very suggestive of demethylation of the major metabolite to produce the minor one. Thus the metabolite in this HPLC fraction appears to be desmethylmethapyrilene glucuronide. Although this compound was apparently not resolved from other major components even using two HPLC separation steps, structural assignment was possible because of the complementary nature of the fragmentation observed in the FAB and TS mass spectra.

CONCLUSION

The fragmentation observed in the thermospray and FAB mass spectra of the glucuronide metabolites of this ethylenediamine antihistamine were utilized advantageously for the identification of the metabolites. The complementary nature of the fragmentation was especially useful in samples showing high levels of extraneous unwanted signals, and we expect this phenomenon will apply to other metabolite identification problems as well. The two mouse-hepatocyte metabolites were identified as methapyrilene glucuronide, and desmethylmethapyrilene glucuronide. Finally, these results are consistent with previous reports that the characteristic fragmentation pathways in the FAB mass spectra of related antihistamines, including other ethylenediamines (3,4), behave more or less independent of either minor structural changes associated with metabolism or the small differences in molecular structure which occur between members of a given antihistamine class. The existence of these antihistamine class-specific fragments in FAB mass spectra significantly aid the interpretation of spectra obtained from metabolism studies, especially in studies such as this one where the presence of unwanted signals from the biological matrix may obscure analytically useful ions.

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BIOACTIVATION OF 1,2-DIBROMO-3-CHLOROPROPANE TO FIVE GLUTATHIONE
CONJUGATES IN VIVO : MECHANISM OF FORMATION AND DETECTION BY TANDEM
MASS SPECTROMETRY

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The haloalkane nematocide 1,2-dibromo-3-chloropropane (DBCP) was used extensively as a soil fumigant prior to the observation of oligospermia and infertility in male pesticide formulators exposed to DBCP. In experimental systems DBCP is a carcinogen, nephrotoxin, testicular-toxin and is mutagenic to *Salmonella typhimurium* TA100. Toxicology studies have inferred a role for glutathione (γ -glutamyl-cysteinylglycine; GSH) dependant metabolism in DBCP-related target-organ toxicities, however, the nature of the reactive intermediate(s) and their mechanism of biogenesis have not been well defined chemically.

Fast-Atom Bombardment Tandem Mass spectrometry (FAB/MS-MS), represents a powerful tool in the structural elucidation of GSH conjugates¹, and when combined with the use of stable isotopically-labelled substrates, the information thus derived provides a unique insight into the chemical nature of the reactive intermediate(s) and their mechanism of biogenesis. Here we report the identification of five GSH conjugates of DBCP by MS/MS techniques and employ a per-deutero- analog of DBCP (D₅-DBCP) as a mechanistic probe to address the metabolic events in the formation of these conjugates.

Experimental: GSH conjugates excreted in the bile of rats administered DBCP (80mg/kg), or D₅-DBCP were converted to their N-benzoyloxycarbonyl-dimethylester derivatives¹, separated by HPLC and examined by FAB/MS-MS. Collision-induced dissociation (CID) daughter ion spectra of the MH⁺ ions of metabolites were recorded on a VG 70 SEQ hybrid tandem instrument of EBQQ geometry, equipped with an Ion-Tech fast atom gun, and a VG11/250 data system. CID was performed in the first (rf-only) quadrupole employing argon as a collision gas (2×10^{-6} Torr) and collision energies of 20-30 eV. Daughter ion spectra were recorded via the data system in the multi-channel-analysis mode and subsequently mass measured.

Results and Discussion: Five biliary GSH conjugates derived from DBCP were identified as: S-(2,3-dihydroxypropyl)glutathione (**1**), S-(3-chloro-2-hydroxypropyl)-glutathione (**2**), 1,3-bis-(S-glutathionyl)-propan-2-ol (**3**), 1-(S-cysteinylglycine)-3-(S-glutathionyl)-propan-2-ol (**4**) and S-(2-hydroxypropyl)glutathione (**5**). Under CID conditions structurally informative fragment ions were observed for **1** - **5**, the origins of which are depicted in Figure 1 and 2. **1** (Figure 1), **2** and **5** displayed characteristic fragment ions arising from expulsion of methylglycine (-89u; ion **a**) or the derivatized γ -glutamyl moiety (-277u; ion **e**). Further fragmentation of ion **e** with elimination of HSX (where X represents the conjugated xenobiotic) afforded an ion at m/z 159 common to **1**, **2** and **5**. The identity of each of these conjugates was confirmed by reference to the CID spectra of authentic standards. For high mass glutathione conjugates **3** and **4**, collision gas pressures of 7×10^{-6} Torr were required to yield structurally informative fragment ions. In the case of **3** (Figure 2) ions arose from two losses of methylglycine (-89u) to afford ions at m/z 906 (ion **a**) and 817 (ion **a'**), or via elimination of the γ -glutamyl moiety (ion **e**). Significant fragmentation was also observed adjacent to either sulfur, and a number of ions at low mass (< 500u) were common to **1** and **3**. Upon administration of D₅-DBCP conjugates **1-4** displayed full deuterium retention. Conversely, **5** retained only three deuteriums indicating an oxidative step in its genesis. The results indicate that the bioactivation of DBCP to GSH conjugates proceeds almost exclusively by conjugative processes. The chemical nature of the conjugates and full deuterium retention in **1-4** implicates the intermediacy of a reactive episulfonium ion in the bioactivation of DBCP.

FAB / MS-MS : CID on m/z 544

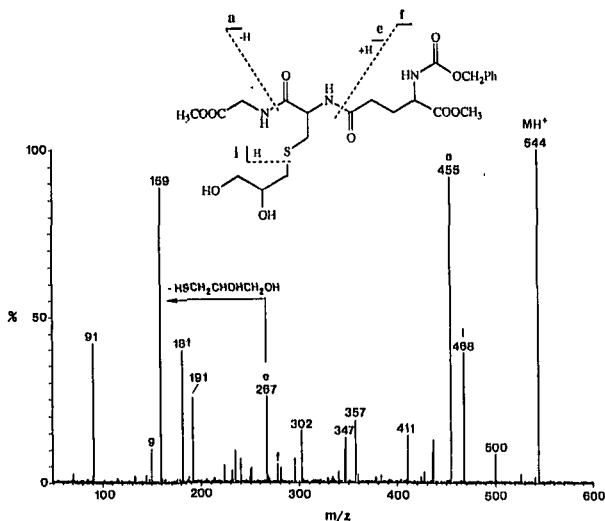


Figure 1. Daughter ion spectrum of **1** obtained by CID on $MH^+ = 544$. The structure of the derivatized metabolite and the proposed origins of the daughter ions are as indicated

FAB / MS-MS : CID on m/z 995

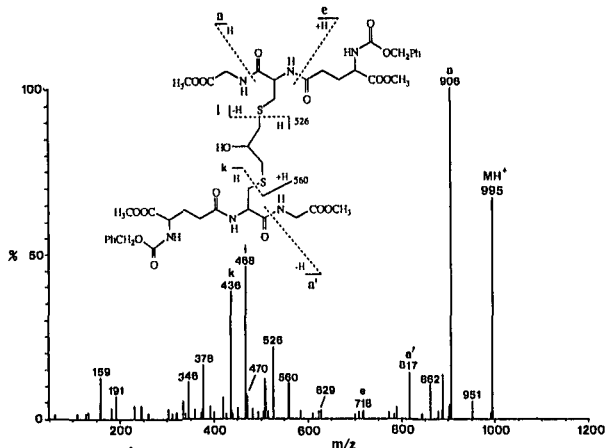


Figure 2. Daughter ion spectrum of **3** obtained by CID on $MH^+ = 995$. The structure of the derivatized metabolite and the proposed origins of the daughter ions are as indicated

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A SCREENING STRATEGY FOR THE DETECTION OF DERIVATIZED GLUTATHIONE CONJUGATES BY TANDEM MASS SPECTROMETRY (MS/MS).

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Conjugates of the endogenous tripeptide glutathione (γ -glutamyl-cysteinyl-glycine; GSH) may arise from the interaction of GSH with reactive electrophilic species, which are generated metabolically from drugs or other xenobiotics. Analytical strategies to isolate and characterize GSH conjugates are important as a knowledge of the structure of the conjugate provides a unique insight into the chemical nature of the transient electrophilic species from which it is derived. We have recently demonstrated the analytical utility of an aqueous phase derivatization procedure to confer excellent chromatographic and collision induced dissociation (CID) MS/MS properties upon GSH conjugates¹

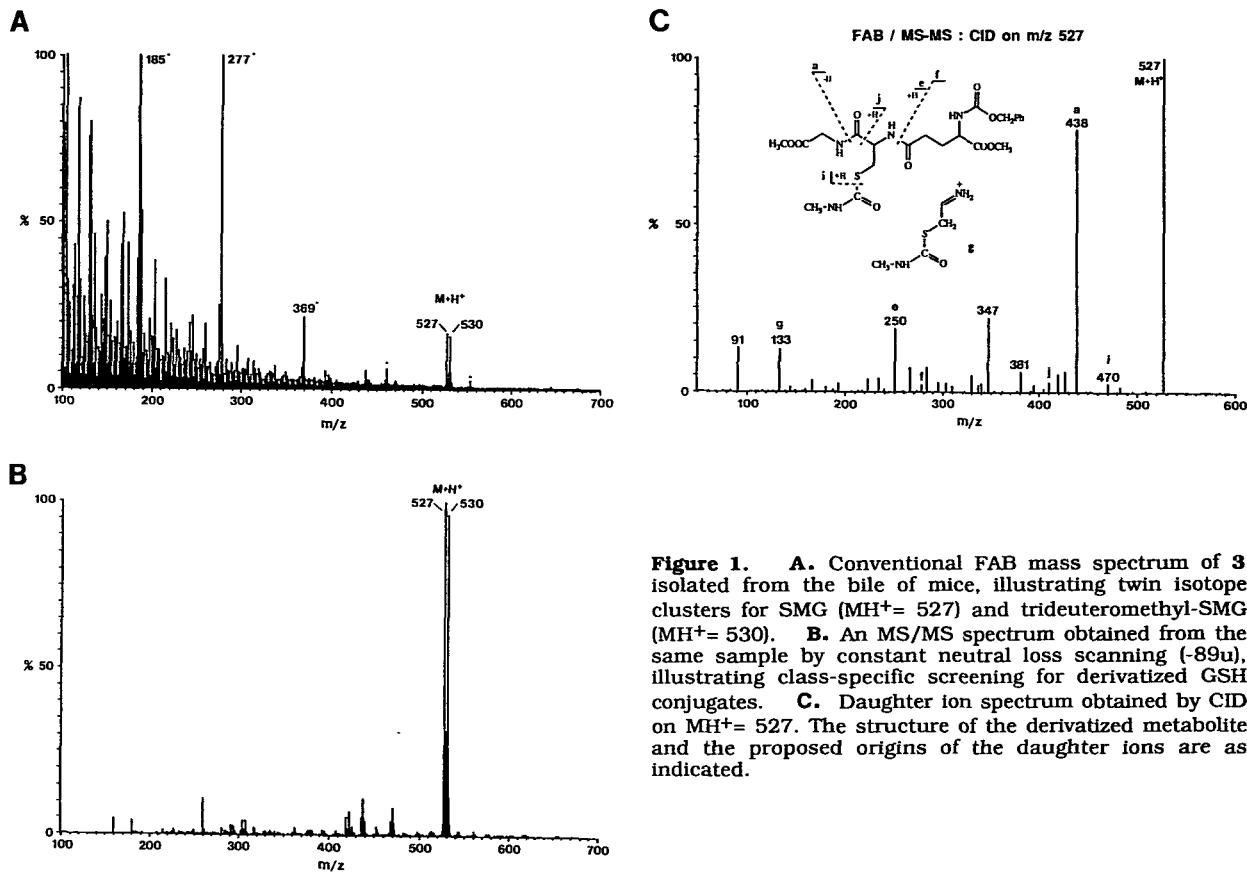
The objective of the present investigation was to exploit a characteristic fragmentation pathway observed for derivatized GSH conjugates (loss of glycine methylester; -89u), to facilitate their identification by a class-specific constant neutral loss screening strategy. As model compounds for this investigation we chose GSH conjugate metabolites of acetaminophen, trichloroethene, N-methylformamide (NMF) and 1,2-dibromo-3-chloropropane (DBCP). The analytical utility of this approach was evaluated by identification of GSH conjugates of NMF and DBCP isolated from biological matrices.

Experimental: 1,2-Dichlorovinylglutathione (**1**), oxidized GSH (**2**) S-(N-methylcarbamoyl)glutathione (SMG; **3**), S-(2,3-dihydroxypropyl)glutathione (**4**), 3-(S-glutathionyl)acetaminophen (**5**) and 1,3-bis(S-glutathionyl)-propan-2-ol (**6**) were converted to N-benzyloxycarbonyldimethylester derivatives. Daughter ion spectra were recorded on a VG 70 SEQ tandem hybrid instrument (EBQQ geometry) as previously described¹. CID was performed in Q₁ and neutral loss screening undertaken by scanning B and Q₂ at a constant mass offset of 89u. NMF and a trideuteromethyl analog (400mg/kg) were co-administered to male BALB/c mice; SMG (**3**) and its trideutero-variant excreted as biliary metabolites were derivatized, purified by HPLC and examined by neutral loss scanning. In a similar experiment, **4** excreted in the bile of rats administered DBCP (80mg/kg) was identified by analogous MS/MS techniques.

Results and Discussion: The general applicability of this technique was established by characterising a total of six derivatized GSH conjugates of diverse chemical structure by MS/MS techniques. Structurally-informative fragment ions were observed in the CID spectra of **1** to **6**; in each case the most intense daughter ion arose by elimination of glycine methylester (-89u) as exemplified for **3** (Figure 1C). Constant neutral loss scanning, for this transition permitted identification of **3** (Figure 1B) and **4**, as biliary metabolites in mice or rats, administered NMF or DBCP respectively. The selectivity of this approach is illustrated by comparison of the conventional FAB spectra of **3** (Figure 1A) with that obtained by constant neutral loss scanning (Figure 1B). In conclusion, the conversion of GSH conjugates to their N-benzyloxycarbonyldimethylester derivatives conferred excellent chromatographic properties and induced characteristic MS/MS (CID) fragmentation pathways. These diagnostic fragments provided detailed structural information and facilitated class-specific neutral loss screening to identify derivatized GSH conjugates derived metabolically from xenobiotics of diverse chemical structure.

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ANALYSIS OF BENZO(A)PYRENE METABOLITES BY
CONTINUOUS-FLOW FAB/MS

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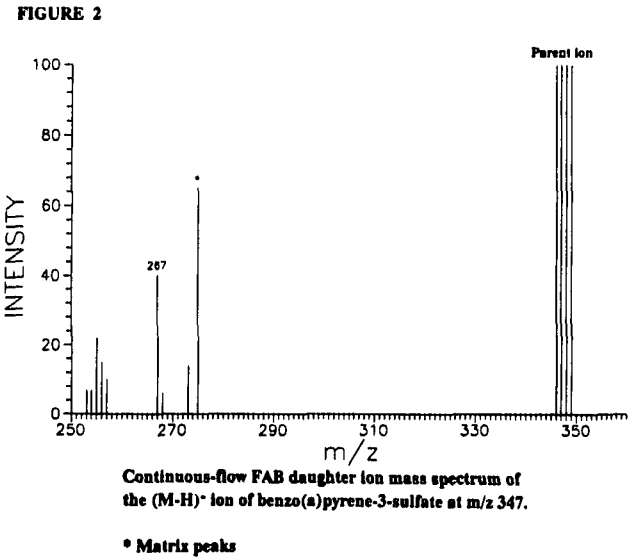
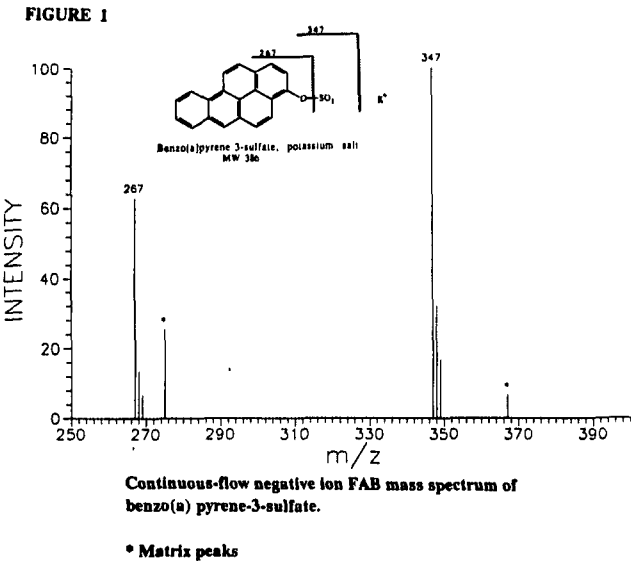
The carcinogen benzo(a)pyrene requires metabolic activation to ultimate carcinogenic derivatives before it binds to macromolecules and causes cancer induction. The study of the multiple pathways of metabolism of benzo(a)pyrene is thus essential to understanding the mechanism of carcinogenesis. The metabolites of benzo(a)pyrene include phenols, diols, tetrols, and the water soluble glucuronic acid, sulfate and glutathione conjugates. Identification of the conjugates by electron impact or chemical ionization mass spectrometry has been difficult because these metabolites are involatile and thermally unstable. Although fast atom bombardment mass spectrometry (FAB/MS) has proven useful for analyzing a wide variety of involatile or thermally labile substances, detection limits are not low because of the characteristically high level of background signals. For this reason, we have investigated the potential of continuous-flow FAB/MS as a means of identifying metabolites of benzo(a)pyrene.

A Kratos MS-50 double focusing mass spectrometer was used for mass spectrometric analyses. An Applied Biosystems microbore pump was used to deliver 2 μ l/minute of a 5% aqueous glycerol solution through a 3 m, 0.075 mm id fused silica capillary tube. The continuous-flow probe tip, which was kept at 45-50°C for all measurements, was constructed in our machine-shop. The standard, benzo(a)pyrene-3-sulfate was dissolved in 5% aqueous glycerol and injected using a one microliter loop injector.

The continuous-flow FAB mass spectrum of the sulfate conjugate shows a strong signal at m/z 347, corresponding to the acid anion as well as another strong fragment ion signal at m/z 267 (Fig. 1). When 100 ng of this sulfate was injected, a signal to noise ratio (S/N) of 60 was achieved. In addition to giving substantially improved S/N, the continuous-flow probe also gives a significant increase in the absolute signal level.

Daughter ion MS/MS spectra have been used frequently to establish parent-daughter relationships which are very useful for determining molecular structures. In addition, MS/MS often give increased selectivity and reduced detection limits. A linked scan (E/B) of the parent ion m/z 347 of the sulfate conjugate gave a daughter ion at m/z 267 as the only daughter ion (Fig. 2). The other peaks in the daughter ion spectrum were background fragment ions from glycerol. The peak at m/z 267 is formed by cleavage of the oxygen-sulfur bond with charge retained on the hydrocarbon portion.

The present studies show that subnanomolar quantities of sulfate conjugates of benzo(a)pyrene can be detected easily by continuous-flow FAB/MS. This provides the sensitivity required for the analysis of these substances when isolated from cell culture. We have also shown that MS/MS can be used to establish definite relationships between fragment ions and parent ions. This feature will be important when we analyze complex mixtures, such as cell extracts. The presence of a strong molecular ion and a characteristic fragment ion enables us to use selected ion monitoring in our LC/MS work.



CHARACTERIZATION OF THE BILIARY METABOLITES OF THE HEPATOTOXIC MONOTERPENE PULEGONE BY TANDEM MASS SPECTROMETRY (MS/MS).

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Conjugation to the tripeptide glutathione (γ -glutamyl-cysteinyl-glycine; GSH) constitutes a major pathway for the detoxification of electrophilic metabolites of protoxic compounds. Thus, structural elucidation of GSH conjugates provides a unique approach to the identification of transient reactive species derived metabolically from foreign compounds.

Pulegone is a hepatotoxic monoterpene constituent of the folklore abortifacient Pennyroyal Oil. Significant hepatocellular GSH depletion is induced by pulegone in experimental systems and its toxicity can be enhanced by prior depletion of hepatic GSH (Gordon *et al*, 1982). Hence, the hepatotoxicity may be mediated by the metabolic generation of electrophilic intermediate(s), which become conjugated with GSH. The objectives of the present investigation was to isolate and characterize biliary GSH conjugates of pulegone by fast-atom bombardment tandem mass spectrometry (MS/MS).

Experimental: GSH conjugates excreted in the bile of rats, following co-administration of ^{14}C -pulegone and a $^2\text{H}_3$ analog (1:1) were converted to their N-benzoyloxycarbonyldimethyl ester derivatives, purified by preparative RP-HPLC and analyzed by FAB / MS (Pearson *et al*, 1988). Metabolites were identified from their MH^+ ion twin isotope clusters (derived from $^2\text{H}_0$ - and $^2\text{H}_3$ -pulegone) observed in their respective FAB mass spectra. Daughter ion spectra of the MH^+ ions were recorded on a VG 70 SEQ hybrid tandem instrument of EBQQ geometry, equipped with an Ion-Tech fast atom gun. Collisionally activated decomposition (CAD) was performed in the first (rf-only) quadrupole, with argon as a target gas (2×10^{-6} Torr) and collision energies of 20-40 eV.

Results and Discussion: Three distinct GSH conjugates were detected, based on HPLC mobility, molecular weights and CAD daughter ion spectra. Molecular species with weights of 617, 619, and 621, corresponded to GSH attached to the elements of oxidized, unchanged and reduced pulegone, respectively. These conjugates displayed uniform fragmentation characteristics, with principle pathways consisting of elimination of glycine methylester (-89u), or the xenobiotic moiety to afford protonated GSH (470u) (see example in figure 1).

In addition to these simple GSH conjugates several metabolites were identified which apparently contained structural elements of glucuronic acid (GLU) as well GSH; mixed diconjugates of this type have not previously been reported. A metabolite with $\text{MH}^+ = 812/815$ (corresponding with $^2\text{H}_0$ and $^2\text{H}_3$ label, respectively) displayed collisionally induced losses of glycine methylester (-89u) and methyl glucuronate (-190u, assignment confirmed by fragmentation of simple glucuronides of hydroxypulegone); ions at m/z 470 (GSH) and 154 (reduced pulegone) were present as well (figure 2). By the use of MS/MS, in conjunction with isotopic labeling, we have determined that pulegone is bioactivated to three distinct electrophilic intermediates, which may each contribute to the hepatic necrosis caused by this agent.

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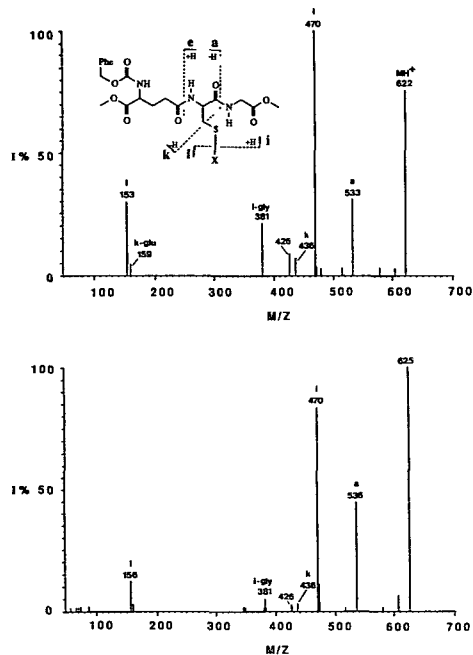


Figure 1. Daughter ion spectra of m/z 622 and 625. This twin ion cluster had been recognized in the regular FAB mass spectrum of a radioactive HPLC fraction obtained from bile of rats treated with $^2\text{H}_0$ - and $^2\text{H}_3$ -labeled pulegone. The moiety X (insert) contains the elements of reduced pulegone. Note that protonated GSH (m/z 470) is the most prominent daughter ion.

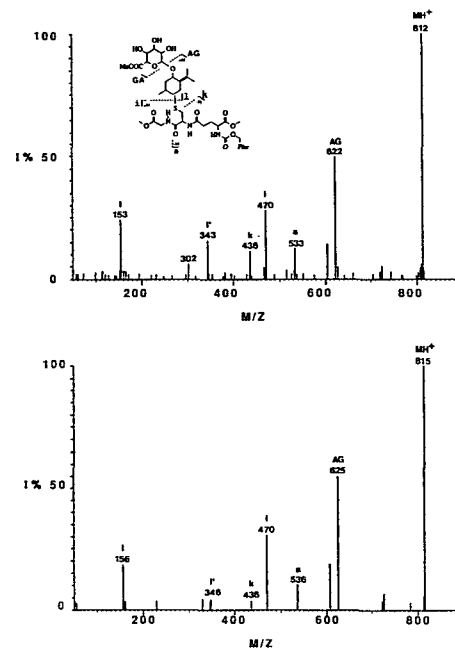


Figure 2. Daughter ion spectra of a diconjugate of $^2\text{H}_0$ (top) and $^2\text{H}_3$ pulegone (bottom), which was isolated from rat bile. The loss of 190u from the respective MH^+ ions is indicative of (methyl ester of) glucuronic acid, whereas m/z 470 corresponds to GSH. The origin of other diagnostic ions is indicated.

RAPID SCREENING OF NEONATAL WHOLE BLOOD FOR VARIOUS METABOLIC
DISORDERS BY FAB-MS/MS

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It is well documented that specific acylcarnitines increase in concentration in biological matrices as a result of inherited disorders of fatty acid and branched chain amino acid catabolism. In this study, a method was developed to obtain diagnostic acylcarnitine profiles from neonatal whole blood adsorbed onto filter paper (PKU cards) and from umbilical cord blood. We also investigated the feasibility of screening for inherited metabolic disorders in the neonatal period.

To recover acylcarnitines from "PKU cards", one dried blood circle, corresponding to approximately 50 μ l whole blood, was cut out, chopped and extracted ultrasonically for 20 minutes with methanol (1 ml). The extract was filtered onto a reverse-phase cartridge (C18, 0.1 g) and eluted with 2 ml methanol. The filtrate was evaporated and the residue treated with methanolic-HCl prior to analysis by FAB-MS/MS. Umbilical cord blood plasma (400 μ l) was mixed with methanol (400 μ l), centrifuged and the supernatant applied to a C18 cartridge (0.6 g) and treated as described for the blood-spot extracts.

Samples were analyzed by FAB-MS/MS using a triple-quadrupole (TRIO-3, VG Masslab, UK). Xenon gas was used for the primary atom beam, argon was employed as the collision gas (0.2 - 0.5 uBar) and the collision energy was 15eV. The instrument was set up to detect precursors of m/z 99; these are predominantly the molecular cations of acylcarnitine methyl ester¹. Thus, the new scan function generates a "metabolic profile" of acylcarnitines which would detect about 12 known inherited metabolic diseases and perhaps identify some new disorders.

Application of the method to neonatal blood spots is shown in Figure 1. Normal blood (Fig. 1A) and post-mortem blood from a child who died of unexplained causes ("SIDS", Fig. 1B) was spotted onto the PKU cards before extraction. The normal blood shows numerous interesting signals, with a weak signal at 218 corresponding to the normal metabolite, acetylcarnitine. The blood from the "SIDS" case showed a profile dominated by the M^+ ions of medium-chain length acylcarnitines, e.g. m/z 274 (hexanoyl) and 302 (octanoyl). This profile is diagnostic of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, one of the most common metabolic diseases and frequently associated with SIDS. The profile in Fig. 1C is from the original PKU card of a child with a confirmed diagnosis of MCAD. A useful diagnostic profile was observed although the card had been stored 6 months at 20° C. The effect of storage on the recovery of acylcarnitines has not been determined.

Normal and diagnostic plasma acylcarnitine profiles of propionic acidemia and isovaleric acidemia are compared in Fig. 2. The profiles from the affected children are very clear. It would appear that FAB-MS/MS is a very efficient screening technique when neonatal blood is available. At present, PKU cards are the most readily available source. The preliminary results are encouraging and studies are in progress to optimize the recovery of acylcarnitines.

Reference 1. D.L. Norwood, N. Kodo, and D.S. Millington, Rapid Commun. Mass Spectrom. 2, 269 (1988).

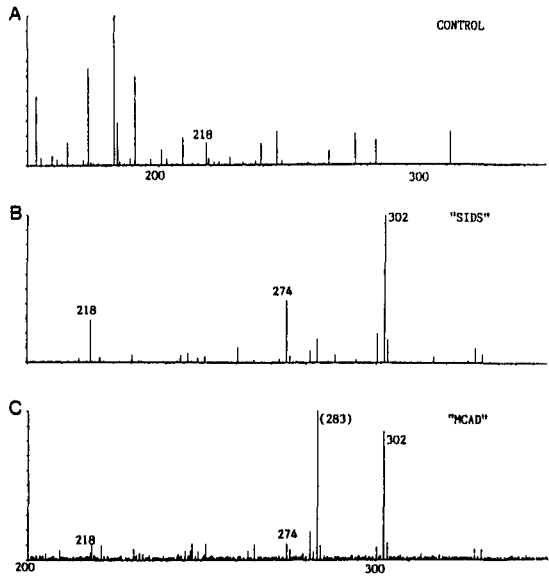


Figure 1: Whole Blood Acylcarnitine Profiles From PKU Cards

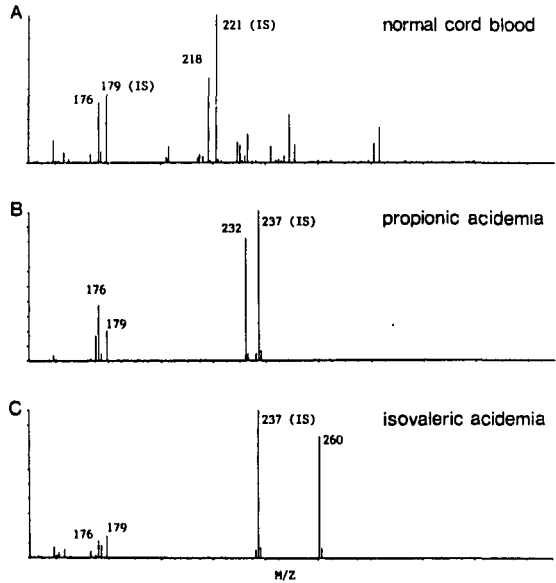


Figure 2: Plasma Acylcarnitine Profiles

URINARY EXCRETION PATTERN OF DICARBOXYLIC ACID AND ACYLCARNITINES IN THE PATIENTS TREATED WITH CALCIUM HOPANTENATE.

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Urine extracts from two patients who were suspected to exhibit acute toxicity of calcium hopantenate [calcium D-(+)-4-(2,4-dihydroxy-3,3-dimethyl-butyramido) butyrate hemihydrate] were analyzed by means of gas chromatography-mass spectrometry (GC/MS) and fast atom bombardment mass spectrometry (FAB/MS). The patients excreted a large amount of medium chain dicarboxylic acids and its carnitine esters, and they were presenting with an episode of Reye like syndrome.

Patient

Case 1. The patient was a 3-year-old boy . On the 2nd year of life, he was admitted to hospital because of his speech delay and hyperactivity, and diagnosed as Autism. He had been treated with 1.5g/day of calcium hopantenate. 3 Months later, he developed a fever and vomiting and further developed to coma in the next morning. He recovered with glucose and sodium bicarbonate treatment.

Case 2. The patient was a 10-year-old girl. At 9-year-old, she was admitted to hospital with abnormal emotion, and had been treated with 1.5g/day of calcium hopantenate. After 3 months, she had developed a fever, vomiting, convulsion and coma, and admitted to hospital. On the next morning, she had developed polypnea and hyperanmonemia (> 400µg/dl). She was doubted Reye's syndrome. After 10 days, her clinical abnormalities were recovered completely.

Methods

Analysis of urinary organic acids.

Urinary organic acids were extracted with diethylether at acidic condition. The organic layer was trimethylsilylated and analyzed using a JEOL DX-300 gas chromatograph-mass spectrometer assisted with a JMA 3500 data acquisition system. The chromatographic separation was carried out on a fused silica capillary gas chromatograph column (25 m x 0.32 mm i.d., 1.0µm film thickness), which was coated with methyl silicone (Quadrex Corporation, Conneticut, USA). The oven temperature was started from 100°C and raised at a rate of 8°C/min to 290°C. Electron impact mass spectra were recorded at an ionizing voltage of 70eV.

Analysis of acylcarnitines

To 1 ml urine was added 10µg D₉-Isovalerylcarnitine as an internal standard, which was mixed and applied to a 0.9 x 4 cm glass column of Bio Rad AG-1X8 (200-400 mesh, Cl⁻ form) anion exchange resin. Acylcarnitine fraction was eluted from the column with 6 ml deionized water. The effluent was collected in a glass test tube and the content was lyophilized. To the dry residue was added 100µl of the methanol and acetylchloride (7:3) mixture for methylation, which was then allowed to stand for 2 hours at room temperature. Sample aliquots of 2µl were analyzed by FAB mass spectrometry at low resolution.

FAB/MS was performed using a JEOL JMS-DX303 mass spectrometer and JMA-DA5000 data acquisition system. Argon was used for the reagent gas. The electron accelerating voltage was 3 KV, GUN voltage 6KV and ionization current was 20mA. Glycerol was used for matrix. Spectra were recorded between m/z80 and 800 at a scan speed of 10s decade⁻¹ and a resolution of 1500. Each ion intensity was calculated from an average of 10 scans.

Result

The excretion of adipic acid, suberic acid, sebacic acid, dodecanedioic acid and 3-hydroxydodecanedioic acid were substantial at clinical conditions, with a concomitant increase of 3-hydroxyhexanoic acid, 5-hydroxyhexanoic acid and dehydrosebacic acid. Lactic acid, 3-hydroxybutyric acid and acetoacetic acid were also excreted in large excess (Fig. 1), and huge amount of acetyl, dehydrosebacyl and sebacylcarnitine were excreted. Suberyl, octanoyl, butyrylcarnitine were also excreted in excessive amounts (Table 1). All the above unusual metabolites disappeared in urine extracts from the patients after recovery.

Discussion

Oxidation of fatty acids is an essential energy supply for cell, when glucose availability is decreased or an impairment of gluconeogenesis. In those hypoglycemic conditions, the massive concomitant excretion of dicarboxylic acids (dicarboxylic aciduria) are observed.

Seven inborn errors of fatty acid catabolism have been described to date:

- (1) systematic carnitine deficiency (SCD);
- (2) hepatic carnitine palmitoyl transferase deficiency (hepatic CPT deficiency);
- (3) long chain acyl-CoA dehydrogenase deficiency (LCAD deficiency);
- (4) medium chain acyl-CoA dehydrogenase deficiency (MCAD deficiency);
- (5) short chain acyl-CoA dehydrogenase deficiency (SCAD deficiency);
- (6) riboflavin responsive defects of β -oxidation;
- (7) multiple acyl-CoA dehydrogenase deficiency (MAD deficiency, GA II)

In our patients, large amount of acylcarnitines were excluded, so the possibility of (1) and (2) are eliminated. The possibility of (3)-(7) are also diminished, because large amount of medium chain monocarboxylic acid carnitine esters are excluded in those patients but not in our patients.

Possible explanation for the dicarboxylic aciduria in the patients treated with calcium hopantenate is the inhibition of coenzyme A synthesis, since calcium hopantenate is known to cause the pantothenic acid (a precursor of coenzyme A) deficiency in animals. The other explanation is the inhibition of mitochondrial acylcarnitine translocation system by hopantenate. In the block of translocation system, fatty acid is oxidized by microsomal α -oxidation system followed by peroxisomal β -oxidation to produce medium chain dicarboxylic acids. Since adipic and suberic acid could not be transport into mitochondria from cytosol. These dicarboxylic acid are accumulated in the cell in the patients. On the otherhand, sebacic acid (C_{10} dicarboxylic acid) can be transported from cytosol to mitochondria as carnitine ester and oxidized by mitochondrial β -oxidation. The disturbance of acylcarnitine translocation causes the accumulation of sebacylcarnitine.

Fig. 1. RIC chromatogram of urinary organic acids (Case 1)

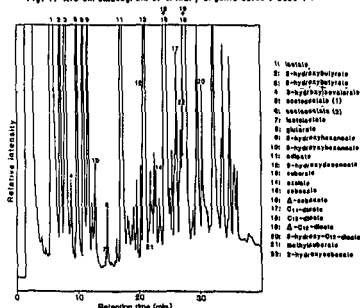


Table 1. Urinary Acylcarnitines (Ion intensity ratio / mg creatinine)

Acylcarnitine	Ion	3 T (male) onset	3 T (male) recovery	10 T (female) onset	Control (S-Ty)	S.D.	
from carnitine	m/z 176	0.323	0.270	2.890	0.273	0.087	
Acetyl	m/z 216	1.351	0.080	7.005	0.076	0.025	
Propionyl	m/z 232	0.514	0.076	2.145	0.054	0.013	
Butyryl	m/z 246	0.757	0.143	1.245	0.322	0.116	
C5	Isovaleryl	m/z 260	0.729	0.120	1.274	0.177	0.052
	Isobutyryl	m/z 304	0.083	0.061	0.151	0.046	0.011
C6	Hexanoyl	m/z 274	0.089	0.058	0.167	0.042	0.035
	Adipyl	m/z 310	0.514	0.114	0.925	0.072	0.011
Dehydrosebacyl	m/z 316	0.168	0.197	0.405	0.233	0.031	
C8	Octanoyl	m/z 302	0.166	0.071	0.384	0.056	0.006
	Octanoyl	m/z 302	1.197	0.252	1.846	0.335	0.101
Suberyl	m/z 346	0.487	0.120	1.992	0.035	0.015	
Dehydrosebacyl	m/z 344	0.200	0.201	0.566	0.070	0.014	
C10	Decanoyl	m/z 330	0.059	0.069	0.167	0.044	0.011
	Decanoyl	m/z 326	0.051	0.102	0.227	0.040	0.017
Suberyl	m/z 374	0.500	0.041	3.594	id		
Dehydrosebacyl	m/z 372	0.351	0.099	4.229	0.052	0.012	
C12	Dodecanoyl	m/z 356	0.030	0.072	0.354	0.034	0.015
	Dodecanoyl	m/z 356	id	0.055	0.159	0.017	0.016

(see supplemental table 1)

(id: undetectable)

CHARACTERIZATION OF ORGANIC NITROXIDE RADICALS BY MASS SPECTROMETRY

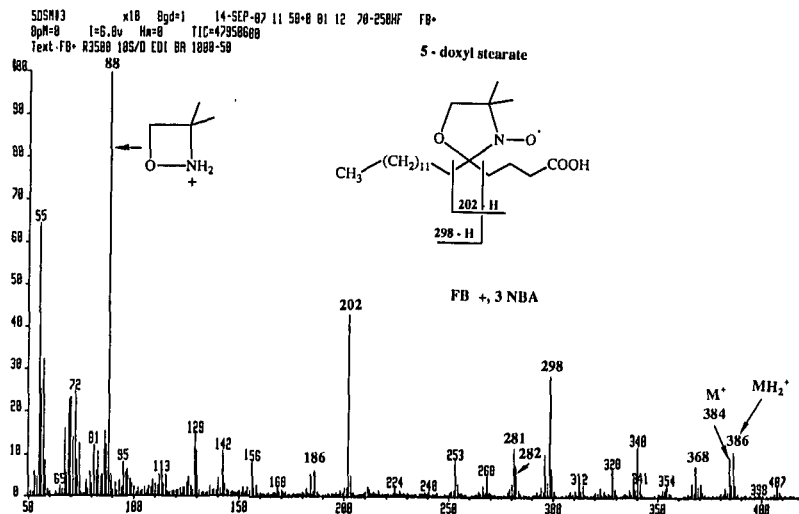
Brian J. Sweetman, P.S.R. Anjaneyulu, Albert H. Beth and James V. Staros
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There are a plethora of stable organic free radical nitroxides available for studies of macromolecular systems of biological importance, such as membranes, for studying drug-receptor interactions in pharmacology and as contrast enhancers in medical NMR imaging applications. Structurally, these stable nitroxides are heterocyclic, secondary amine N-oxides which have no hydrogen atoms attached to the carbon atom α to the nitrogen. Because of this structural feature, disproportionation of the nitroxide to nitron and hydroxylamine is minimized and stability of the nitroxide is enhanced. Many of the stable nitroxides available commercially fall into a small number of structural classes, such as 2,2,6,6-tetramethylpiperidinoxyl nitroxides (TEMPO spin labels), the 4,4-dimethyl-2,2-disubstituted-oxazolidinoxyl nitroxides (DOXYL spin labels) and the 5,5-dimethyl-2,2-disubstituted-pyrrolidinoxyl nitroxides (PROXYL spin labels). There is a need to characterize adequately new spin labels which have been synthesized for specific applications in biochemistry, pharmacology and medicine. Little has been described concerning the mass spectrometry of such nitroxides.

Nitroxide spin labels are stable organic radicals which are detectable by EPR spectroscopy and have seen wide use in biochemistry as probes of the dynamic state of macromolecules or of molecular aggregates, e.g., membranes. Many substituted nitroxides are available to address a diversity of biological questions. We have been interested specifically in characterizing by mass spectrometry compounds containing the DOXYL moiety. Simple 1,1 dialkyl DOXYLS, under electron impact ionization, give spectra showing abundant structural information. Their FAB spectra also yield much structural information and show a unique molecular ion cluster pattern containing M^+ and MH_2^+ as the abundant species, which are attributed to the nitroso oxonium radical cation and the protonated hydroxylamine, respectively. We have also characterized using FAB-MS, several labile, very polar bifunctional bis (sulfosuccinimidyl) DOXYL derivatives as their di-sodium salts, and their stable isotope-labelled analogs. These polar, membrane-impermeant bifunctional spin-labelled DOXYLS, bis(sulfo-N-succinimidyl) doxyl-2-spiro-4'-pimelate (BSSDP), bis(sulfo-N-succinimidyl) DOXYL-2-spiro-5'-azelaate (BSSDA) and [^{15}N , $^2H_{16}$] BSSDP have been synthesized as probes to study the rotational dynamics of the anion-exchange channel in intact human erythrocytes using EPR spectroscopy. These polar di-sodium sulfonates have been characterized by FAB-MS using glycerol as matrix and yield meaningful molecular ion data in both the positive and negative ion modes. The isotopic distribution in the dicarboxylic acid precursor to BSSDP, [^{15}N , $^2H_{16}$]-2-spiro-4'-pimelic acid was determined using high resolution electron impact mass spectrometry. This was synthesized from 4-keto pimelic acid.

The positive ion FAB spectrum of BSSDP shows low abundance ions at m/z 660 (MH_2^+) (2 sodiums) and m/z 682 ($MHNa^+$) (3 sodiums) which apparently arise by conversion of the nitroxide to the hydroxylamine in the FAB process. The spectrum of the homologous azelaate BSSDA shows corresponding peaks. The corresponding negative ion FAB spectrum show peaks having m/z values at $[M-Na+H]^-$ (one sodium) and $[M-Na]^-$ (one sodium), attributed to hydroxylamine sulfonate and sulfonate anions, respectively. [^{15}N , $^2H_{16}$] BSSDP gave a negative ion FAB spectrum (not shown), showing the appropriate mass increment of 17 daltons, compared to BSSDP, with a major cluster of peaks centered at m/z 653.

We have acquired and studied the mass spectra of 3 isomeric DOXYL stearic acids substituted at the 5, 12, and 16 positions on the stearic acid chain. Spectra obtained using high resolution electron impact ionization or fast atom bombardment with positive or negative ion extraction were compared. The EI spectra are characterized by very low abundance molecular ions, ions arising by the loss of the elements of the DOXYL ring, and in each case, ions formed by cleavage of the carbon-carbon bond alpha to the ring junction with the loss of either sidechain and with substantial concomitant loss of the nitroxide oxygen. The corresponding FAB+ spectra obtained using 3-nitrobenzyl alcohol as matrix also show the same cleavages alpha to the ring junction as do the EI spectra, but loss of the nitroxide oxygen is not a major process. The base peak in each spectrum at m/z 88 was attributed to the C_4H_9NO cation. Each FAB spectrum showed a relatively abundant peak at MH_2^+ , attributed to the protonated hydroxylamine, together with a peak at M^+ , attributed to the nitroso oxonium radical cation. MH_2^+ increases substantially during the bombardment suggesting that the reduction of nitroxide radical occurs as a consequence of the FAB process. The negative ion FAB spectra show M^- and $[M-H]^-$ as the most abundant peaks with little or no structural information, except for ions at m/z 312 and 297 arising by the loss of C_4H_8O and C_4H_8O-O from M . Despite the formation of MH_2^+ , the FAB spectra are preferable to the EI spectra, since they do give clear molecular ion information and fragment ions are more easily related to the parent structure than in the EI spectra. These spectral features are illustrated below in the FAB+ spectrum of 5-DOXYL stearic acid using 3-NBA as matrix.



FAB-MS is the preferable ionization mode both for the characterization of more polar or polycyclic nitroxides, which are generally not amenable to EI-MS and for the characterization of small nitroxides which are amenable to EI-MS, because the peaks observed in FAB-MS relate more closely to the parent structure.

Supported by Grants DK31880, HL34737, CA43720, and RR01688 from the National Institutes of Health.

DETERMINATION OF INTACT STYRENE-7,8-OXIDE IN BLOOD USING AUTOMATED GAS CHROMATOGRAPHY-MASS SPECTROMETRY. PATRICK W. LANGVARDT and RICHARD J. NOLAN, The DOW CHEMICAL COMPANY, MIDLAND, MI., 48674.

Styrene-7,8-oxide (SO) is a putative metabolite of styrene, an important monomer used in the production of plastics and resins. Styrene is thought to undergo enzymatic oxidation to SO via microsomal cytochrome P-450. SO then reportedly undergoes spontaneous and enzymatic hydrolysis to phenylethylene glycol (PG) or is conjugated with glutathione. PG is then further oxidized, or conjugated with glucuronic acid. SO is not an excreted metabolite of styrene, but has been reported to have been measured in blood after exposure of individuals to styrene vapors.¹

A rapid, specific method for the determination of SO in rat blood was needed to support pharmacokinetic studies. These studies required sequential, small (~0.1 ml) blood samples from individual animals. This sampling regimen would allow the kinetics to be defined in individual animals and minimize the number of animals needed. In addition, the analytical method needed selectivity, particularly in regard to possible or known metabolites of styrene such as phenylacetaldehyde or PG. Previously reported methods either did not have the desired 10 ng/ml detection limit, required larger volumes of blood, or required time-consuming derivatization procedures, possibly compromising the specificity of the measurement (reported derivatization procedures call for liquid-liquid extraction of the minor metabolite, SO, from samples containing the major metabolite, PG, followed by conversion of SO to PG prior to derivatization).

The method described here involves a single extraction of SO from whole blood samples as small as 100 µg and subsequent analysis by gas chromatography/mass spectrometry. Whole blood is obtained from the animal through an indwelling silastic cannula and placed directly into a tared vial containing ~100 mg anhydrous sodium sulfate. Benzene containing 0.3 µg/ml 1-phenylpropylene oxide (PPO) as an internal standard is added immediately. Samples are mixed vigorously and centrifuged. The liquid is then transferred to autosampler vials for GC/MS analysis.

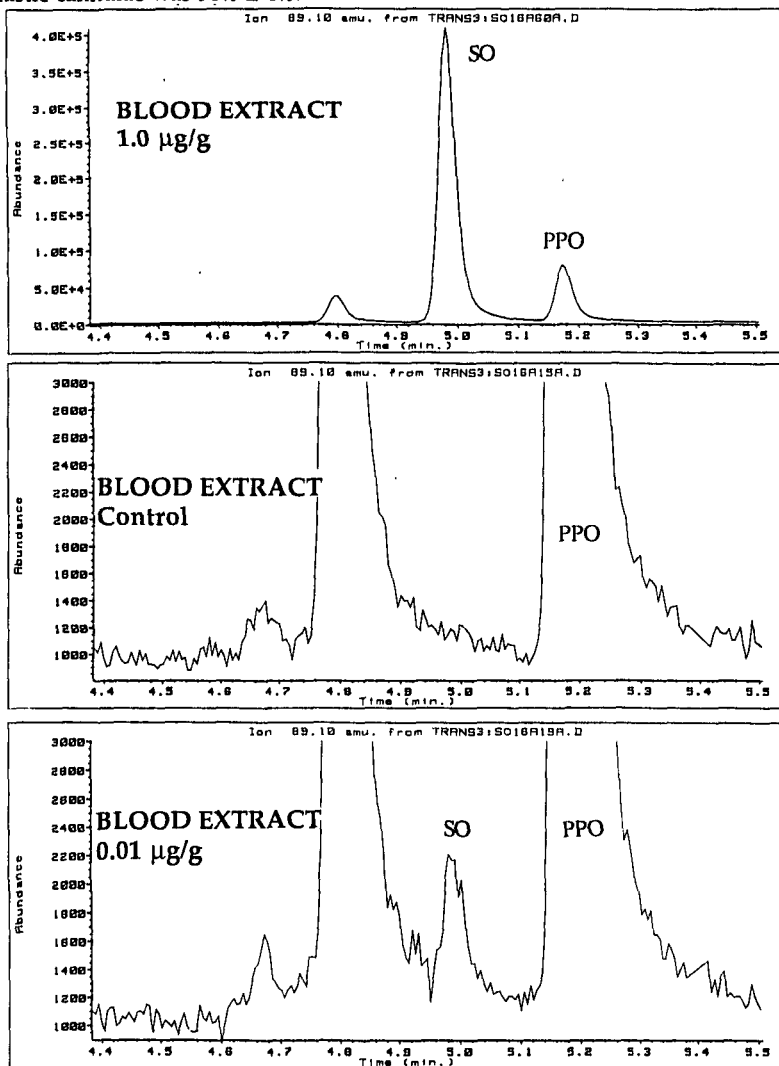
During method development studies SO was found to undergo some thermal rearrangement to phenylacetaldehyde when using conventional heated split or splitless injection techniques. The use of cold on-column injections (1 µl) eliminated that problem. A 4 m x 0.53 mm retention gap was used ahead of a 10 m x 0.25 mm DB-5 (1 µ film) analytical column. The column temperature was held at 35°C for 0.25 min, then programmed at 40°C/min to 220°C. SO eluted at 4.98 min and PPO at 5.17 min (see figure).

The 70 eV electron impact mass spectra of aromatic epoxides contain characteristic fragment ions at m/z 89 and 90 which have been attributed to the formation of ring expansion products.² The relatively unique and abundant fragment ion at m/z 89 was chosen for quantitation to improve the selectivity of the method. A Hewlett-Packard 5970B GC/MS system equipped with a 7673 autosampler and a reduced-pressure interface device was used.

¹ E. Wigaeus et. al., *Scand. J. Work. Environ. Health*, 9, 479-488, (1983).

² H. E. Audier et. al., *Tetrahedron Letters*, 2077-2082, (1966)

The internal standard response factors at m/z 89 were equivalent from 0.01 to 1.0 $\mu\text{g/ml}$. If possible, higher samples were diluted into that range, otherwise bracketing calculations were necessary. Recovery of SO spiked into whole blood was $91\% \pm 6\%$ (SD, $n=21$). The half-life of SO in whole rat blood at 22.5 and 37°C was 84 and 26 min, respectively., but SO concentrations in benzene extracts were unchanged after six days. SO was also found to be stable ($94\% \pm 2\%$) in corn oil dosing solutions under ambient conditions for 39 days. Recovery of SO in blood (1.0 $\mu\text{g/g}$) passed through silastic cannulae was $91\% \pm 4\%$.



CHARACTERIZATION OF AN INTERMEDIATE IN THE BIOSYNTHESIS OF CHOLESTEROL

B.S. Larsen, R.G. McKay, R. T. Fischer, S.S. Ko, C. Brosz, R. L. Magolda, and J. M. Trzaskos

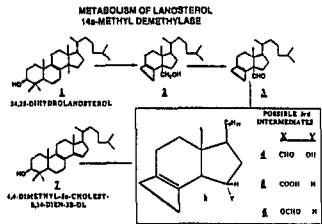
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Experimental Station
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Wilmington, Delaware 19880-0228.

Cholesterol is a precursor to important endocrine hormones as well as bile acid surfactants, and a component of cellular membranes. All the enzymes required for the synthesis of cholesterol from lanosterol, the first steroid intermediate, are bound to the endoplasmic reticulum. The net change from lanosterol to cholesterol encompasses the loss of three methyl groups at the 4 α , 14 α , and 14 β positions, the reduction of the side chain double bond and the isomerization of the nuclear double bond from carbon 8 to carbon 5. These five modifications to lanosterol are accomplished with molecular oxygen, NADPH, NADH and NAD⁺ as the required substrates and cofactors. A plausible 19-step sequence involving nine distinct enzymes can now be written as a result of organic synthesis of presumed intermediates, generation of intermediates in microsomes, further enzymatic conversion of these intermediates to cholesterol, solubilization and purification of membrane bound enzymes and reconstitution of groups of enzymes into artificial membranes.

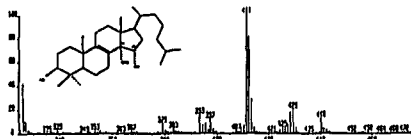
The initial attack upon the lanosterol precursor is by a cytochrome P-450 mono-oxygenase enzyme complex that demethylates the 14 α -methyl group from lanosterol. The 14 α -methyl demethylation occurs in three consecutive oxidative steps first to a C-32 alcohol, then to a C-32 aldehyde and then to a final undefined intermediate which undergoes decarbonylation (see Figure 1). To study this mechanism of oxidation and elimination of the 14 α -methyl substituent of lanosterol a labeled substrate, 3 β -hydroxy-[3 α -(³H)]-5 α -lanost-8-en-32-al (3), was metabolized with a rat liver microsomal preparation of the lanosterol demethylase P-450 enzyme. The metabolism was stopped with methanol and the metabolite was extracted with petroleum ether and purified by a normal phase HPLC method. The chromatogram contained 4,4-dimethyl-5 α -cholest-8,14-dien-3 β -ol(Z) and an unknown polar peak.

Initial attempts to analyze the unknown intermediate by GC/MS was inconclusive because the material decomposed on the column. Direct probe EI analysis resulted in the spectrum shown in Figure 2C which is dramatically different from the spectrum for 4,4-dimethyl-5 α -cholest-8,14-dien-3 β -ol(Z) and the two synthetic standards (4) and (5), the postulated structures for the intermediate. The molecular ion was observed at m/z 458 for both synthetic standards (4) and (5) and at m/z 412 for (Z), but no molecular ion was apparent for the unknown. In addition, the fragmentation patterns are sufficiently different to indicate that the isolated intermediate has a different structure than those proposed.

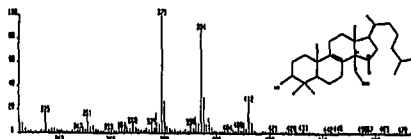
For further verification of the structure, the polar intermediate was analyzed by an LC/MS interface, continuous flow fast atom bombardment (FAB) mass spectrometry (MS) interfaced to an LC. Continuous flow FAB was required because of its increased sensitivity (a factor of 10 over direct probe FAB). To further improve the sensitivity selected ion monitoring (SIM) was used to analyze significant ions [e.g. (M+H)⁺, (M+H-CH₄)⁺ and (M+H-H₂O-CH₄)⁺] (see Figure 3). The SIM experiment provided molecular weight information for the isolated intermediate.



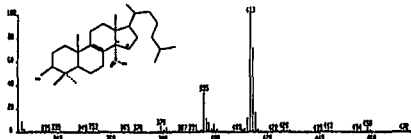
A 3 β , 15 α -Dihydroxy-5 α -lanost-8-en-14 α -aldehyde



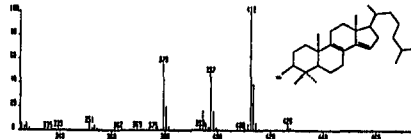
C 3 β -Hydroxy-5 α -lanost-8-en-14 α -formate ester



B 3 β -Hydroxy-5 α -lanost-8-en-14 α -carboxylic acid



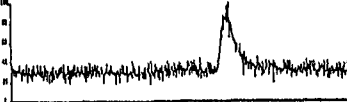
D 4,4-Dimethyl-5 α -Cholest-8, 14-dien-3 β -ol



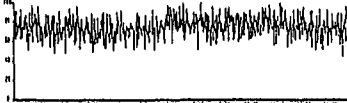
3 β -Hydroxy-[3 α -(3H)]-5 α -lanost-8-en-32-al
Starting Material 100 nanogram injection
Mass 425 (M+H $_2$ O) $^+$ AREA 2638



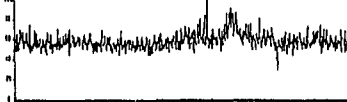
Mass 443 (M+H) $^+$ AREA 5881



Mass 459



Mass 420 Background Ion



3 β -Hydroxy-5 α -lanost-8-en-14 α -formate ester
Isolated intermediate 8 nanogram injection
Mass 425 (M+H-CH $_4$ -H $_2$ O) $^+$ AREA 310



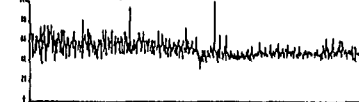
Mass 443 (M+H-CH $_4$) $^+$ AREA 409



Mass 459 (M+H) $^+$ AREA 785



Mass 420 Background



Determination of Radiation Induced Cholesterol Oxides by On-Column²
Capillary GC-MS.² Thomas G. Hartman¹, Robert T. Rosen¹, Gerhard Maerker²
and Leon Lakritz²; Center for Advanced Food Technology¹, Cook College,
Rutgers University, New Brunswick, NJ 08903 and USDA Eastern Regional
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The safety evaluation of food irradiation as an alternate means of sterilization or preservation has initiated a search for unique radiolytic products to act as indicator or tracer compounds. The search for such unknown compounds in an authentic food is extremely difficult due to its complex matrix. Therefore, model systems are being used to identify such compounds which can then be targeted in real foods.

¹³⁷Cs gamma source at a rate of .125 kGy/min. for 6-7 hours at 0-2°C. The cholesterol radiodecomposition products produced were then fractionated by TLC and analyzed using on-column injection capillary GC-MS with EI detection. This technique is the preferred method for the determination of underivitized sterol oxidation products since it is highly inert and results in library searchable EI spectra.

Compounds identified include various combinations of diol, triol, keto, epoxy and unsaturated analogues of cholesterol. The analytical data is summarized in table # 1. At least one of the compounds identified, cholesta-3,5-diene-7-one is produced by both radiolysis and as an artifact by dehydration of 7-ketocholesterol in the gas chromatograph. The use of cold on-column injection technique was shown to minimize the production of this artifact.

None of the compounds detected in this investigation were found to be useful as radiation induced "indicators" since they are also produced in foods through enzymatic or nonenzymatic lipid peroxidation pathways and in normal cooking. Apparently, the free radical reactions induced by irradiation fail to produce unique radiolytic products but rather are very similar to hydroxy radical propagation reactions which occur during lipid oxidation in foods.

NJAES Publication No. J10535-2-89, CAFT is an initiative of the New Jersey Commission on Science and Technology.

Table # 1 Radiodecomposition Products of Cholesterol

<u>Compound</u>	<u>M.W.</u>
cholestatriene	366
cholesta-3,5-diene-7-one	382
7-ketocholesterol	400
7-ketocholestanol	402
6-ketocholestanol	402
cholesta-5-ene-3,7-diol	402
cholestanol-5 α ,6 α -epoxide	402
cholestanol-5 β ,6 β -epoxide	402

CONTINUOUS FLOW L-SIMS, GC/MS AND BIOASSAY IN THE ISOLATION AND IDENTIFICATION OF PLANT TOXINS

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INTRODUCTION

The high incidence of a variant of amyotrophic lateral sclerosis - Parkinsonism complex (ALS-PD) in three foci in the Pacific region (the Mariana island of Guam, the Kil peninsula of Japan and Irian Jaya) may be associated with consumption and/or medicinal use of cycads. β -methylamino-L-alanine (BMAA), a minor constituent of cycads, has recently been shown to cause a behavioral dysfunction, clinical signs of motor-neuron deficiency and, pathological changes consistent with those seen in the human disease (1). To examine the link between cycads and ALS-PD we have developed assays for the quantification of BMAA in a variety of samples including plant material, foods prepared from cycads, brain tissue, CSF and plasma. We have employed 1) GC/MS of the di-N-pentafluoroacyl derivative of BMAA *iso*-butyl ester and 2) continuous flow L-SIMS MS/MS of BMAA as the *iso*-butyl ester

MATERIALS AND METHODS

GC/MS ASSAY Formation of the *iso*-butyl ester of BMAA (*via* the methyl ester) before analysis permitted acid-base extraction as well as concentration of the sample prior to acylation. An Hewlett-Packard 5890 gas chromatograph and 5970 mass selective detector were used. Chromatography was performed with a split/splitless capillary inlet on a fused-silica capillary column (12 m X 0.22 mm i.d.) with a cross-linked methyl silicone stationary phase (HP1, 0.33 μ m film thickness). Samples were run in the selected-ion monitoring mode (SIM) with the base peak (m/z 190) and a second ion (m/z 365) used for quantification.. A representative SIM chromatogram for the analysis of a cycad flour extract is shown in figure 1.

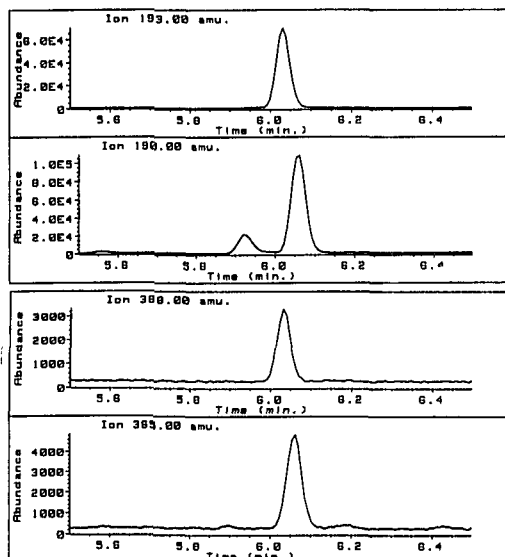


Figure 1
Representative SIM chromatograms for the quantification of the di-N-pentafluoroacyl derivative of BMAA *iso*-butyl ester in a plant sample. Two ions (i.e. m/z 190 & 365) are monitored for BMAA while the others (i.e. m/z 193 & 368) correspond to the same fragments for the stable isotopomer added as standard.

L-SIMS ASSAY The *iso*-butyl ester of BMAA was formed and analyzed directly. Data were acquired on a Finnigan Mat TSQ-70 triple stage quadrupole mass spectrometer equipped with a Finnigan Mat prototype Bioprobe ion source and a Phrasor cesium ion gun. An HPLC pump with a microflow adaptor and pulse dampener was used to deliver mobile phase through a 0.5 μ L loop injector, through the vacuum manifold flange and into the ion source. Cesium ions (5-6 keV) were used to bombard the surface of the probe tip (where capillary tubing was continuously delivering mobile phase). A water/glycerol mobile phase (80/20) was used and delivered at a rate of $\approx$ 8 μ L/min. Argon was used as the collision gas with a pressure of 0.5 millitorr in the collision chamber. For quantification, the BMAA *iso*-butyl ester parent ion $[M+H]^+$ (i.e. m/z 175 for BMAA and m/z 178 for the deuteriated isotopomer) were selectively transmitted through the first quadrupole every 0.1 secs and the corresponding daughter fragments (m/z 44 & 47) were then alternatively monitored. Centroid data were collected and stored for analysis. Representative integrated ion currents (figure 2) were obtained by using routine chromatographic data processing techniques.

RESULTS AND CONCLUSIONS

Comparison of Assay Methods Comparison of the results show an excellent quantitative correlation ($r^2 > 0.98$). However, at this stage, GC/MS offers a significantly lower limit of detection (i.e. $\approx$ 10 pg injected on column) when compared with continuous flow L-SIMS MS/MS (maximum sensitivity for the detection of BMAA *iso*-butyl ester was approximately 1 ng injected). Recent findings from our laboratory suggest that the application of a focused cesium ion beam will result in a significant improvement in the detection limit of the L-SIMS approach. (See abstract by Wang T-C *et al.* - this meeting.)

BMAA in Cycads and ALS Although we have demonstrated the presence of BMAA in the seeds of cycads levels are relatively low. Processing of the seeds results in a fall in BMAA content to levels of 1-100 μ g/g of flour. Based on these data, consumption of 1 kg of processed flour would only deliver a maximum dose of about 1 mg/Kg. However, when demonstrating BMAA neurotoxicity in primates, oral administration of doses of 100-350 mg/kg were employed. We conclude that diet is unlikely to deliver neurotoxic quantities of this compound to the central nervous system. Further, studies with neurons in culture suggest that BMAA levels in toxic cycad extracts are orders of magnitude lower than those required to demonstrate toxicity when the pure compound is employed. If a causal link does exist between cycads and ALS-PD on Guam, other constituents are likely the key neurotoxic agent(s).

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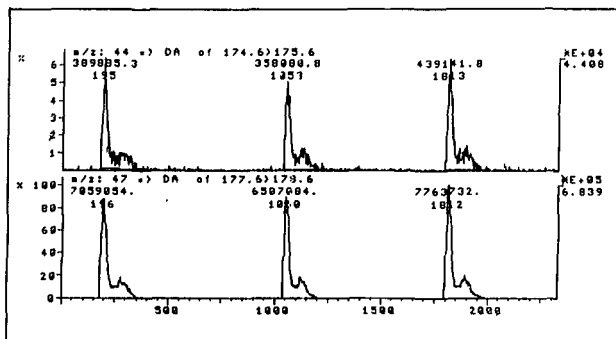


Figure 2: Quantification of BMAA as the *iso*-butyl ester in a plant sample by continuous flow L-SIMS selected reaction monitoring. Three repeat injections are shown. The parent ion $[M+H]^+$ (i.e. m/z 175 for BMAA and m/z 178 for the deuteriated isotopomer) were selectively transmitted through the first quadrupole every 0.1 secs and the corresponding daughter fragments (m/z 44 & 47) were then alternatively monitored.

Determination of Ethyl Carbamate (Urethane) in Commercial Protein Based Condiment Sauces (Soy Sauces) by Gas Chromatography-Mass Spectrometry.
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Center for Advanced Food Technology, Cook College, Rutgers University, New Brunswick, N.J. 08903

Commercial protein based condiments such as soy and teriyaki sauces are available as traditional fermented products or as synthetic nonfermented substitutes. Both types of products were analyzed for the presence of ethyl carbamate, a well known carcinogen, since the potential exists for its formation in the fermentation process. Initial attempts to analyze these products using GC with FID or nitrogen selective detection were unsuccessful due to the presence of interfering matrix derived compounds. Therefore, we have developed a mass spectrometric method for the determination of this compound in these matrices.

Samples were spiked with *t*-butyl carbamate internal standard, extracted with methylene chloride and concentrated prior to GC-MS analysis. Experiments were conducted using electron ionization and chemical ionization (methane and isobutane) modes. The characteristic ion masses from ethyl carbamate produced by electron ionization could not be reliably used for detection or quantitation due to the presence of matrix derived interferences. The methane CI spectrum of ethyl carbamate contained a protonated molecular ion at m/z 90 and a prominent fragment ion at mass 62. However, the mass chromatogram for m/z 62 contained numerous interferences and hence there was no advantage in monitoring this ion. Isobutane CI produced a strong protonated molecular ion exclusively and was interference free. Therefore, for quantitation, the ratio of the protonated molecular ions produced during isobutane chemical ionization for ethyl carbamate and *t*-butyl carbamate (m/z 118) were monitored. Samples testing positive were confirmed by EI. Table # 1 summarizes the analytical data obtained from several commercially obtained fermented and nonfermented products.

The analytical GC-MS methodology developed provides for rapid and reliable determination of ethyl carbamate in protein based condiment sauces. The method proved interference free in spite of the simple extraction procedure employed. A detection of ethyl carbamate at twice the signal to noise ratio in the m/z 90 mass chromatogram provides sensitivity down to at least 1 ppb. Confirmation limits in the EI mode are 10 ppb. Selected ion monitoring provides increased sensitivity over mass chromatography, but a limit of detection of 1 ppb was deemed adequate. The extraction efficiency, recovery of internal standard and response factors were determined. The extraction efficiencies for the *t*-butyl carbamate internal standard and ethyl carbamate averaged 87.6 % and 88.7 % respectively ($n=12$). Fermented products were found to contain ethyl carbamate at levels ranging from 36 ppb for a "lite" soy sauce to 102 ppb for a traditional soy sauce. No trace of ethyl carbamate was found in the nonfermented substitute condiments.

NJAES Publication No. J10535-1-89, Caft is an initiative of the New Jersey Commission on Science and Technology.

Table # 1 Analysis Results on Condiment Samples

<u>Sample</u>		<u>Conc. Ethyl Carbamate ppb</u>
Traditional Soy Sauce (fermented)	---	86 - 102
"Lite" Soy Sauce (fermented)	---	36 - 42
Teriyaki Sauce (fermented)	---	86 - 89
Nonfermented Soy Sauce	---	ND

AMMONIA CHEMICAL IONISATION GCMS ANALYSIS OF
POLYHYDROXY AMINES FROM PLANT SOURCES

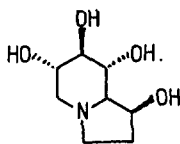
Robin T. Aplin, Daniel P.M. Coffey, George W.J. Fleet,
The Dyson Perrins Laboratory, Oxford University, Oxford, U.K., and
Linda E. Fellows, The Royal Botanic Gardens, Kew, London, U.K.

A number of polyhydroxy cyclic amines e.g. castanospermine (I) have shown potential for the control of the AIDS virus.^{1,2} As part of an ongoing plant screening program based on both taxonomic considerations and enzyme inhibition studies a number of potential sources of natural polyhydroxy cyclic amines have been identified.

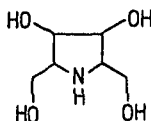
We have developed a routine procedure for the analysis of crude plant extracts for these compounds. Following ion-exchange chromatography the crude extract is silylated using Sigma Silyl A and the resulting O-trimethyl silyl ethers are analysed by ammonia chemical ionisation capillary GCMS on a VG Masslab TRIO 1 "Bench Top" mass spectrometer. In contrast to their EI mass spectra which fail to show the molecular ion the ammonia CI mass spectra show abundant MH⁺ ions together with three characteristic fragmentations corresponding to the type of substituent present.

- (i) MH⁺ - 90 Secondary hydroxyl group.
- (ii) MH⁺ - 104 CH₂OH alpha to nitrogen
- (iii) MH⁺ - 206 CHOCH₂OH alpha to nitrogen

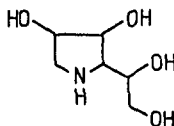
Such fragmentations allow the ready identification of the common structural isomers: the pyrrolidines II and III; the hydroxy piperidines IV and V and the isomeric castanospermines VI and alexins VII as well as providing molecular weight and structural data on new compounds (e.g. VIII) obtained during the crystallisation of an alexin from Castanospermum australe.



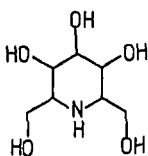
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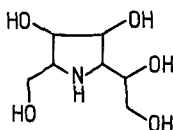
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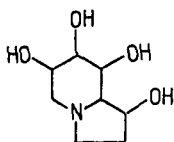
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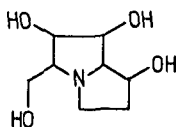
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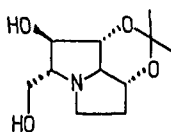
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VI



VII



VIII

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DETERMINATION OF MONO (2-ETHYLHEXYL) PHTHALATE IN HUMAN SERUM ALBUMIN.
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Canada, Bureau of Drug Research, Ottawa, Ontario, K1A 0L2.

The Canadian Red Cross Society requested the Health and Welfare Canada to determine levels of mono (2-ethylhexyl) phthalate (MEHP) and bis (2-ethylhexyl) phthalate (DEHP) in commercial preparations of Human Serum Albumin (HSA) [1-8]. HSA is prepared from blood plasma by either older precipitation fractionation or newer gel permeation chromatography. The latter products were suspected of having high concentrations of MEHP. Whole blood is usually collected into polyvinylchloride bags, which contain 30-40% by weight of DEHP (not very toxic) [5]. DEHP leaches into the collected blood and is enzymatically converted to more toxic MEHP [5]. A GC-MS assay was developed to confirm MEHP values, obtained through a published HPLC method.

GC-MS assay: Standards were prepared by spiking the phthalate free HSA solutions (1 mL) with MEHP at concentrations 0.2, 2, and 20 µg/mL. Phenobarbital (0.5 µg/mL) was used as an internal standard. The mixture was acidified with 0.1M citric acid buffer (pH 2) and extracted with 3 mL of toluene, spun at 2500 rpm to break an emulsion, dried under stream of nitrogen at 40°C. An excess amount of ethereal diazomethane was added and the mixture evaporated and reconstituted with methanol for injection in GC-MS. Samples were treated in a similar fashion, 1 mL of HSA was spiked with aqueous internal standard (0.5 µg of phenobarbital). The extraction and derivatization were done as described above. Finnigan 4610 B GC-MS was used. GLC: 15 m fused silica capillary column from J&W Scientific, 0.25 mm I.D., DB-5 0.25 µm thick stationary phase; helium as a carrier gas. The injector (split 1:30) was kept at 250°C, the interface oven was at 260°C. The GLC oven was programmed from 150°C at 10°C/min. to 260°C. MS: electron impact (EI) and multiple ion detection (MID) mode. Fragments 149, 163 and 181 m/z were monitored for MEHP methyl ester and 232 m/z for methylated phenobarbital (scanned 0.25 sec./mass; 45 eV; emission current of 0.32 mA). The ion source was kept at 150°C and manifold at 80°C. A ratio of areas under fragments 163 and 232 m/z was plotted against the standard concentrations. A typical curve was linear with an equation $Y = 1.849 X - 0.313$ with a correlation coefficient 0.99995. Samples were assayed for intra- (6.34 % C.V.; n=10) and inter- (8.4% C.V.; n=10) assay variation statistics.

The HPLC assay [1,4]: Standards were prepared in acetonitrile : 1% acetic acid (9:1), with 0.2, 2, 10 and 50 µg/mL standard points per a curve. Samples (1 mL) were extracted after addition of 2 mL of citrate buffer (pH 2) and 5 mL of toluene. The mixture was shaken, spun to break the emulsion and 3 mL aliquot was removed and dried under N₂ (@40°C). The sample was redissolved in 1 mL of acetonitrile : 1 % acetic acid (9:1). 20 µL of standard solutions and samples were injected onto a HPLC column (Dupont Zorbax ODS 4.6 mm x 15 cm). The UV detector (Milton Roy/LCD SpectroMonitor D) was set at 230 nm. The system was used in a isocratic mode with mobile phase flow of 1 mL/min. and mobile phase acetonitrile : 1% acetic acid (65:35).

The two assays for MEHP were plotted against each other (GC-MS - X and HPLC - Y). The resulting line had an equation $Y = 0.5341 X + 0.2455$ with a correlation coefficient 0.99646. The HPLC assay produced consistently lower results than GC-MS method over a tested range 0.1-50 µg/mL. A typical values found in the high MEHP content HSA ranged from 5-20 µg of MEHP per mL of HSA. The products obtained by older fraction precipitation techniques had values 10 times lower than contaminated HSA. This discrepancy has to be addressed before the HSA obtained by gel permeation technology can be released on the market.

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ANALYSIS AND MASS SPECTROMETRIC IDENTIFICATION OF FOOD DYES AND THEIR SUBSIDIARY COLORED MATERIAL

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Introduction

Many dyes are used for foods all over the world and the following eleven dyes are only permitted in Japan; Erythrosine, Phloxine, Rose Bengale, Acid Red, Tartrazine, Sunset Yellow, Amaranth, New Coccine, Brilliant Blue FCF, Fast Green FCF and Indigo Carmine. However, non-permitted dyes are also frequently detected in foods. Usually, food dyes are synthetically prepared and contain many similar components. Few mass spectrometric studies of food dyes has been reported to date because of their nonvolatility. Consequently, a combination of precise and simple chromatography and appropriate mass spectrometry is greatly required for identification of small amount of food dyes. In this paper we would like to describe the mass spectrometric features of these compounds under SIMS conditions and the optimization for TLC/MS.

Experimental

SIMS and TLC/SIMS were performed using a double focusing Hitachi M-80B mass spectrometer fitted with a M-0101 data system. Operation conditions were: primary ion, Xe^+ ; accelerating voltage, 8 kV (primary) and 3 kV (secondary). Samples were dissolved in a solution of water and each sample solution (1-5 μl) was loaded on a silver target. About 3 μl of matrix material was added to the sample on a target. Aluminum TLC precoated plates impregnated with 10% liquid paraffin in petroleum ether were used for TLC/SIMS. The employed mobile phase were methanol:methyl ethyl ketone:10% sodium sulphate (1:1:3.5) for xanthene dyes and methanol:acetonitrile:10% sodium sulphate (3:3:10) for triphenylmethane dyes. The TLC/SIMS were carried out as follows. Thioglycerol was applied around the developed spot on a TLC plate. After a few minutes magic bullet was dropped on the condensed sample zone. The plate was cut (0.6 x 0.5 cm) and introduced into ion source.

Results and Discussion

Synthetic food dyes are classified into three groups, xanthene, triphenylmethane and azo (Fig. 1). In general they have conjugated chromophores and highly polar functional groups such as carboxylic or sulphonic acid groups, as their sodium salts. Many matrices, glycerol, a mixture of dithioerythritol and dithiothreitol (magic bullet), 15-crown-5, sulfolane, diethanolamine and thioglycolic acid were attempted. Among them, magic bullet and glycerol were only effective and showed differently characteristic mass spectrometric behavior. Table 1 shows the molecular ion species of the three type food dyes using glycerol and magic bullet as the matrices under SIMS conditions. While sodium ion-eliminated ions such as $(\text{M}-2\text{Na}+3\text{H})^+$ and $(\text{M}-2\text{Na}+\text{H})^+$ were mainly formed using glycerol, normal protonated molecules $(\text{M}+\text{H})^+$ were found in magic bullet assisted spectra, which retain completely sodium ion and are very convenient for determination of their molecular weights. An additional and unfavorable feature that glycerol reduced the sample molecule to give the hydrogenated molecular ion species was observed in the spectra using glycerol as the matrix. As the results of these experiments magic bullet proved to be the most suitable for identification of these food dyes by SIMS.

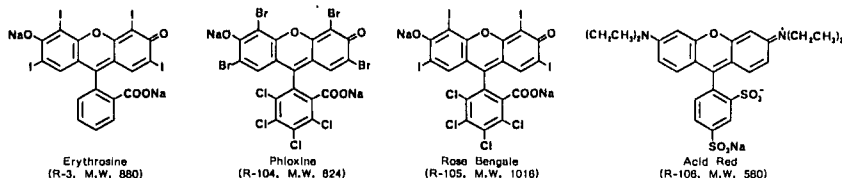
TLC is the simplest chromatography and does not require special equipments. So TLC/SIMS has been widely and successfully applied to analysis of various nonvolatile and unstable compounds, and we also wished to use it. To effectively perform TLC/SIMS with good sensitivity, selection of suitable matrix, optimization of TLC conditions and prevention of diffusion of spots on TLC plate must be carried out. We have already made sure that magic bullet is suitable for ionization of food dyes under SIMS conditions and reversed phase TLC is quite effective for separation of food dyes using the two mobile phases described in Experimental section.¹⁾ Accordingly, we devised a method to reconcentrate the developed spot on TLC plate. For this purpose thioglycerol is most suitable because it has faster diffusion speed than other matrices such as glycerol and threitol. In our method thioglycerol was applied around the developed spot on TLC plate and after a few minutes the dyes was condensed to center of the spot in line with the penetration of thioglycerol. And then magic bullet was dropped on the condensed zone. The present method can provide satisfactory SIMS spectra of the above food dyes with good sensitivities (0.1-0.5 $\mu\text{g}/\text{spot}$).²⁾

Finally the established results and method were successfully applied to identification of the subsidiary colored materials of Brilliant Blue FCF and an unknown food dyes from an imported candy.

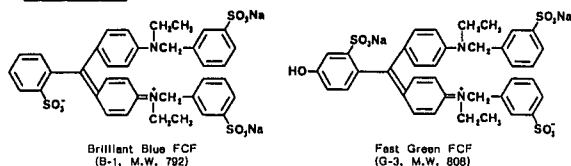
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1. Xanthenes



2. Triphenylmethanes



3. Azo

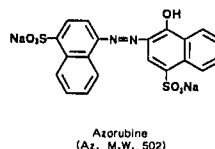


Fig. 1

Table 1

	Positive		Negative	
	Glycerol	Magic bullet	Glycerol	Magic bullet
Xanthene	(M-Na+2H) (M-2Na+3H)	(M+Na) (M+H)	(M-2Na+H)	(M-Na) (M-2Na+H)
Triphenylmethane	(M+Na) (M+H)	(M+Na) (M+H)	—	(M-Na) (M-2Na+H)
Azo	—	(M+Na) (M+H) (M-Na+2H)	—	(M-H) (M-Na) (M-2Na+H)

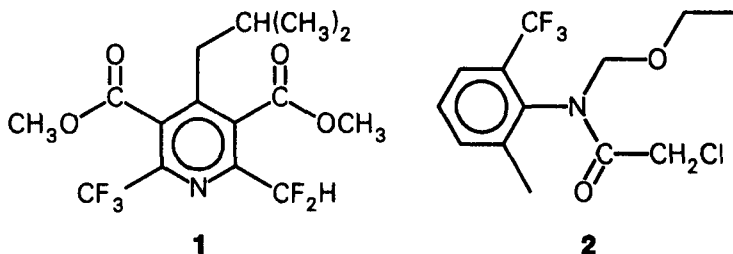
MASS SPECTROMETRIC ANALYSIS OF *IN VITRO* METABOLITES OF FLUORINATED HERBICIDES

Hideji Fujiwara*, R. Thomas Solsten, Robert C. Chott,
and Eugene W. Logusch

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The *in vitro* metabolism of herbicides with rat liver enzyme preparations yields important information on the types of metabolites that may be produced during *in vivo* metabolism studies. Such information can be obtained rapidly, since *in vitro* incubations can usually be completed within a few hours.

This study was focused on the *in vitro* transformation of two experimental Monsanto herbicides: dimethyl-2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarboxylate **1**; and 2-chloro-N-(ethoxymethyl)-N-[2-methyl-6-(trifluoromethyl)phenyl]acetamide **2**.



The *in vitro* incubations were carried out using S-9 rat liver enzymes in the presence of NADPH and glutathione. Radiolabeled substrate was used in the incubations to allow the progress of metabolism to be monitored by HPLC with radioactivity detection (HPLC/RAD). To facilitate identification, the substrate also contained an appropriate amount of ¹³C-label to provide ¹²C/¹³C "doublets" in the mass spectra of metabolites.

Metabolite structure elucidation was performed using both positive and negative ion liquid chromatography mass spectrometric (LC/MS) techniques, as well as static fast atom bombardment mass spectrometry (FAB/MS). Negative ion LC/MS can be a convenient analytical method for metabolites of fluorinated herbicides since many are readily ionized by electron capture. Most microsome constituents do not undergo electron capture and are essentially transparent to detection.

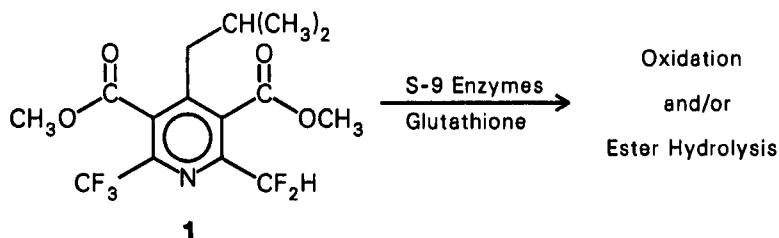
Although **1** and **2** both contain a fluorinated methyl group, their relative negative ion sensitivities were very different. Negative ion sensitivity was found to be excellent for **1** and its metabolites. Negative ion analysis of **2** and many of its metabolites provided very poor sensitivity. The latter compounds were therefore analyzed in positive ion mode. An HPLC injection typically contained 100ng-2μg of each metabolite. A simple, rapid sample clean-up and concentration greatly improved

the spectra obtained during the positive ion analyses of **2** and its metabolites.

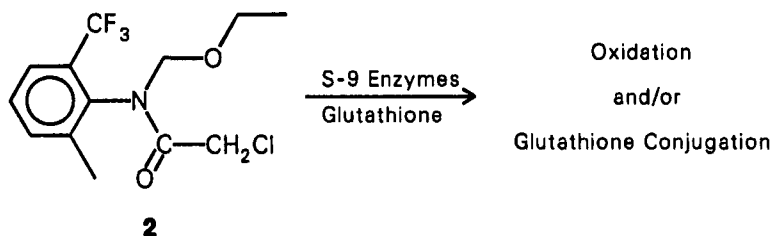
The pyridine **1** was metabolized to six metabolites which were identified by LC/MS. The significant metabolic reactions were oxidation and ester hydrolysis of the substrate. There was no mass spectral evidence to suggest that glutathione conjugation had occurred. See Scheme 1.

The chloroacetanilide **2** was rapidly and extensively metabolized by rat liver S-9 enzymes to give nine metabolites. Seven of these were identified by LC/MS. FAB/MS was also used to confirm the structure of one of the metabolites. The significant metabolic reactions included glutathione conjugation and oxidation of the substrate. Many of the initial oxidation products underwent glutathione conjugation as the incubation progressed. See Scheme 2.

Scheme 1. Metabolism of **1**



Scheme 2. Metabolism of **2**



ANALYSES OF ACIDIC AND NEUTRAL CATECHOLAMINE METABOLITES IN BRAIN TISSUES,
CSF, PLASMA AND URINE.

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A simple extraction method combined with high resolution EI SIR GC/MS was used to analyze homovanillic acid (HVA), 3-methoxy-4-hydroxyphenylethylene-glycol (MHPG) and 5-hydroxyindolacetic acid (5HIAA) in 1 to 10 mg of brain tissues, 200 μ l of plasma, 100 μ l of CSF or 50 μ l of urine samples.

Reagents and Chemicals - Buffer solution was 1 M sodium acetate (pH 6.0) containing 10 mM EDTA. $^{13}\text{C}_6$ -HVA (phenyl carbon) and $^{13}\text{C}_6$ -MHPG (phenyl carbon) were obtained from Cambridge Isotope Laboratories, Woburn, MA. The 5HIAA-d₂ (alpha H) was purchased from Merck Isotopes, St. Louis, MO.

Brain Tissue Homogenization - One to 30 mg of wet tissue was sonicated in 1 ml of sodium acetate buffer using an Ultrasonic Processor (W-375) at 3°C. The homogenate was centrifuged at 3,000 rpm (Servall, RT6000B) at 3° to remove tissue debris.

Extraction and Derivatization - Approximately 15 ng each of $^{13}\text{C}_6$ -HVA and $^{13}\text{C}_6$ -MHPG and 45 ng of 5HIAA-d₂ were added to brain homogenate (500 μ l), plasma (200 μ l plasma and 300 μ l sodium acetate buffer), CSF (100 μ l and 400 μ l sodium acetate buffer) or urine (50 μ l urine and 450 μ l sodium acetate buffer). To the sample mixture which contained IS and sodium acetate buffer, 60 μ l of 8 N HCl and 2 ml of 5% ethylacetate in hexane were added, mixed thoroughly, centrifuged and the organic layer was discarded. The organic solvent wash step was repeated. All neutral and acidic catecholamine metabolites (HVA, MHPG, 5HIAA and others) were extracted from this organic solvent (5% ethylacetate in hexane) washed aqueous layer with 2 ml of ethylacetate-hexane (3:2) mixture. The ethylacetate-hexane layer was transferred to 1 ml Reactivial and the solvent was removed in a nitrogen stream. The dried residue was reacted with 50 μ l of BSTFA-CH₃CN-CH₂Cl₂ (2:1:0.01) mixture at 100°C for 30 min.

Instrumentation - The instrument was operated in EI SIR mode at 6,000 resolution (10% valley). Ions were divided into 3 groups. HVA was monitored with m/z 326.14 (M⁺) and m/z 332.16 (M⁺, IS). MHPG was monitored with m/z 297.13 (M-TMSOCH₂) and m/z 303.15 (M-TMSOCH₂, IS). 5HIAA was monitored with m/z 407.18 (M⁺) and m/z 409.19 (M⁺, IS). The VG70S mass spectrometer was interface with a HP5890 GC equipped with a J&W on-column injector. The column used was DB5-30W (0.25 μ thickness).

Results - Twenty-five anatomically specific regions of cynomolgus monkey brain tissues were examined before and after 1-methyl-4-phenyl-1,2,4,5-tetrahydropyridine (MPTP) treatments. A significant change in the levels of HVA and MHPG were noted (Table 1). The method was also used to analyze brain levels of HVA, MHPG and 5HIAA in rats treated with the antidepressant bupropion which is undergoing clinical trials (Table 2).

Conclusions - A simple extraction method combined with high resolution EI SIR gave a reproducible and sensitive method for determining neutral and acidic metabolites of catecholamines in brain tissues, plasma, CSF, and urine. TMS derivatives are stable for 2 to 3 weeks and show no change in sensitivity. The method was successfully applied to numerous analyses of brain samples and other matrices.

Table 1. Catecholamine Metabolites in Cynomolgus Monkey Brain Before and After MPTP Treatment

Control	ng/mg tissue & SD*		
	HVA	MHPG	5HIAA
<u>Caudate</u>			
mCdl	7.8 ± 2.0	0.6 ± 0.3	1.5 ± 0.7
cCdl	9.7 ± 0.3	2.7 ± 1.7	1.7 ± 0.4
<u>Putamen</u>			
mPdl	13.7 ± 0.9	0.7 ± 0.5	1.4 ± 0.2
cPdl	6.8 ± 0.9	1.5 ± 1.2	2.3 ± 1.0
<u>MPTP treated</u>			
<u>Caudate</u>			
mCdl	0.7 ± 0.2	0.005 ± 0.0008	0.8 ± 0.3
cCdl	0.3 ± 0.2	0.006 ± 0.002	0.6 ± 0.3
<u>Putamen</u>			
mPdl	1.4 ± 0.5	0.02 ± 0.02	0.7 ± 0.3
cPdl	0.8 ± 0.1	0.007 ± 0.004	0.7 ± 0.3

* Average of 3 control vs. 4 MPTP treated animals.
Tissue size: 1 to 30 mg.

Table 2. Effects of Bupropion* in Whole Rat Brain

	pg/mg tissue & SD**		
	HVA	MHPG	5HIAA
Control	99 ± 5	15 ± 3	518 ± 63
Bupropion 12 mg/kg	83 ± 10	8 ± 2	508 ± 32

* An antidepressant, 2-t-butylamino-3'-chloropropionphenone.

** Average and standard deviations of 6 control vs. 6 treated animals.

QUANTIFICATION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND THREE OF ITS METABOLITES IN RAT BRAIN BY GAS CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY.

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Introduction

MDMA (3,4-methylenedioxyamphetamine) is now known to be a serotonergic neurotoxin in several animal models such as mice, rat and nonhuman primates. We have recently reported the identification of several biotransformation pathways of MDMA in rat (1) and human (2). To further understand the contributory roles of metabolites of MDMA in MDMA-induced neurotoxicity, we now report a disposition study of MDMA in the rat brain.

Experimental

A capillary GC/PCI-MS assay for simultaneous quantification of MDMA, 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMM) and 4-hydroxy-3-methoxyamphetamine (HMA) in brain tissue was developed. Isobutane was the PCI reagent gas. The assay was performed on an ion trap mass spectrometer by repetitively scanning a narrow mass range (m/z 275-290) which encompasses the ion currents for the MH^+ of the trifluoroacetyl derivatives of MDMA, MDA, HMM, HMA and the internal standard, 3,4-methylenedioxyethylamphetamine (MDE). A manifold temperature of 120°C was selected to minimize fragmentation of MH^+ ions of MDMA, MDA and MDE.

Results and Discussion

The mean recoveries of MDMA, MDA, HMM and HMA over the concentration range examined (5 and 250 ng/g) are 83.4, 86.0, 86.6 and 83.2, respectively. Calibration curves for MDMA, MDA, HMM and HMA are linear from 1 to 500 ng/g wet brain tissue (Figure 1 A-D). In each case, a correlation coefficient of 0.999 was obtained. The measured concentrations of MDMA, MDA, HMM and HMA in fortified brain tissues were within 10% of the target concentrations (Table 1). The mean coefficient of variation, both intra- and inter-assays, was less than 12% in each case. The limit of quantification of MDMA and metabolites is 1 ng/g.

Figure 2 shows ion current profiles from analysis of rat brain tissue at 3 hours after a subcutaneous dose of 10 mg/kg MDMA. The rat brain concentration-time profiles of MDMA and its metabolites are shown in Figure 3. Maximum brain concentrations of MDMA, MDA, HMM and HMA were obtained at 1, 3, 2 and 2 hours, respectively. The reported maximum decrease and recovery of the serotonergic markers following a subcutaneous dose of 10 mg/kg MDMA (3) correlate with the brain concentration-time profiles of HMM and HMA. This suggests that these phenolic amine metabolites may be responsible for MDMA neurotoxicity via oxidative stress.

Acknowledgement: This work is supported by NIDA grant 1R01 DA 05860-01

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TABLE 1. PRECISION AND ACCURACY FOR DETERMINATION OF MDMA, MDA, HMM, AND HMA IN FORTIFIED BRAIN TISSUES

target concn. ng/g brain	measured concn. ng/g brain (% C.V.)			
	MDMA	MDA	HMM	HMA
Intra-assay (n = 4)				
2.0	2.2(11.8)	2.1(9.2)	2.1(10.4)	2.0(10.0)
50.0	49.5(10.3)	53.7(6.9)	55.3(10.0)	49.8(9.6)
Inter-assay (n = 4)				
2.0	2.0(8.6)	2.1(6.3)	2.1(6.7)	2.0(2.5)
50.0	50.1(7.6)	47.9(11.3)	48.6(10.9)	48.3(6.0)

C.V. = coefficient of variation

FIGURE 1A. CALIBRATION CURVE FOR QUANTIFICATION OF MDMA IN BRAIN

Calibration Plot (Int Stds) Filename: DISPOS Compound: 2 of 5 Correlation Coeff: 0.999
MDM Standard Deviation: 0.633
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin/Lin)

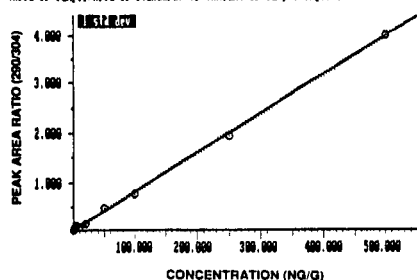


FIGURE 1B. CALIBRATION CURVE FOR QUANTIFICATION OF MDA IN BRAIN

Calibration Plot (Int Stds) Filename: DISPOS Compound: 3 of 5 Correlation Coeff: 0.999
MDM Standard Deviation: 0.939
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin/Lin)

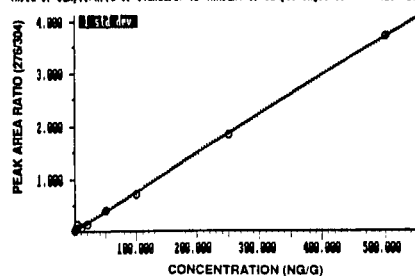


FIGURE 1C. CALIBRATION CURVE FOR QUANTIFICATION OF HMM IN BRAIN

Calibration Plot (Int Stds) Filename: DISPOS Compound: 4 of 5 Correlation Coeff: 0.999
HMM Standard Deviation: 0.974
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin/Lin)

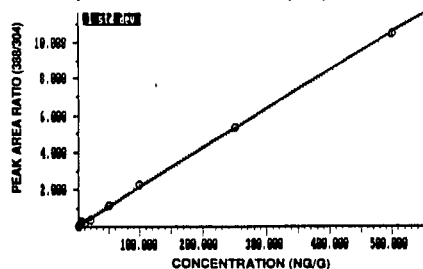


FIGURE 1D. CALIBRATION CURVE FOR QUANTIFICATION OF HMA IN BRAIN

Calibration Plot (Int Stds) Filename: DISPOS Compound: 5 of 5 Correlation Coeff: 0.999
HMA Standard Deviation: 0.987
(Area of Sample/Area of Standard) vs (Amount of Sample Injected) (Lin/Lin)

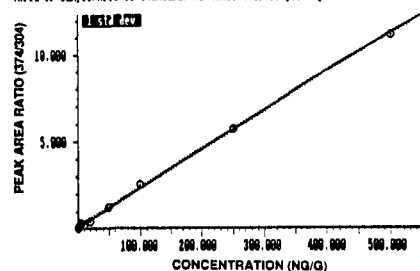


FIGURE 2. ION CURRENT PROFILES OF MH⁺ IONS OF MDMA, MDA, HMM AND HMA FROM ANALYSIS OF BRAIN TISSUES AT 3 HR AFTER A SUBCUTANEOUS DOSE OF 10 MG/KG MDMA

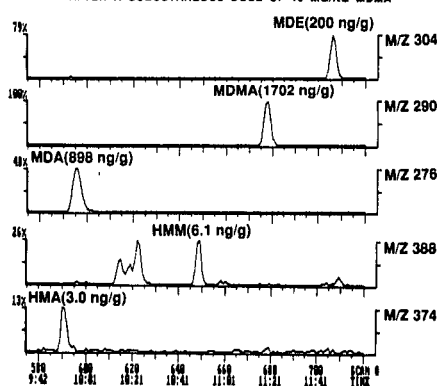
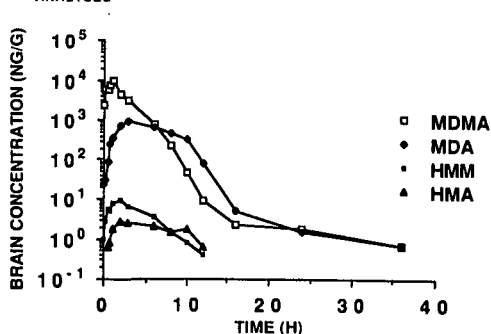


FIGURE 3. RAT BRAIN CONCENTRATION-TIME PROFILES OF MDMA, MDA, HMM AND HMA AFTER A SUBCUTANEOUS DOSE OF 10 MG/KG MDMA. EACH POINT IS AN AVERAGE OF 2 ANALYSES

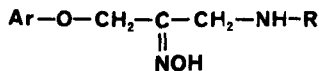


**METABOLISM OF KETOXIME ANALOGS OF BETA-ADRENERGIC BLOCKERS:
GC-MS AND THERMOSPRAY LC-MS STUDIES**

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INTRODUCTION

The site-specific action of the ketoxime analogs of several β -adrenergic antagonists (1) in the reduction of the intraocular pressure, which is a beneficial effect in glaucoma treatment, has recently been achieved by the topical administration of the compounds [1,2].



(1)

The mechanism of action appears to be ultimately connected to their metabolic properties, and relies on the biotransformation of these substances by enzymes present in the eye. More specifically, the iris-ciliary body is one of the major sites of the intraocular enzymatic processes [3] that may be responsible for the observed pharmacological effect of the title compounds. Propranolone oxime (Ar = 1-naphtyl, R = *i*-Pr) and alprenolone oxime (Ar = 2-allylphenyl, R = *i*-Pr) have been selected as models for more detailed studies, since these compounds have proved to be the most potent ones regarding the reduction of the intraocular pressure. The direct aim of our approach was to identify the principal route(s) of bioactivation by determination of the products of the proposed enzymatic reactions in the related ocular tissues. Mass spectrometry, especially in combination with on-line chromatographic methods of analysis such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), appeared to be a sensitive and specific method that can provide means to assess the underlying metabolic processes.

Specific problems associated with the application of mass spectrometry to the identification and determination of the ketoximes and their metabolites *in vivo* include, however, the possible geometrical (E- and Z-) isomerism of the oximes, the low volatility of the compounds, and the chemical/thermal lability of certain possible intermediates. Therefore, GC-MS and thermospray (TSP) LC-MS methods have been developed and evaluated for these studies.

EXPERIMENTAL

The mass spectrometer used in the experiments was a KRATOS MS80RFA double focusing instrument (operated at 4 kV accelerating voltage), and the electron impact/chemical ionization (EI/CI) source was interfaced to a Carlo Erba MEGA Series GC (Model 5160) by direct coupling. 25 m x 0.22 mm i.d. DB-1 (bonded methylsilicon stationary phase, 25 μ m film thickness) fused silica capillary column was applied for separations. The column oven temperature was programmed during the GC-MS analyses, generally from 80 to 280 $^{\circ}$ C at 10-30 $^{\circ}$ C/min rate. On-column injections were made, and the mass spectrometer scan rate was selected to be 1 s/decade and the spectra were recorded in the *m/z* 50-500 mass range under the control of data system (DS90). Different derivatives of the analytes were prepared in order to improve their chromatographic properties and/or increase their chemical/thermal stabilities for GC. Trifluoroacetylations (TFA) were carried out with the anhydride or with N-methyl-bis(trifluoroacetamide) (MBTFA), trimethylsilylations (TMS) were achieved by the application of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA). By carefully selecting the appropriate reaction conditions, the derivatization techniques could be used in combination, and even selective protection of the relevant functional groups was achieved. N-, and N,O-trifluoroacetyl, N,O-trimethylsilyl, and O-TMS-N-TFA derivatives were prepared and evaluated for practical applications.

The TSP LC-MS experiments were performed by the KRATOS Thermospray Option. The HPLC apparatus consisted of a Spectroflow 400 solvent delivery system and a Spectroflow 480 injector module (with 20 μ l loop). 5 cm x 4.6 mm i.d. SUPELCOSIL LC-18-DB column was applied for the separation of the analytes. The mobile phase contained an aqueous buffer solution (0.1 M ammonium acetate, 0.02 M acetic acid, and 0.02 % triethylamine), and acetonitrile (30 %, v/v) as an organic modifier. At 1.0 ml/min flow rate, optimal thermospray conditions were achieved by setting the probe, vaporizer, source, and jet temperature to 135,

165, 175, and 220 °C, respectively. The spectrometer was scanned at 3 s/decade, the calibrated mass range (PEG 600) was m/z 150-600.

In vivo experiments were carried out by topical administration of the solutions containing the ketoximes to rabbits. The animals were sacrificed 1-1.5 h after the treatment, the respective eye tissues were removed, homogenized in alkaline buffer (pH 10.0), and extracted with dichloromethane/diethyl ether.

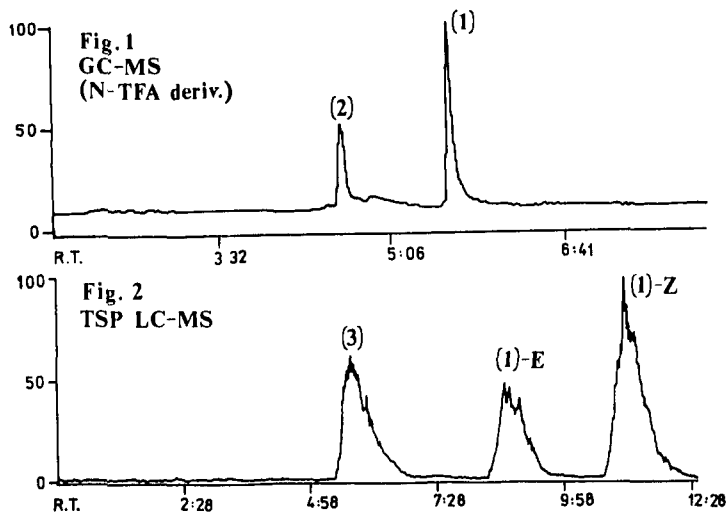
RESULTS AND DISCUSSION

The developed GC-MS methods are useful for the identification and sensitive determination of the biotransformation product. However, separation of the oxime isomers has not been achieved under routine capillary GC conditions, although derivatives leaving the oxime hydroxyl groups intact for possible regiospecific interactions (i.e., N-TFA derivatives) have also been prepared (Fig. 1). On the other hand, both EI and CI give readily interpretable spectra. (Derivatization may even improve the fragmentation properties of these substances). TSP LC-MS provides, however, the separation of the E- and Z-isomers of the title oximes (application of a column packing material deactivated for the analysis of basic compounds is a must, and addition of a competing base to the mobile phase is advisable in order to achieve adequate chromatographic resolution), and it can also be used for the assay of the possible intraocular metabolites (Fig. 2), although (ca. an order of magnitude) lower sensitivities can be achieved, as compared to GC-MS.

The combined results have allowed the identification of the dominant intraocular enzymatic processes of the ketoxime analogs. Significant oxime isomerase activity of the tissues was found. The hydrolysis of the oximes, making the ketone (2) available to further metabolic process, and the subsequent reduction result in the formation of the aminoalcohol (3) which exerts the desired effect.

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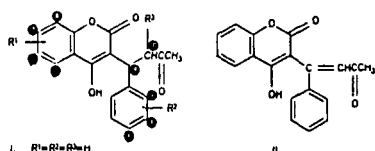


FAST ATOM BOMBARDMENT OF WARFARIN AND WARFARIN METABOLITES

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We have proposed that urinary analysis for warfarin metabolite profiles will provide information on hepatic cytochrome P-450 composition, and its susceptibility to environmental pollutants. This and other applications underscore the importance of the analysis of warfarin (I) and its metabolites, dehydrowarfarin (II), 4-hydroxywarfarin (III), 6-hydroxywarfarin (III), 7-hydroxywarfarin (V), 8-hydroxywarfarin (VI) and 10-hydroxywarfarin (VII).

WARFARIN AND WARFARIN METABOLITES



I. $R^1=R^2=R^3=H$

III. $R^1=R^2=H$, $R^3=4-OH$

IV. $R^1=5-OH$, $R^2=H$

V. $R^1=7-OH$, $R^2=H$

VI. $R^1=8-OH$, $R^2=H$

VII. $R^1=10-OH$, $R^2=H$

Current widely used methods involve GC/CIMS of the diazomethane derivatives of warfarin and its metabolites¹. A method utilizing multiple metastable peak monitoring of warfarin in plasma has been published². Recently we reported a Thermospray LC/MS method for (I) through (VII) which does not require derivatization with carcinogenic and explosive diazomethane. This method required only a two step separation before Thermospray ionization³.

In this work we show data on the FAB mass spectrometry of (I) and its metabolites on a high resolution mass spectrometer. These investigations were undertaken to provide a convenient means of confirming identifications by Thermospray LC/MS, to compare FAB analysis of warfarin with other currently used methods and to identify new or unusual metabolites.

The desorption of (I) and (II) was tested in triethanolamine, TEA, and glycerol. Both the oxygen content and the nearly completely conjugated structure of (I) and (II) suggested that these structures would stabilize a negative charge resulting from proton abstraction. The negative FAB spectra of (I) and (II) in TEA are shown in Figures 1 and 2. Both (I) and (II) gave relatively intense molecular ions. The only fragmentation is the loss of acetone from the warfarin molecule to give m/z 250. Dehydrowarfarin gives m/z 263 which represents a loss of acetyl from its molecular structure. The base peak in both Figs. 1 and 2 is due to the matrix, TEA, and this material gives an intense dimer ion at m/z 297. Other intense ions are due to a trace of CsI and RbI which remained on the probe tip after calibration. The spectra were taken on a Kratos MS-50 at 1000 RP (10X valley) scanning at 3 sec/dec from m/z 700 to n/z 30. Warfarin and dehydrowarfarin and TEA were dissolved in acetone, the acetone was evaporated and 2 μ l of the remaining TEA solutions were added to the probe. The FAB probe tip was bombarded by 5 keV Xe from a saddle field gun described in Reference 4. Data were collected as voltage readings into a Kratos DS-90 data system for subsequent processing.

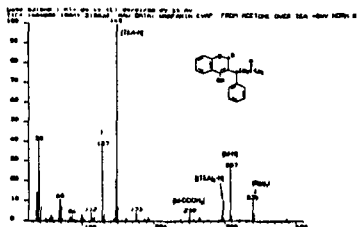


Figure 1

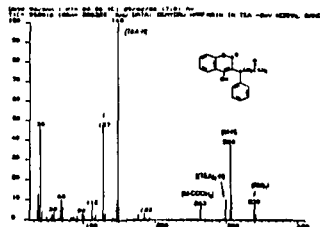


Figure 2

Addition of a proton from glycerol or acidified glycerol to the generalized warfarin structure (I) or (II) produces a $[M+H]^+$ ion in positive ion mode. As in negative ion mode little fragmentation occurs. The ion at m/z 251 in the spectrum of warfarin represents loss of CH_3COCH_3 from the $[M+H]^+$ ion at m/z 309. The spectrum of (III) also shows a loss of CH_3COCH_3 from m/z 325, m/z 267. The ion at m/z 289 in the spectrum of (II) represents the loss of H_2O from the $[M+H]^+$ ion at m/z 307. This loss of water which also occurs to a very limited extent in the FAB spectrum of warfarin is observed in the spectra of all three compounds, (I), (II) and (III) in positive ion Thermospray³. The FAB conditions for the positive ion spectra were similar to those used for the negative ion spectra. Although no quantitative measurements were made (I) and (II) appear to desorb more efficiently as negative ions from TEA.

In order to push the detection limit of these compounds below a clinically significant level high resolution selected ion monitoring experiments were attempted. The mass range, m/z 261 to m/z 357 was calibrated using a saturated solution of CsI and RbI in glycerol. An ion at nominal mass 277, although weak occurs in both the mass calibrant and the glycerol matrix. Using the CsI/RbI calibration and m/z 277 as a lock mass 13.4 ng of warfarin were easily detected by desorption from a conventional FAB probe. The detected signal when compared against background in blank glycerol represented a detection limit of less than 2 ng of warfarin. Unfortunately this performance was not reproducible. The accurate mass of highly resolved (10,000 RP, 10% valley) m/z 305 ions of (II) was measured as 305.0818834 with 3 ppm rms error in a mixture with TEA, CsI and RbI. This measurement can be made on 2 μ g of (II) in a classical peak matching experiment. Data on further refinements in the accurate mass measurements of these compounds and on their analysis in mixtures will be presented at a later date.

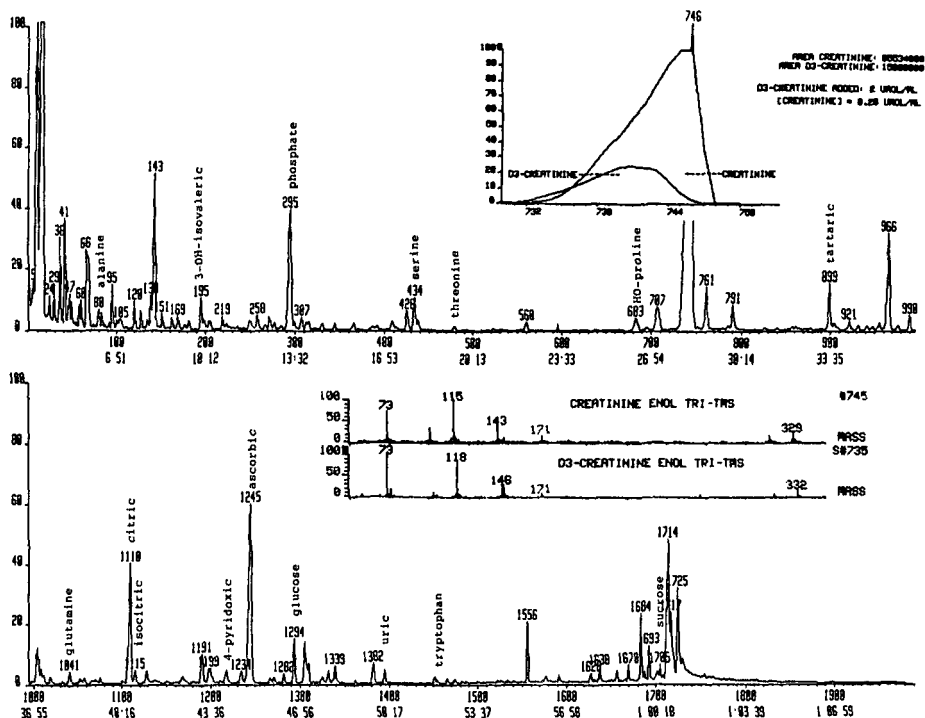
Further work will involve investigating high resolution selected ion monitoring with continuous flow FAB in both positive and negative ion modes.

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SIMULTANEOUS PREPARATION OF ACIDIC BASIC AND NEUTRAL URINARY CONSTITUENTS FOR GC/MS. Shoemaker, J.D., Elliott, W.H., E.A. Doisy Dept. of Biochemistry and Molecular Biology, St. Louis Univ., St. Louis, MO 63104

Urinary organic acids, amines, amino acids, sugars, purine and pyrimidine metabolites were prepared simultaneously for derivatization and gas chromatography. Urease was used to decrease the urea concentration below detectable levels. Samples were desiccated by azeotropic evaporation and triethylamine trifluoroacetate was used to maintain solubility in the anhydrous state. After derivatization with N-methyl-N-(trimethylsilyl) trifluoroacetamide, capillary GC-MS on DB-5 from 80 to 240°C with the VG Trio 2 in EI+ mode revealed creatinine tri-TMS to be a suitable internal standard. The creatinine-TMS derivative was identified by co-elution with the (methyl) tri-deuterated compound and by comparison of the mass spectra below. The d₃-creatinine was formed from d₃-creatine in the aqueous phase of the procedure as described by Edgar and Shiver (1). In contrast to their results, however, dehydration at pH 9-10 in the presence of trifluoroacetate and triethylammonium ions yielded creatinine exclusively. The method has been applied to urine samples from subjects with hydroxymethyl glutaric, propionic, methylmalonic, and 'medium chain' fatty aciduria, and generalized amino aciduria and confirmed these diagnoses. Software compatible with the multi-task RSX 11M Plus V3.0 Program supplied with the DEC Micro PDP 11/73 computer system was developed to identify peaks using the NBS Library during the 60-90 minute GC run, so that by the end of the run a printout is available of peak identities, heights and areas. Thus GC-MS metabolic screening required no more time than GC alone. Supported by NIH Training Grant HL-07050 and NIH Grant HL-07878.

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SIMULTANEOUS DETERMINATION OF ACIDS AND AMINES BY GC-NCIMS.

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Aliphatic amines have been successfully derivatized to imines directly in water using pentafluorobenzaldehyde (PFB) [1]; the imines were isolated using solid phase extraction and separated and measured using GC-MS [2]. This direct procedure can be extended by adding pentafluorobenzyl bromide (PFBzBr) to form esters directly of any aliphatic acids present in the water sample. Preliminary test data are presented to show that aliphatic amines and acids in water samples can be simultaneously determined with high sensitivity and selectivity.

EXPERIMENTAL

Derivatization Procedure. --A 0.5 mL aliquot of in situ oil shale retort water or test water in a 5 mL vial was spiked with 5 μ g each of CD_3NH_2 AND CD_3COOH and the pH adjusted to 8 with 0.5M borate. Then 300 μ L of acetonitrile containing 1.0 mg PFBzBr and 2.0 mg PFB was added to the vial and heated to 80°C and held at that temperature for 40 minutes. After cooling to ambient, the derivatives were isolated using a solid phase extraction cartridge containing 100 mg of C-18 bonded porous silica. The bonded silica was dried by aspirating room air through the cartridge for 5 minutes and the amine and acid derivatives eluted with 200 μ L hexane.

Instrumentation. --A Finnigan model 4000 combination GC-MS with an INCOS 2300 data system was used for all analyses of the hexane extracts. The GC-MS was operated in the negative chemical ionization (NCI) mode using methane as the buffer gas at 0.3 torr and an ion source temperature of 250°C. The acid and amine derivatives were measured using multiple-ion-monitoring and quantitated using response factors generated from the appropriate deuterated internal standard.

NCIMS behavior. -- The amine derivatives formed predominantly molecular anions under the NCI conditions of our instrument. These anions appear at m/z 209, 223 and 237 for methyl, ethyl and propylamines, respectively. The acid derivatives formed characteristic carboxylate anions. These anions appear at m/z 59, 73 and 87 for acetic, propionic and butyric acids, respectively. The deuterated internal standards produced anions three mass units higher than the respective unlabeled compounds.

RESULTS AND DISCUSSION

The sensitivity of the method for 50 ppb of ethylamine and propionic acid is illustrated in Figure 1. The high S/N and lack of any background peaks suggest that this procedure could be used at much lower concentrations.

The quantitative performance of the method was tested by spiking propionic acid and ethylamine into pure water. Deuterated acetic acid and methylamine were used as internal standards. For triplicate determinations, the precision was 3% RSD for the acid and 16% RSD for the amine. Accuracies were 11% and 26% respectively. These preliminary figures are promising and it is expected that fine-tuning of the reaction conditions can be used to improve the quantitative data.

The developed method was applied to a waste water from an in situ shale retorting process. This water sample contained aliphatic acids and amines as well as a complex variety of other compound classes, including pyridines, phenols, aldehydes, ketones and nitriles. Mass chromatograms for the derivatives of three acids and three amines are shown in Figure 2. The two peaks in the mass chromatogram labelled 'C4' correspond to the derivatives of iso- and n-butyric acids. The resolution of the fused silica capillary column used for the separation ensures that other isomeric acid and amine derivatives can be measured selectively. Quantitative values for selected acids and amines in the sample are listed in Table 1. These values are consistent with the reported composition of shale oil retort waters [3].

The demonstrated applicability of the method to low levels of aliphatic acids and amines present in waste waters and the high sensitivity and selectivity using GC-NCIMS suggest that successful application to biological fluids may be possible.

1. Anal. Chem., 1985, 57, 790.
2. J. Chromatogr., 1987, 420, 379.
3. Environ. Sci. Technol., 1982, 16, 714.

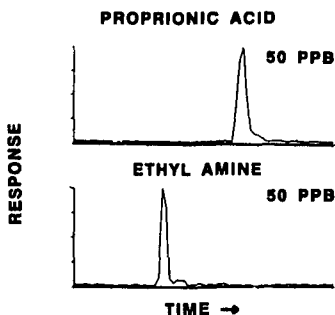


Figure 1. GC-NCIMS-MIM of an extract from a treated sample containing propionic acid and ethylamine.

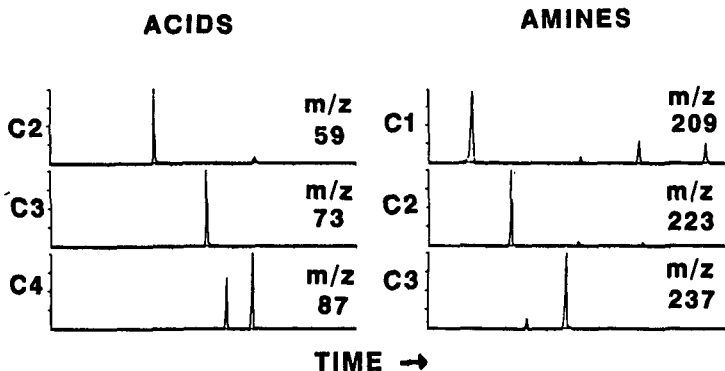


Figure 2. GC-NCIMS-MIM of an extract from a treated sample of a shale oil retort water.

Table 1 . Quantitative values for selected acids and amines present in a waste water sample.

SHALE OIL RETORT WATER	
ANALYTE	CONCENTRATION IN mg/L
ACETIC ACID	228
PROPIONIC ACID	303
n-BUTYRIC ACID	151
METHYLAMINE	51
ETHYLAMINE	16
n-PROPYLAMINE	3

IDENTIFICATION OF MERCAPTURIC ACID METABOLITES OF ACRYLONITRILE BY TANDEM MASS SPECTROMETRY

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Acrylonitrile (AN) is widely used in the manufacture of plastics, rubber, and acrylic fibers. Oral administration of AN to rats causes tumors of the brain, stomach, and Zymbal's gland. Current data on the metabolism and carcinogenicity of AN do not allow an accurate assessment of risk to exposed humans.

Metabolism studies of AN in rats and mice have demonstrated that most of the administered dose is excreted as urinary metabolites. The two major biotransformations are conjugation with hepatic glutathione resulting in excretion of N-acetyl-S-(2-cyanoethyl)cysteine, and a cytochrome P-450-mediated oxidation to 2-cyanoethylene oxide which is further metabolized to N-acetyl-S-(2-hydroxyethyl)cysteine. The epoxide metabolite 2-cyanoethylene oxide has been shown to alkylate DNA.

We are studying the metabolic disposition of AN in rats and mice and have identified the urinary metabolites of AN, quantitated these metabolites as a function of dose, and compared the metabolite profiles of the two species. The data presented here describe a method to identify the two major mercapturic acid metabolites of AN using minimal sample preparation and tandem mass spectrometry. It is hopeful that this method can be extended to monitoring these metabolites in occupationally exposed humans.

¹⁴C Male Fischer-344 rats and male B6C3F1 mice rats were administered [2,3-¹⁴C]acrylonitrile p.o. (rats 0.1-28 mg/kg, mice 0.1 - 11.7 mg/kg). Animals were placed in individual glass metabolism cages and urine and feces were collected for 72 hr. Urine was mixed with an equal volume of methanol and centrifuged to remove protein.

Mass spectral analysis was performed on a Kratos MS90MS hybrid mass spectrometer of geometry EBQQ. Pseudomolecular ions, MH^+ , were generated by methane positive ion chemical ionization. Sample introduction was by solids probe. Samples were prepared by evaporating 5 μ l aliquots of deproteinized urine in glass capillaries. The MH^+ ions were selected at 1000 resolving power by MS-I (EB), and focussed into the collision quadrupole containing xenon (2×10^{-5} torr). The daughter ions generated by CID (30 eV) were mass analyzed by scanning the analytical quadrupole from m/z 300-50 in 3 seconds. Multiple scans were acquired and combined/integrated using the raw data software facility in DS-90.

HPLC analysis of urine samples from AN dosed animals indicated the presence of four qualitatively similar metabolites in rats and mice. Peak 3 was identified as N-acetyl-S-(2-hydroxyethyl)cysteine and Peak 4 as N-acetyl-S-(2-cyanoethyl)cysteine, by comparison with spectra of authentic compounds. Figures 1 and 2 show the daughter ion spectra of the MH^+ ions of the respective hydroxyethyl and cyanoethyl mercapturic acid metabolites in mouse urine along with the spectra of the authentic compounds. The daughter ion spectra indicate that most of the ions produced by CID come from fragmentation of the cysteine portion of the molecule. Both compounds produce analogous ions from loss of H_2O (18), $COCH_2$ (42) from the N-acetyl group, $HCOOH$ (46), and $H_2NHC(O)CH_3$ (59), although the relative abundances of these ions are different in each compound.

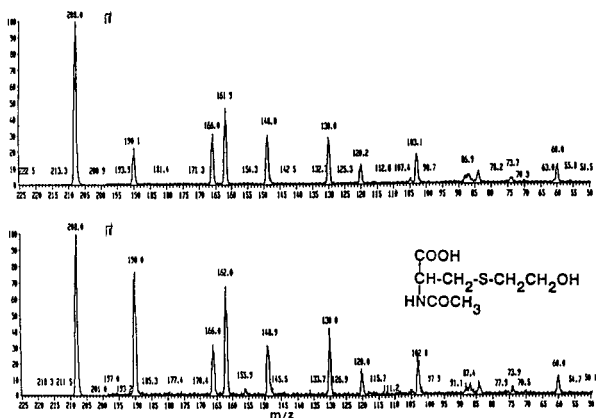


Figure 1

Daughter ion spectra of the MH^+ ion from authentic N-acetyl-S-(2-hydroxyethyl)cysteine (lower) and from the m/z 208 ion in urine from a mouse given AN (upper).

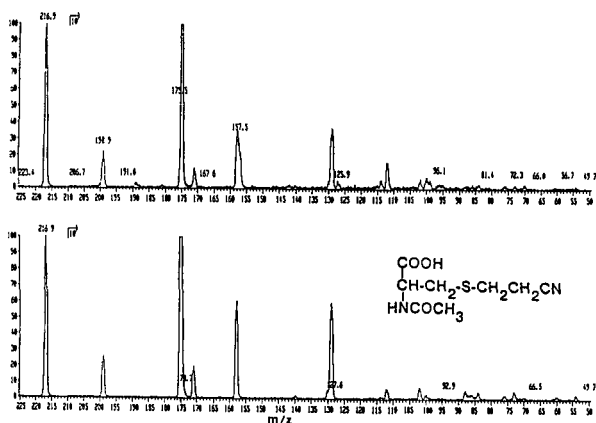


Figure 2

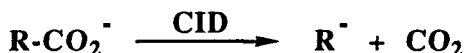
Daughter ion spectra of the MH^+ ion from authentic N-acetyl-S-(2-cyanoethyl)cysteine (lower) and from the m/z 217 ion in urine from a mouse given AN (upper).

Conclusion: This method provides a rapid and specific means to screen urine for mercapturic acid metabolites of AN. By selectively monitoring one or two daughter ions from each metabolite, it should be possible to qualify/quantify these metabolites (if present) in the urine of occupationally exposed humans. Along with a better understanding of the disposition of acrylonitrile in other species, this method could provide a better assessment of exposure of people to acrylonitrile.

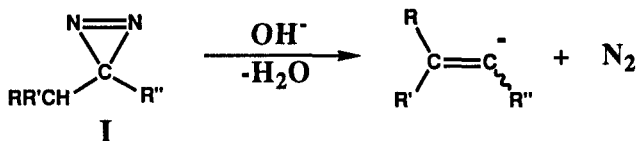
VINYL ANION FORMATION AND REACTIVITY:

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Strong bases such as alkyllithiums and lithium dialkylamides are common reagents for generating carbanions in solution.¹ In the gas phase, amide ion is the strongest base which can readily be prepared ($PA = 403.6 \text{ kcal mol}^{-1}$)², and it is frequently used to produce other ions by proton abstraction. Compounds which are less acidic than ammonia can not be readily deprotonated by NH_2^- and the formation of their conjugate bases presents a considerable synthetic challenge. Several ions of this type such as CH_3^- , $c\text{-}C_3H_5^-$, and $C_2H_3^-$ have been prepared but only one general method, decarboxylation by collision-induced dissociation has been reported to date.³



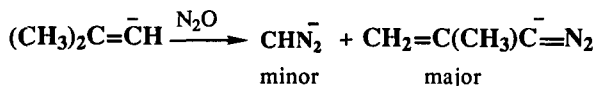
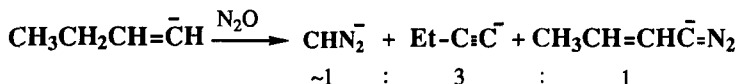
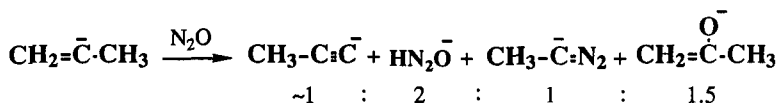
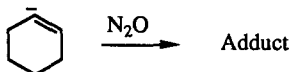
We have recently discovered a convenient chemical ionization procedure for the preparation of highly reactive vinyl anions in our flowing afterglow device. In particular, the reaction of OH^- and appropriately substituted diazirines (I), which are readily synthesized in two steps, leads to the elimination of molecular nitrogen and the formation of vinyl anions.



R, R', and R'' = H or alkyl

Using this this method we have prepared a number of mono and disubstituted vinyl anions. They are all exceedingly reactive and sufficiently basic to readily deprotonate ammonia. The

reactivity of these ions has been explored and the reactions with N_2O are given:



1. For example see: Brandsma, L.; Verkruijsse, H. "Preparative Polar Organometallic Chemistry 1", Springer-Verlag: New York, 1987.
2. Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; and Mallard, W. G. *J. Phys. Chem. Ref. Data* 1988, **17**, Supplement 1.
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DETERMINATION OF THE FRAGMENTATION MECHANISMS OF ORGANOPHOSPHORUS IONS
BY H₂O AND D₂O ATMOSPHERIC PRESSURE CHEMICAL IONIZATION
TANDEM MASS SPECTROMETRY

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The literature relates that methyl phosphonates, phosphates and phosphites, e.g., dimethylmethylphosphonate (DMMP), trimethylphosphate (TMP) and trimethylphosphite (TMPI), produce fragments primarily by direct radical loss (-CH₃ and -OCH₃) from the M⁺ ion under conventional electron impact mass spectrometry (MS) or collision-induced dissociation (CID) MS/MS (1,2). Formaldehyde loss by a McLafferty-type rearrangement has also been reported (1,2,3). Isobutane chemical ionization (CI) of DMMP followed by CID has reportedly resulted in methanol, methyl and methoxy losses from the pseudomolecular ion, MH⁺ (1).

These observations have been compared with those of the daughter ion mass spectra (see Figure) of the MH⁺ from DMMP, dimethylphosphite (DMPI), TMPI and TMP. H₂O and D₂O atmospheric pressure CI tandem MS/MS of the MH⁺ and MD⁺ species show that the majority of the fragmentation mechanisms of parent ions can be described by stable molecule (not radical) loss events and a summary is presented in the Table, where H⁺ denotes the ionizing proton or deuteron.

All four species exhibit methanol and dimethylether losses from the MH⁺ and MD⁺ species; water loss is observed from DMPI. Methanol, ether, formaldehyde, and water losses were observed from various daughter ions. All four compounds form m/z 79 from MH⁺ and m/z 80 from MD⁺ in the MS/MS spectra. However, the MD⁺ MS/MS mass spectra of DMPI, TMPI and TMP also show m/z 79 indicating that there are two parallel mechanisms for the fragmentation of the pseudomolecular ions of these two species. The m/z 79 from the MH⁺ of TMP can be explained by a consecutive loss of MeOH⁺ and CH₂O. The other mechanism constitutes consecutive methoxy losses, the first being unobserved (an alternative mechanism might be a rearrangement and loss of CH₃OOCH₃) while retaining the source proton to yield either an m/z 79 or 80. From DMPI the formation of m/z 79 involves a methanol loss with either MeOH⁺ directly or MeOH by a rearrangement; the latter mechanism would not have been obvious without the D₂O experiment.

The daughter ion mass spectra showed that the m/z 47 ion was produced from the MH⁺ and MD⁺ species from all four compounds. In addition, the D₂O analysis showed the presence of m/z 48 from TMPI and TMP with intensities equal to that of m/z 47. High resolution Fourier Transform MS/MS mass spectra of (TMP)H⁺ produced two ions having a nominal mass of 47 amu with a Δm of 0.03 amu. The indications are that m/z can be PO⁺ and (HPCH₃)⁺; the mechanism(s) of the formation of the latter species are still under investigation.

MS/MS analyses of MD⁺ confirm that the ionizing proton is contained in other fragments, e.g., m/z 31, 33, 49, 65 (except TMPI) and 95 (except DMPI and TMPI). The ions at m/z 15, 29, 45 (DMMP and TMPI), 63, 93, and 109 (TMP) do not contain the ionizing proton. Methyl and methoxy neutral losses were not directly observed from the MH⁺/MD⁺ of the four compounds.

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TABLE OF NEUTRAL LOSSES AND RESULTING IONS

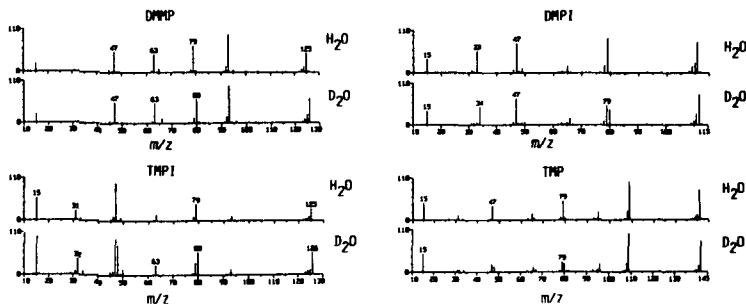
Neutral Loss	DMMP 125/126	DMPI 111/112	TMPI 125/126	TMP 141/142
MeOH*	93	79	93	109
MeOH		79/80		
ether	79/80	65/66	79/80	95/96
HOH*		93		
ethane	95/96			
MeOH*-CH ₂ O	63	49	63	79
MeOH*-MeOH		47		
MeOH-CH ₂ O		49/50		
MeOH-MeOH*		47		
ether-HOH*		47		
ether-CH ₂ O	49/50		49/50	65/66
ether-MeOH*	47		47	63
MeOH*-ether	47		47	63
MeOH*-ethane	63			
HOH*-CH ₂ O		63		
HOH*-ether		47		
MeOH*-O				93
H ₃ COOCH ₃				79/80
H ₃ COOCH ₃ -MeOH*				47
MeOH*-CH ₂ O-H ₂ O	45			
MeOH*-CH ₂ O-MeOH				47
ether-CH ₂ O-HOH*				47
ethane-CH ₂ O	65/66			
ethane-CH ₂ O-HOH*	47			
[MeO(H)H*] ^a	33/34	33/34	33/34	33/34
[H*O-CH ₂] ^a	31/32	31, 32	31/32	31, 32
OCH ₃ ⁺	31	31		31
?			47/48	47/48
?			79	

A slash between two numbers indicates that the first ion is observed in the MH⁺ MS/MS mass spectrum and only the second ion is found in the equivalent MD⁺ mass spectrum. A comma between two numbers indicates that the first ion is observed in the MH⁺ MS/MS mass spectrum and both ions are observed in the equivalent MD⁺ spectrum.

*denotes either ion source proton or deuteron

^astructure of the tabulated ion from the MH⁺/MD⁺ species

API DAUGHTER ION MASS SPECTRA



**A COMPARATIVE STUDY OF ISOELECTRONIC AND ISOGYRIC REACTIONS:
MOLECULAR ORBITAL CALCULATIONS OF DIATOMIC HYDRIDES AND**

HALIDES. Carol A. Deakyne, Ionospheric Physics Division, Air Force Geophysics

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Biochemistry, University of Maryland, Baltimore County Campus, Baltimore, MD 21228;

Gernot Frenking, Fachbereich Chemie, Universität Marburg, Hans - Meerwein - Strasse, D -
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Qualitative comparison of isoelectronic molecules has provided a useful conceptual framework for the understanding of many species.¹ Indeed, a recent round of computational studies on noble gas fluoride cations^{2,3} made heavy use of isoelectronic reasoning, both "vertical" and "horizontal". In the current paper we wish to quantitate the use of isoelectronic comparisons to aid in the computational study of reaction enthalpies for isogyric and nonisogyric reactions involving HeF^+ , NeF^+ , ArF^+ , ArH^+ , HCl , HCl^+ , ClF , Cl_2 , HF , HF^+ , F_2 , H_2 and H_2^+ . We will provide a general conceptual framework and other studies, in progress, are designed to test the qualitative and quantitative utility of our approach.

The calculations were carried out ab initio using the Gaussian 82 and 86 programs.⁴ Fully optimized geometries were obtained utilizing the HF/6-31G*, MP2(fu)/6-31G** and CID(fu)/6-31G** approximations employing analytical gradients.⁴ The largest difference in the MP2/6-31G**³ and CID/6-31G** geometries is 0.044 Å; the smallest is 0.001 Å. Most of the calculated values are within 0.015 Å of the available experimental values.⁵

Normal mode vibrational frequencies were computed with the HF/6-31G* and MP2/6-31G** equilibrium structures. The MP2 and HF harmonic vibrational frequencies are larger than the experimental frequencies.⁵ The greatest overestimation is 322 cm^{-1} for F_2 ; the others are all within 250 cm^{-1} .

The CID and MP2 equilibrium structures were used to compute single - point energies with fourth (MP4SDTQ) order Møller-Plesset theory.⁴ Reaction energies were corrected for zero-point energies, basis-set superposition errors, the pressure-volume work term and temperature effects.⁴ The ZPEs were scaled by 0.89 (HF/6-31G*) and 0.93 (MP2/6-31G**) ⁴

Calculated and experimental⁶ reaction enthalpies for eight representative gas phase reactions are listed in Table I. Forty reactions were examined in all. The reactions have been divided into four categories. The reactions in category 1 (reactions 1 and 2) are isogyric *and* the reactant and product pairs are isoelectronic. In isogyric reactions the number of unpaired electrons is identical on both sides of the equation. The reactions in category 2 (reactions 3 and 4) are isogyric *and* the reactant and product pairs are valence isoelectronic. The reactions in category 3 (reactions 5 and 6) are *only* isogyric. The reactions in category 4 (reactions 7 and 8) are *not even* isogyric.

The deviations in the MP4/6-31G** and MP4/6-311G(2df,2pd) enthalpies of reaction for category 1 are generally small, ranging from about 0.5 - 3 kcal/mol. With two exceptions, both basis sets yield $\Delta H^\circ_{\text{calc}}$ values within 2.5 kcal/mol of $\Delta H^\circ_{\text{exp}}$. For the reactions in categories 2 and 3, the situation is less promising. The deviations in the MP4/6-31G** and MP4/6-311G(2df,2pd) enthalpies are as large as 11 kcal/mol. The MP4/6-311G(2df,2pd)

enthalpies of reaction are generally within 3.5 kcal/mol of the experimental enthalpies; the MP4/6-31G** values differ from the experimental values by up to 16 kcal/mol. The worst results are obtained for the nonisogyric reactions (category 4). The MP4/6-31G** enthalpies differ from the MP4/6-311G(2df,2pd) enthalpies by as much as 17 kcal/mol. The deviations in the experimental and MP4/6-311G(2df,2pd) and MP4/6-31G** enthalpies of reaction are as large as 10 and 25 kcal/mol, respectively.

Table I. Reaction Energies (kcal/mol)

Reaction ^a	Basis ^b	ΔE	$\Delta H^\circ_{\text{calc}}^c$	$\Delta H^\circ_{\text{exp}}^d$
1. $\text{Ar} + \text{HCl}^+ \rightarrow \text{ArH}^+ + \text{Cl}$	MP4/6-31G**	34.7	34.7	34.1
	MP4/6-311G(2df,2pd)	32.0	32.0	
2. $\text{Ar} + \text{HF}^+ \rightarrow \text{ArH}^+ + \text{F}$	MP4/6-31G**	-6.7	-7.1	-8.9
	MP4/6-311G(2df,2pd)	-8.4	-8.8	
3. $\text{Cl}_2 + \text{HF} \rightarrow \text{HCl} + \text{ClF}$	MP4/6-31G**	19.9	18.8	31.0
	MP4/6-311G(2df,2pd)	29.6	28.5	
4. $\text{Cl}_2 + \text{HF}^+ \rightarrow \text{HCl}^+ + \text{ClF}$	MP4/6-31G**	-51.1	-51.2	-45.0
	MP4/6-311G(2df,2pd)	-43.1	-43.2	
5. $\text{ClF} + \text{H} \rightarrow \text{Cl} + \text{HF}$	MP4/6-31G**	-77.1	-72.8	-76.2
	MP4/6-311G(2df,2pd)	-81.8	-77.3	
6. $\text{ClF} + \text{H} \rightarrow \text{F} + \text{HCl}$	MP4/6-31G**	-46.5	-43.6	-43.2
	MP4/6-311G(2df,2pd)	-49.5	-46.4	
7. $\text{F}_2 \rightarrow 2 \text{F}$	MP4/6-31G** ^e	28.2	27.5	38.0
	MP4/6-311G(2df,2pd)	33.9	33.5	
8. $\text{H}_2 + \text{F}^+ \rightarrow \text{HF} + \text{H}^+$	MP4/6-31G**	-96.6	-97.0	-120.1
	MP4/6-311G(2df,2pd)	-111.3	-111.8	

^aAll reactants and products are gas phase species. ^bMP4/6-31G**/CID/6-31G** or MP4/6-311G(2df,2pd)/MP2/6-31G** calculations. ^c ΔE corrected for the zero-point energy, temperature term, and pressure-volume work term differences. ^dReference 6. ^eMP4/6-31G** and MP4/6-311G(2df,2pd) energies corrected for BSSE.

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CROSSED-BEAM INVESTIGATIONS OF $\text{Ar}^+ (2P_{3/2}) / \text{O}_2 (X^3\Sigma_g^-, v=0)$ CHARGE TRANSFER AT 2.75 AND 5.70 eV CM COLLISION ENERGY: Julie A. Biggerstaff and Jean H. Futrell, Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

INTRODUCTION

Crossed beam experiments performed at 2.75 and 5.70 eV CM (center of mass) collision energy on the Ar^+/O_2 charge transfer system show that the coupled complex reaction surfaces result in behavior anomalous to most other charge exchange reactions previously studied. The O_2^+ formed at 2.75 eV, found in all three available product surfaces, is produced by both long and short range collisions. At the higher energy the most exothermic product channel disappears. The role of Frank-Condon factors, energy resonance principles, and direct and intimate collision mechanisms in Ar^+/O_2 charge transfer and the effect of translational energy on these parameters is examined.

EXPERIMENTAL

The crossed beam instrument used here has been described in great detail elsewhere.¹

DISCUSSION

Our experiments at 2.75 and 5.70 eV demonstrate that charge exchange in the $(\text{Ar}-\text{O}_2)^+$ system is rather complex. This system differs from many previously-studied charge transfer systems in that there are five potential energy surfaces (neglecting vibrational states) to provide multiple reaction pathways. Clearly this plays a most significant role in the $(\text{Ar}-\text{O}_2)^+$ system. Specifically, the $\text{Ar}^+ (2P_{3/2}, 2P_{1/2})$ surfaces are capable of interacting with both the quartet ($a^4\Pi_u$) and doublet ($X^2\Pi_g$ and $A^2\Pi_u$) O_2^+ product surfaces of the reactant $\text{O}_2 (X^3\Sigma_g^-, v=0)$ ground state surface. As is seen from the scattering diagrams O_2^+ is formed along all three product surfaces.

The mechanisms responsible for the formation of O_2^+ in these product states cannot be rationalized on the basis of any simple theory. Concepts of energy resonance (or energy defect), the Frank-Condon (FC) principle and elements of both long range and intimate collision processes all appear to play a role in Ar^+/O_2 charge transfer.

Figure 1 represents the scattered O_2^+ product from charge transfer with argon at 2.75 eV CM collision energy. Within the ground potential surface ($X^2\Pi_g$) the O_2^+ product is found scattered at large angles and in highly excited vibrational levels (ie., found predominantly in $v \geq 12$). The $v=19$ to $v=22$ level of the X^2_g state are nearly energy resonant with the $\text{Ar}^+ (2P_{3/2})$ state. However, the FC overlap probability of the $\text{O}_2 (X^3\Sigma_g^-, v=0)$ with these levels is on the order of $10^{-22.2}$. Such a poor overlap implies that an electron jump from the $\text{O}_2 (X^3\Sigma_g^-)$ ground state is highly unlikely. A small impact parameter collision is inferred from the large scattering angles, indicative of large angular momentum exchanges.

Kato suggests that the doublet potential product surfaces ($X^2\Pi_g$ and $A^2\Pi_u$) of O_2 interact and stabilize the $\text{Ar}^+ (2P_{3/2}$ and $2P_{1/2})$ until small internuclear distance typical of intimate collisions are reached. At this point a non adiabatic transition (ie. a Landau-Zener type transition) occurs at an avoided curve crossing.² He concludes that FC overlap and energy resonance principles should not influence the outcome of these intimate collisions since at small internuclear separations these curves are highly perturbed. This interpretation is supported by the observation of Marx et. al. They found from ICR experiments that O_2^+ is formed predominantly in the $v=9$ level in the ground state for near thermal collision energies.³ In contrast with these predictions, our results demonstrate a definite preference for forming the $X^2\Pi_g$ product in vibrational levels nearly resonant with the $2P_{3/2}$ state even though the $v=0$ level is exothermic by ≈ 3.7 eV.

The $\text{O}_2^+ a^4\Pi_u$ product formed in 2.75 eV collisions has a maximum in intensity on the relative velocity vector at $v=0$ and again in $v \approx 4 - 5$. In addition, a significant amount of intensity is found scattered at large CM angles (Θ). After considering the $\sin(\Theta)$ factor in the conversion of

the plotted differential cross sections into total reaction probabilities, it is clear that the dominant reaction channel is formation of O_2^+ ($a^4\Pi_u$). This surface lies closer in energy to the $Ar^+(^2P_{3/2})$ than any other electronic surface. Thus it has the smallest energy defect and is the most probable surface for formation of O_2^+ product. The FC factor for the $v=0$ level is about 8×10^{-3} , rising to a maximum of 0.35 at the $v=5$ level.⁴ Since the product is formed with both zero and a range of scattering angles we infer it is formed over a large range of impact parameters and is a result of both short and long range interactions.

The O_2^+ ($A^2\Pi_u$) channel exhibits scattering behavior very similar to that seen for the O_2^+ ($a^4\Pi_u$). However, the $A^2\Pi_u$ product is both lower in intensity and formed predominantly in the $v=0$ state. These differences can be rationalized on the basis of both reduced overlap and by the larger energy defect for this product surface relative to that for the O_2^+ ($a^4\Pi_u$).

At 5.7 eV CM collision energy (figure 2) the scattered O_2^+ product exhibits mechanistic behavior similar to that observed for both the $a^4\Pi_u$ and $A^2\Pi_u$ product surfaces at 2.75 eV, with the exception that product scattered from the relative velocity vector is more structured and of somewhat lower intensity. Additionally there is very little, if any, O_2^+ ($X^2\Pi_g$) product. Clearly translational energy is a very important variable in the Ar^+/O_2 charge transfer system.

For Ar^+/O_2 charge transfer it can be concluded that the extent of FC overlap and the size of the energy gap play an important role in both short- and long-range collisions. The extent of influence these variables have on intimate collisions is clearly related to the relative translational energy of the collision. It appears that the greater the interaction time available the more probable it is to yield product from more highly perturbed surfaces. Therefore the lower the relative translational energy, the less influence FC factors and energy resonance will have on the outcome of small impact parameter collisions.

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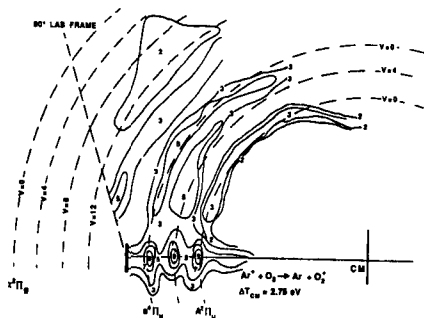


Figure 1

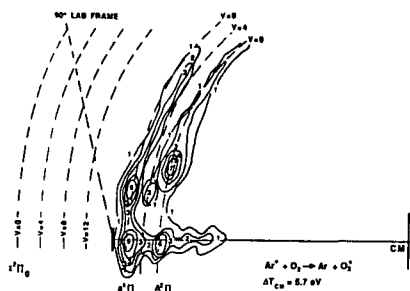


Figure 2

CROSS-SECTION FOR PROTON TRANSFER FROM LARGE BIOLOGICAL IONS TO SMALL MOLECULES IN THE GAS PHASE

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The enormous body of literature on the subject of ion-molecule reactions concerns almost exclusively ions of relatively low masses [1,2]. We report results of a study of proton transfer between ions of the cyclic peptide valinomycin (relative molecular mass 1110.6) and ammonia molecules. The ion beam set up used in this work is shown in Figure 1. Collisions of valinomycin ions with ammonia molecules were carried out in the source region of a large-scale reverse-geometry mass spectrometer. Valinomycin ions were produced by field desorption (FD). Collisions of these ions were carried out without prior mass selection. The electrostatic lens system designed especially for these experiments [3] is shown in Figure 1. The performance of the lens system was evaluated from a detailed study of the well characterised reaction between $[\text{NH}_3]^+$ and NH_3 [4-11].

The rates of reaction were determined as a function of incident $[\text{NH}_3]^+$ energy, and from comparison with literature data used to draw up a correction curve accounting for the transmission characteristics of the lens system. The structure of the correction curve can be explained in terms of ion-optical effects [3].

An initial difficulty in the valinomycin experiments was interference from $[\text{NH}_3]^+$ generated in the FD source from a background pressure of NH_3 . The problem was alleviated by using ND_3 instead of NH_3 . In this case, $[\text{ND}_4]^+$ will be formed from $[\text{ND}_3]^+$, whereas $[\text{ND}_3\text{H}]^+$ will be formed by proton transfer from valinomycin ions. The yields of $[\text{ND}_3]^+$ and $[\text{ND}_3\text{H}]^+$ from the collision cell were monitored using ion counting techniques. The collision cell pressure of ND_3 was 1.3×10^{-3} torr.

RESULTS

Results are shown in Figure 2 for the percentage yields of $[\text{ND}_3\text{H}]^+$ as a function of incident valinomycin ion energy. There is a maximum yield of $[\text{ND}_3\text{H}]^+$ of 0.07 + or - 0.015% at an ion kinetic energy of about 1500 eV (cell voltage 6500). After correcting for losses of $[\text{ND}_3\text{H}]^+$ ions due to extraction at the front of the collision cell (i.e. in the reverse direction to the source slit) and losses due to the reaction $[\text{ND}_3\text{H}]^+ + \text{ND}_3 \rightarrow [\text{ND}_4]^+ + \text{NHD}_2$ in the time taken for extraction out of the cell, this figure becomes 0.13 + or - 0.03%. The cross-section for proton transfer from valinomycin to ammonia calculated using this value is $3.0 + \text{or} - 0.5 \times 10^{-17} \text{ cm}^2 \text{ molecule}^{-1}$. The measured cross-section for proton transfer from the $[\text{NH}_3]^+/\text{NH}_3$ experiments was $4.9 + \text{or} - 0.6 \times 10^{-18} \text{ cm}^2 \text{ molecule}^{-1}$ (for ion energy 1 eV). The measured cross-section for collision induced dissociation (CID) of the valinomycin ion was $1.5 \times 10^{-14} \text{ cm}^2 \text{ molecule}^{-1}$.

DISCUSSION

It has been assumed that the proton transfer observed has been due to the $[\text{M} + \text{H}]^+$ ion of valinomycin. It is possible that transfer also occurs from the $[\text{M}]^+$ ion of valinomycin. Since $[\text{M} + \text{H}]^+$ and $[\text{M}]^+$ are produced in about equal intensities, the experimental cross-section would then represent an average of the cross-sections for these ions.

In order for a proton transfer reaction to occur, it could be argued that the ND_3 molecule would have to interact with a specific part of the valinomycin ion where the proton is situated. Interaction with other parts of the ion structure could be expected to be more likely to result in CID processes. The measured cross-section for proton transfer from $[\text{NH}_3]^+$ to NH_3 ($4.9 + \text{or} - 0.6 \times 10^{-18} \text{ cm}^2 \text{ molecule}^{-1}$) is much larger than the measured cross-section for proton transfer from valinomycin ions to ND_3 ($3.0 + \text{or} - 0.5 \times 10^{-17} \text{ cm}^2 \text{ molecule}^{-1}$). The greater momentum in the case of a valinomycin ion (a factor of 8 larger than the momentum of an ammonia ion with the same translational energy) would inhibit orbiting and complex formation. Shielding effects from other parts of the ion structure would also be larger in the case of valinomycin ions.

Apart from the differences in magnitudes of the cross-sections for proton transfer from valinomycin and from ammonia, a major difference arises in the ion kinetic energies at which the cross-sections attain their maximum values. For ammonia the maximum occurs at room temperature. The cross-section at 1 eV ion kinetic energy is lower by a factor of about 0.85. For valinomycin the maximum cross-section occurs at 1500 eV ion energy. Consider an $[\text{NH}_3]^+$ ion travelling with 0.1 eV kinetic energy and interacting with an NH_3 molecule. The maximum amount of energy available for conversion into internal energy is 0.05 eV. Since $[\text{NH}_3]^+$ has 6 vibrational modes the maximum amount of energy available per vibrational mode is 0.008 eV. As some indication of the interaction time, the time taken for the $[\text{NH}_3]^+$ ion to travel 3 Å can be calculated i.e. a distance equal to the size of the ion. At 0.1 eV kinetic energy, $[\text{NH}_3]^+$ takes 3×10^{-13} seconds to travel 3 Å. Consider now an $[\text{M} + \text{H}]^+$ ion of valinomycin travelling with 1500 eV kinetic energy and interacting with an ND_3 molecule. The maximum amount of energy available for conversion into internal energy is 26.5 eV. $[\text{M} + \text{H}]^+$ of valinomycin has 501 vibrational modes, giving a maximum amount of energy available per mode of 0.05 eV. This is a similar magnitude to a typical vibrational quantum (0.1 eV). The interaction time with an amine group of $[\text{M} + \text{H}]^+$ of valinomycin can be estimated by calculating the time taken for the ion to travel 3 Å (1×10^{-14} seconds). This interaction time is of the same order of magnitude as a typical vibrational period (10^{-14} seconds).

CONCLUSION

In view of the results for proton transfer from valinomycin to ammonia, it would seem that the cross-sections for ion-molecule reactions between large ions and small molecules are very small. A "clean" interaction at the position necessary for reaction is apparently required, otherwise the ion will tend to fragment. The maximum amount of energy available per vibrational mode, at the ion energy giving the maximum cross-section for proton transfer to ammonia was found to be greater for valinomycin ions than for ammonia ions. The amount of this energy actually converted into internal energy of the ion would be expected to depend on the structure of the ion as a whole. In the case of valinomycin the interaction time is very short, and sufficient energy would need to be available in the appropriate vibrational modes in the collision. The ion kinetic energy giving the maximum cross-section for an ion-molecule reaction between a large multiatomic ion and a small molecule presumably reflects balancing conditions where there is both sufficient energy for conversion into internal energy in the appropriate bonds of the ion and a sufficiently long interaction time. This being the case, it might be expected that the larger is the ion, the smaller would be the cross-section for an ion-molecule reaction.

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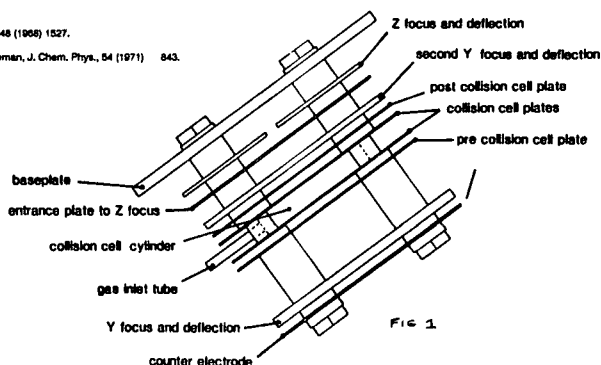


FIG 1

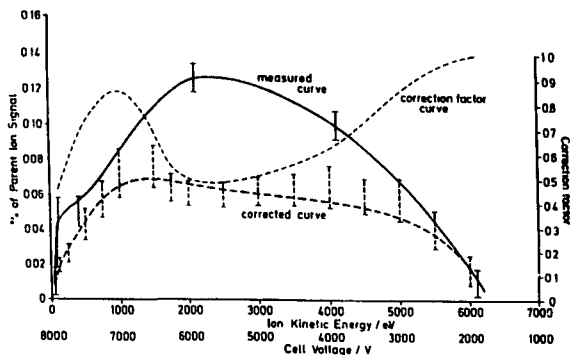


FIG 2 Yield of $(M+H)^+$ product ions from the collision cell as a function of kinetic energy of the incident $(M+H)^+$ ions of valinomycin. The pressure of ND_3 in the collision cell was 1.3×10^{-5} torr.

FAB INDUCED CONDENSATION OF GLYCEROL WITH TETRAALKYL AMMONIUM SURFACTANTS.

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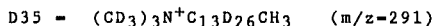
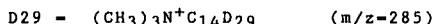
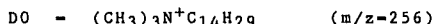
INTRODUCTION

The FAB spectrum of the tetraalkyl ammonium cation "D0" (structure below), from a glycerol matrix shows numerous peaks at higher mass than the naked analyte at m/z 256. These result from the reaction of radical glycerol artifacts with the analyte in the liquid phase or in the selvedge. An understanding of the reaction mechanism governing formation of these adducts may provide insight into the mechanism of the FAB process itself.

Keough¹ has concluded that $[M+14]^+$ of cetyl pyridinium chloride is formed by methylation at the para-position of the aromatic "head" following primary hydrogen atom abstraction from that site.

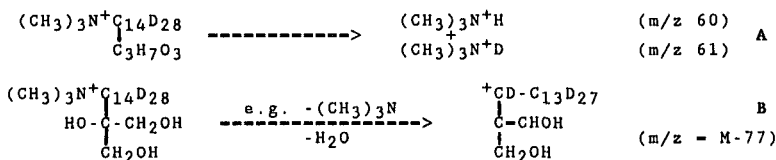
RESULTS AND DISCUSSION

The abundant oxygen-containing adducts of "D0", and its deuterium labelled analogues "D29" and "D35" were examined to detect possible regio-selectivity of glycerol (G) adduct formation.

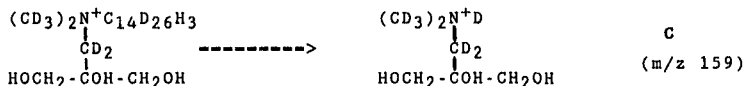


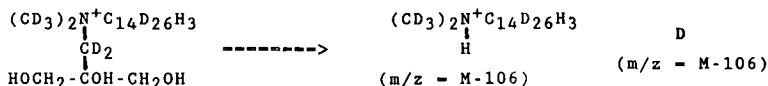
For the chain labelled surfactant (D29), the most abundant adduct peak (m/z 374) represents the addition of 89 Daltons, indicating loss of one deuterium, and predominant attack at the long chain of the surfactant. The adduct therefore has the composition D29+G-HD. The abundance of m/z 375 (58% of 374) is more than twice that expected for ^{13}C of m/z 374. This indicates competitive attack at N-CH₃ (producing D29+G-2H).

Low energy CID (10 eV collision with Argon on a hybrid VG-ZAB-EQ) of the glycerol adducts for all three products produced daughter spectra interpretable because of the known original position of the isotopes. Two of the prominent daughter masses derive from glycerol attack at the long chain, as illustrated by reactions A and B for the D29 adduct (D29+G-HD). The excess of hydrogen migration over deuterium (60>61 for D29 and 69>70 for D35) indicates attachment close to N (possibly at the alpha carbon).

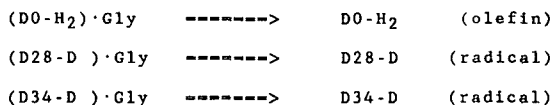


Reactions C and D below indicate parents with glycerol attached at an N-methyl group, as illustrated for the D35+G-HD parent.





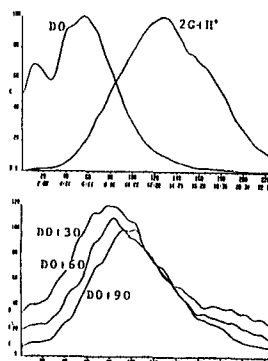
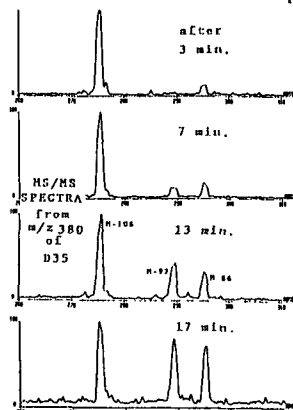
Abundant daughter peaks at M-92 in all three spectra cannot be explained as the loss of neutral glycerol from the adducts mentioned above, since the D35 product would have to lose 93 Daltons. Instead, decomposition of complexes formed between neutral glycerol and surfactant radical or olefin are invoked. These complexes are isobaric or isomeric with the "normal" adducts.



D28 and D34 refer to surfactant with imperfect labelling; e.g. $(\text{CH}_3)_3\text{N}^+\text{C}_{14}\text{D}_{28}\text{H}$. These account for up to 30% of the surfactant present. The mislabelling is known² to be random.

Adduct formation is TIME DEPENDENT:

- 1) Relative intensities of certain daughter ions vary dramatically with FAB irradiation time (Fig. 1). This reflects a change in the relative abundances of different isobaric and isomeric parents with FAB irradiation time.
- 2) When the FAB target is loaded with a thin film of D0 plus glycerol, the intensity of adduct ions passes through a maximum over time (Fig. 2). This effect is less pronounced or not observed when the mixture is applied as a thicker film or as a convex droplet.



CONCLUSIONS

1. Adduct formation is not regio-specific, and ions at the nominal masses representing the adducts are not homogenous.
2. The relative abundance of different glycerol adducts varies as a function of FAB irradiation time.

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FT-ICR Determination of the Ionization Potentials
of Carbon Clusters via Charge Transfer

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Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry is an ideal method for studying the reactivity of cluster ions. These ions can either be formed externally and injected into the FT-ICR analyzer cell, or formed directly in the cell by laser ablation from suitable solids. In either case, the use of a pulsed valve to introduce a burst of unreactive collision gas (argon, 7.5×10^{-7} torr peak pressure) to remove excess translational and/or internal energy in the cluster ions is desirable. We have begun our FT-ICR studies of the properties of cluster ions by examining charge transfer reactions of carbon cluster cations in order to determine the ionization potentials of neutral carbon clusters. An accurate determination of the I.P.'s of the clusters may also lead to a better understanding of the structures of these species and may help resolve the question as to the cluster size at which the transition from cyclic to linear structure occurs. Previous photoionization work by Kaldor and co-workers led to I.P.'s for carbon clusters with up to seven atoms in the range 12.84 to 9.98 eV and between 12.84 and 6.42 eV for clusters with up to 30 atoms¹.

A variety of compounds have been employed, for charge transfer reactions, with I.P.'s ranging from 7.50 to 11.00 eV². Most of these neutrals are substituted benzenes. These molecules ionize via one of the delocalized electrons in the aromatic cloud, and often do not rearrange structurally upon ionization. This means that the ionization energy of the neutral does not need to be corrected because of structural changes between the neutral and the ion. These compounds are leaked into the system at a pressure of $1-2 \times 10^{-8}$ torr above the background pressure. By observing the occurrence or non-occurrence of charge transfer reactions with the neutral molecules of known ionization potential, the ionization potentials of carbon clusters containing from 6 to 24 atoms have been determined to an uncertainty of only a few tenths of an eV. This has substantially lowered the uncertainties in the I.P.'s of the carbon clusters.

The accompanying graph shows (black bars) the ionization potential range determined for each carbon cluster. The area above this (light hash marks) is where no charge transfer occurs and therefore the ionization potential of the carbon cluster is lower than that of the neutral species being leaked in. The area below the black bars (heavy hash marks) is where charge transfer between the carbon cation and the neutral charge transfer agent is observed and the ionization potential of the cluster is then greater than that of the neutral being leaked in. A general decrease of the carbon cluster ionization potential with increasing number of atoms in the cluster is seen, but pronounced differences from one cluster to the

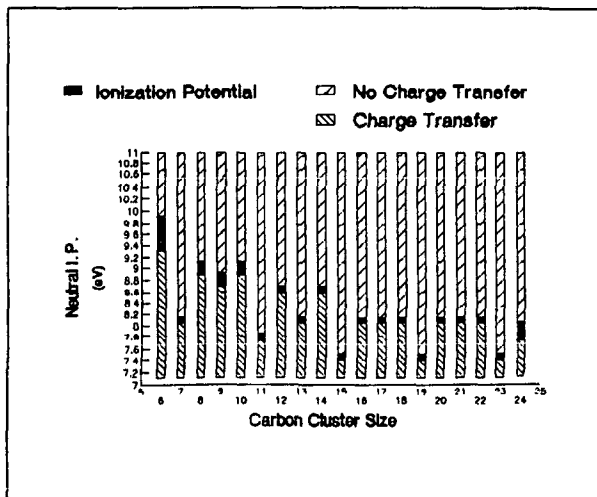
next are also apparent. These differences could be the result of significant structural variation between the clusters.

The lower ionization potentials seen for clusters with 7 and 11 atoms relative to the I.P.s for clusters with 6, 8, and 10 atoms supports earlier speculation³ that the former have cyclic structures, while the latter have linear structures. The low ionization potentials seen for clusters with 11, 15, 19, and 23 atoms suggest that the higher intensities of these clusters ("magic numbers") found in earlier experiments¹ by other groups may not be due to any special stability of these particular size clusters, but rather is due to the ease of ionizing these clusters relative to those with more or fewer carbon atoms. The relatively lower I.P. of these cluster may also indicate that these species are aromatic.

Experimental Results

Cluster	I.P. (eV)
C7	8.07±0.1
C8	9.00±0.2
C9	8.79±0.2
C10	9.00±0.2
C11	7.85±0.3
C12	8.63±0.1
C13	8.07±0.1
C14	8.63±0.1
C15	7.4*
C16	8.07±0.1
C17	8.07±0.1
C18	8.07±0.1
C19	7.4*
C20	8.07±0.1
C21	8.07±0.1
C22	8.07±0.1
C23	7.4*

* lower limit is not firmly established.



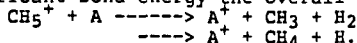
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A CHARGE TRANSFER REACTION OF EXCITED CH_5^+

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CH_5^+ is used extensively as a proton transfer chemical ionization reagent, but it is energetically possible for CH_5^+ to undergo charge transfer with some molecules. Since CH_5 neutral does not have a significant bond energy the overall reaction would be



The enthalpy change for either process would be $\text{IP}(\text{A}) - 7.89\text{eV}$. Thus species with low IP might undergo charge transfer with CH_5^+ . It is of interest to know how successfully charge transfer competes with proton transfer.

The para magnesium bromide of anisole effectively immobilizes active H and D substituents and can be used to determine the extent of D for H exchange in solution. Detecting D substituted anisole is therefore important. This led us to a particular interest in the methane CI mass spectrum of anisole. Anisole has a relatively low ionization potential, and if charge transfer competes with proton transfer that would complicate the analysis of mixtures of anisole and D-anisole. Another problem is that proton transfer from CH_5^+ to D-anisole accompanied by H for D exchange would be indistinguishable from charge transfer except with very high mass resolution.

We report here that charge transfer is the dominant product where A is anisole. Furthermore this reaction is observed only for CH_5^+ that has not suffered collisions after it has been formed in the reaction of CH_4^+ and CH_4 . This is a reaction of excited CH_5^+ and is not observed for collisionally cooled species. Indeed the reaction is only exothermic for excited reactants since $\text{IP}(\text{anisole}) = 8.20\text{ eV}$. The reaction was identified using labelled species and a Nicolet FTMS-2000 ion cyclotron resonance spectrometer. This makes it possible to do a kinetic analysis of the effect of collisional cooling on the competition between charge transfer and proton transfer.

The reactions of CD_5^+ with anisole produce anisole parent ion (m/z 108) and deuterated parent (m/z 110). If isotopic exchange accompanied deutron transfer and ion at m/z 111 would be expected. The m/z 111 intensity is just that appropriate for the ^{13}C peak of the deuterated parent. Any exchange must be less than 1% of the deutron transfer process.

To monitor the effect of collisional relaxation of CD_5^+ on its chemistry with anisole a two cell experiment was done. Methane was admitted to the source cell at a nominal pressure of 2×10^{-7} Torr. A 5 ms electron beam pulse formed CD_4^+ which reacts for time T_1 with methane. The resulting CD_5^+ is transferred to the analyzer cell which has a nominal background pressure of 3×10^{-9} Torr. All ions but m/z 22 are ejected and anisole is admitted through a computer controlled valve. After a delay of 1 s a mass spectrum is acquired. Plotted in the figure is the relative

intensity of the observed anisole parent ion as a function of T_1 . At short time the parent ion is the only product of reaction between CD_5^+ and anisole indicating that nearly all the CD_5^+ has enough energy (0.3 eV) to undergo charge transfer. At long times after the CD_5^+ has had an opportunity to collide before it is transferred to the analyzer, charge transfer disappears in favor of proton transfer.

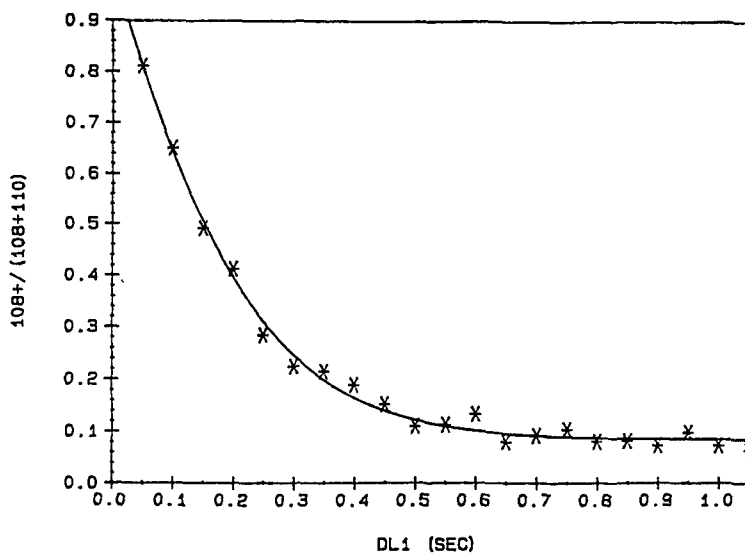


FIGURE 1

ON THE USE OF NEGATIVE ION CLUSTERING BY DMSO FOR
THE ANALYSIS OF SUBSTITUTED NITROBENZENES BY APIMS:

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The use of electron capture (EC) reactions in the ion source of an Atmospheric Pressure Ionization Mass Spectrometer (APIMS) provides the basis for the sensitive detection of numerous environmentally interesting compounds, including the nitroaromatic hydrocarbons. The mass spectra thereby obtained are very simple, often consisting of the molecular anion, only, of the compound of interest.



In many cases, however, it would be desirable to have spectra which are more complex and contain more information concerning the identity of the analyte. In this study we have explored the use of dimethylsulfoxide (DMSO) in the buffer gas of an APIMS in order to cluster with the molecular anion, as shown in reaction 2.

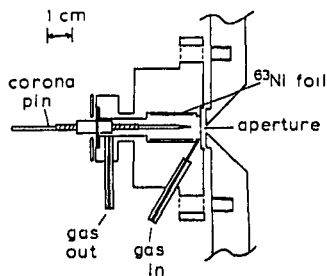


Along with the initial electron capture product formed by reaction 1, the cluster ion has been shown to be a potential source of additional information.

Chowdhury and coworkers have recently reported a collection of thermodynamic parameters for clustering processes involving several isomeric sets of substituted nitrobenzenes. That study showed that a particular structural isomer may exhibit a unique, distinguishing propensity for clustering with a given solvent. (see Ref. 1)

We likewise investigated the majority of compounds studied by Chowdhury et al which had been found to cluster most favorably with DMSO. We found that, despite the free jet expansion conditions associated with ion sampling from our APIMS that with use of He buffer gas and a 50 um sampling orifice, the clustering chemistry clearly followed known trends characteristic of the electron affinity of the compound tested. Those APIMS results are shown in Figure 1.

APIMS Ion Source



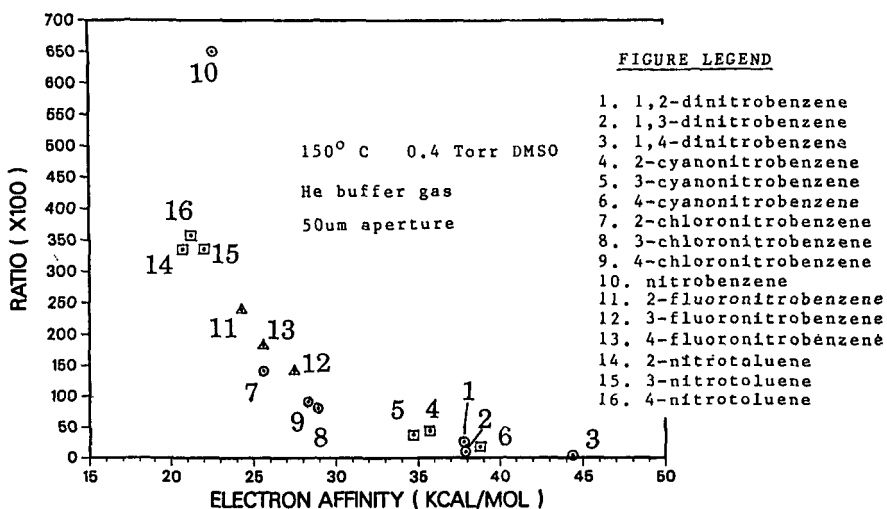
continued

Through the course of our study we found generally that the pre-sampling isotopic stability patterns were mirrored at a standard condition of 150°C, and 0.4 Torr DMSO. An exception appeared to exist for the fluoronitrobenzenes. This non-equilibrium isotopic pattern was however quite reproducible, and was found to be invariant with changes in the partial pressure of DMSO. The effect of temperature was also found to be ineffective at altering the APIMS isotopic pattern for the fluoronitrobenzenes, from 125 to 225°C. The effect of temperature must be more carefully examined prior to making generalizations, however.

Use of a 50um aperture with He appeared to result in enhancement of the cluster ion while providing the same isotopic pattern. Due to the very temperature sensitive "DMSO" this enhancement attributed to adiabatic cooling was apparently insufficient to greatly increase cluster ion signal at higher temperatures, although significant enhancement is achieved at temperatures below 100°C.

The use of negative ion clustering appears to be of a controllable nature under carefully controlled conditions, and may be of analytical utility for providing additional information for isomer identification in APIMS.

OBSERVED CLUSTERING RATIO VS. EA



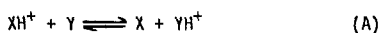
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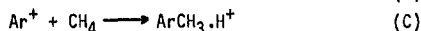
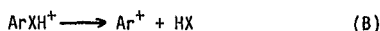
PROTON TRANSFER AND HYDROGEN HALIDE ELIMINATION
REACTIONS OF HALOGENATED BENZENES

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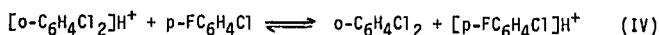
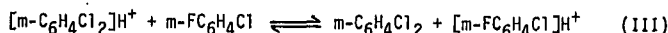
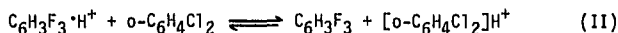
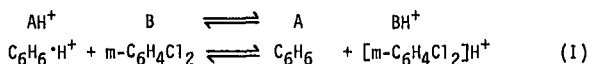
In the study of proton transfer equilibria of the type



in a pulsed electron beam high pressure ion source, it is essential to ensure that competing reactions do not interfere with the establishment of equilibrium. In systems containing partly halogenated benzenes the major competing reaction is loss of HX (X = halogen) from the protonated species. The mechanism of this reaction has been determined by Harrison and co-workers¹:



This reaction has been studied as a function of temperature over the range 350 - 600 K. No HF is lost from $[\text{o-C}_6\text{H}_4\text{F}_2]\text{H}^+$ but HF loss from the meta and para isomers increases with temperature. This may be rationalised in terms of site of protonation. In the ortho isomer, the proton is bound between the two fluorine atoms to form a stable ion. In the meta and para isomers, protonation occurs at one of the fluorine atoms or directly on the ring and as the temperature rises, HF loss becomes more favourable. For the dichlorobenzenes, no HCl loss was observed, due to unfavourable energetics. For the chlorofluorobenzenes, HF loss is preferred over HCl loss. The ortho isomer shows greatest loss and increases with temperature for all isomers. Proton affinities of four dihalogenated benzenes were determined from the following systems:



	$\Delta H^\circ/\text{kJ mol}^{-1}$	$\text{PA(B)}/\text{kJ mol}^{-1}$	$\Delta S^\circ/\text{JK}^{-1} \text{ mol}^{-1}$	$\Delta S_{\text{calc}}/\text{JK}^{-1} \text{ mol}^{-1}$
(I)	-2.24 $\pm$ 1.0	756.3	-13.09	-14.9
(II)	-5.2 $\pm$ 0.9	751.3	-11.85	- 3.37
(III)	-1.5 $\pm$ 0.5	754.8	- 0.2	0
(IV)	-10.6 $\pm$ 1.8	740.6	-18.5	0

Absolute proton affinities were determined from $\text{PA}(\text{benzene}) = 758.5 \text{ kJ mol}^{-1}$.
Entropy changes were calculated using the relationship

$$\Delta S_{\text{calc}} = R \ln \frac{\sigma_A \sigma_{\text{BH}^+}}{\sigma_B \sigma_{\text{AH}^+}} \text{ where } \sigma_x = \text{symmetry number}$$

of species X. The entropy changes determined constitute supporting evidence for the dynamic protonated arene ion model, in which the proton has a degree of mobility within the plane of the ring, proposed by Fernandez et al². The results indicate that in meta disubstituted compounds, protonation occurs at a specific site ortho to one substituent and para to the other. For ortho and para disubstituted compounds there is no specific site of protonation.

¹ A G Harrison et al J.A.C.S. 1978, 100, 2479 - 2484.

² M T Fernandez, K R Jennings and R S Mason, J. Chem. Soc., Faraday Trans 2., 1987, 83, 159 - 171

DIFFERENTIATION OF OLEFINS AND NAPHTHENES USING
ION-MOLECULE REACTIONS IN THE COLLISION CELL OF A
HYBRID MASS SPECTROMETER

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Collisionally activated reactions between selected parent ions and isoprene were used to distinguish olefins and naphthenes commonly found in petroleum products. Parent ions selected by the EB analyzer of a hybrid mass spectrometer (EBQQ configuration) were introduced into the quadrupole collision cell at low energy and subjected to collisional activation with isoprene target gas. The addition products thus formed were subsequently mass separated in the quadrupole analyzer. A substantial increase in the slope of the ion energy ramp was required to detect addition products, due to the relatively low velocity that these compounds possess following collision (Figure 1). Previous studies (1) with vinylethyl ether and butadiene suggested that the reaction between an olefin and diene proceeds via a gas phase Diels - Alder mechanism.

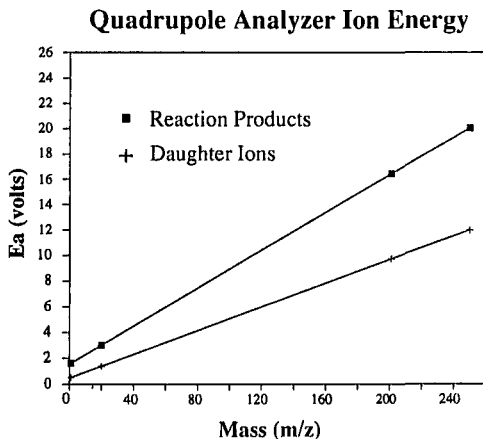


Figure 1. Ion energy program ramps required for
detection of collision products

Both olefinic and naphthenic hydrocarbons were found to produce addition products with isoprene. Naphthenes such as cyclohexane are known to undergo ring opening upon ionization in the source and produce radical-ions similar to olefins. Parent ions thus formed would be expected to have different internal energies than those formed by olefins, and could be expected to yield dissimilar reaction spectra. Figure 2 clearly shows the marked differences between relative ion intensities of major reaction products formed by isomeric olefins and naphthenes.

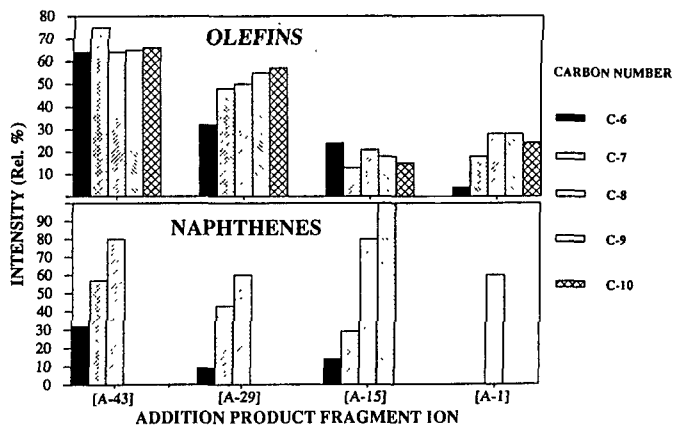


Figure 2. Major addition product fragment ions from collisional reaction with isoprene. Intensities normalized to [A-57] ion.

Discriminant analysis was used to determine if sufficient differences exist between reaction spectra to enable differentiation of olefins and naphthenes (Figure 3). Of the compounds examined, only the C-7 isomers were difficult to distinguish. All olefins examined were grouped in a narrow region of the graph, while naphthenic isomers tended to be widely distributed. Additional studies will need to be performed to determine the reactivity of other chemical species such as tetralins and polynuclear aromatic hydrocarbons.

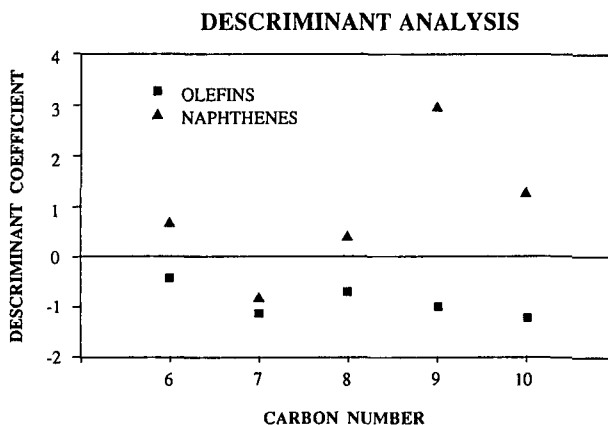
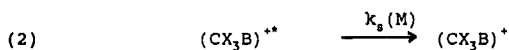
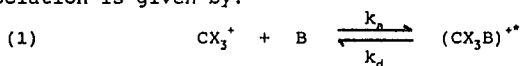


Figure 3. Discriminant analysis of addition product spectra from olefins and naphthenes.

GAS PHASE ION-MOLECULE REACTIONS OF CARBOCATIONS:
RADIATIVE ASSOCIATION REACTIONS.

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Radiative association reactions are of central importance in models of interstellar chemistry due to the low number densities that preclude synthesis of large ions by three-body association. The amount of experimental evidence supporting radiative association is, at best, minimal due to the low temperatures and pressures that are required experimentally to allow the ruling out of three-body association. The general ion-molecule association is given by:



where k_a is the rate coefficient for association, k_d is the rate coefficient for unimolecular decay, k_s is the rate coefficient for collisional stabilization, and k_r is the rate coefficient for radiative stabilization. Recently halide ion additions to the boron trihalides^{1,3} have been shown to possess a radiative stabilization component that competes with the three-body process and unimolecular decay back to reactants even at the relatively high pressures of the flowing afterglow and SIFT experiments. While BX_3 is not an important interstellar species it is interesting to note that CX_3^+ is isoelectronic with BX_3 and carbocations are some of the most important species found in space. We have studied the pressure and temperature dependencies of the reactions of CX_3^+ ($X=H,D,F,Cl$) with SO_2 and NO . The energetics of the reactions calculated from ionization potentials⁴ are given in table I.

Table I. Energetics of Reactions Studied

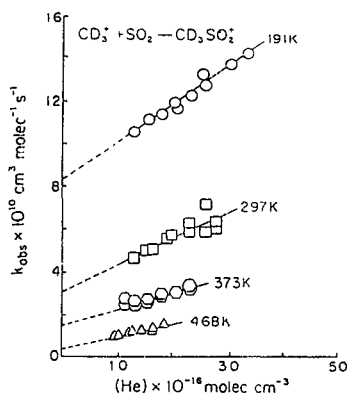
	A^+	$CH_3^+ + B$	$CD_3^+ + B$	$CF_3^+ + A$	$CCl_3^+ + A$
B					
NO		-0.58	-0.59	+0.09	+0.48
SO_2		+2.48	+2.49	+2.61	+3.54

The goals of the present study are:

- 1) to find and characterize carbocation systems which proceed via a radiative association mechanism
- 2) to examine reactions where charge transfer is near thermo-neutral or energetically unfavorable (eg. NO with CF_3^+ and CCl_3^+ and all SO_2 reactions) to see whether association will occur when no bimolecular channel is available
- 3) to calculate the temperature dependence of k_d and compare the results with theory
- 4) to gain information about the magnitude of k_r with the hope of gaining insight into the type of transition involved.

We have studied the reactions of CX_3^+ ($X=H,D,F,Cl$) with SO_2 and NO in helium buffer gas over a temperature and pressure range of 191 K to 468 K and 0.25 torr to 0.9 torr respectively in a Selected Ion Flow Tube (SIFT). The reactions of CH_3^+ and CD_3^+ demonstrated a positive pressure dependence and a negative temperature dependence. The presence of a non-zero intercept suggests the possibility of a radiative stabilization pathway in both reactions, as has been seen in the BX_3 work^{1,3}. The data for CH_3^+ is quantitatively similar to the CD_3^+ data shown below in figure 1, but in general k_{obs} for CD_3^+ is larger than the corresponding k_{obs} for CH_3^+ by a factor of two.

Figure 1. Temperature and pressure dependence of SO_2 association with CD_3^+



The reaction of CF_3^+ with SO_2 was found to be anomalously slow and CCl_3^+ did not appear to react with SO_2 . The temperature dependence of k_d was determined for both the CH_3^+ and CD_3^+ reactions with SO_2 , using the method developed previously⁵, and the values obtained were compared with the predictions based upon the theories of Herbst and Bates^{5,7}. The experimentally obtained temperature dependences are given in table II.

Table II. Temperature dependence of unimolecular decay rate coefficient: $k_d(T) = A \cdot T^n$

Reaction	n
$\text{CD}_3^+ + \text{SO}_2$	2.71
$\text{CH}_3^+ + \text{SO}_2$	1.79

The reactions of CD_3^+ , CH_3^+ , and CF_3^+ with NO produced only charge transfer products and again the CF_3^+ reaction appeared to be anomalously slow. No reaction was observed between CCl_3^+ and NO . Although table I indicates that charge transfer will not occur, no association product was observed even under the most favorable conditions (i.e. low temperature and high pressure) for ion-molecule association.

In summary the possibility exists for radiative association in CH_3^+ and CD_3^+ association reactions with SO_2 . The data are quantitatively similar to the isovalent BX_3 systems where we have good evidence for such a process, and the values of k_r are consistent with such a mechanism. When charge transfer is not energetically feasible association does not occur in the reactions of CCl_3^+ and CF_3^+ with NO . We determined the temperature dependence of k_d and k_{obs} in the reactions of CH_3^+ and CD_3^+ with SO_2 . Estimates of k_r for association of SO_2 with CH_3^+ ($k_r = 2.3 \times 10^6 \text{ s}^{-1}$) and with CD_3^+ ($k_r = 3.4 \times 10^6 \text{ s}^{-1}$) with SO_2 have been determined as well.

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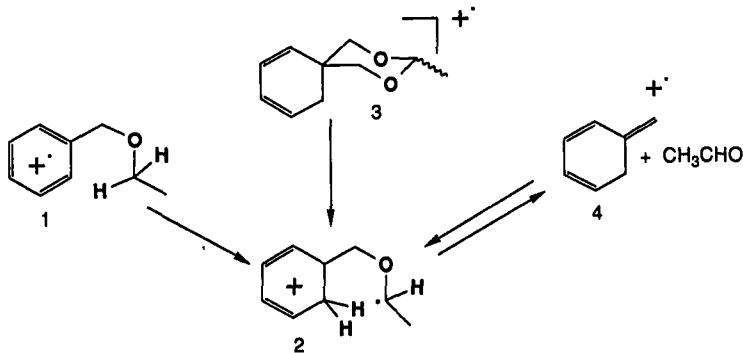
Neutralization-Reionization Studies of Isomeric Ions and Neutrals

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Neutralization-Reionization ($^+NR^+$) combined with collisionally activated dissociation of fast neutral species ($^+NCR^+$) has been used to differentiate isomers. CAD of neutral cyclobutadiene induces ring cleavage to give C_2H_2 as the most abundant product. The biradical $^{\bullet}HC=CH-CH=CH^{\bullet}$ is proposed as a common intermediate for dehydrogenation and ring cleavage to acetylene. Concerted cycloreversion to acetylene is disfavored. CAD of neutral vinylacetylene produces $C_4H_2 + H_2$ as major products, though the fragmentation is less abundant than with the less stable cyclobutadiene. Isomerization of cyclobutadiene to vinylacetylene is disfavored against dissociation to C_2H_2 .

Phenol and its less-stable keto-form, cyclohexa-2,4-dienone [1,2], are distinguished by CAD and $^+NR^+$ mass spectra. CAD spectra obtained at unit mass resolution allow for accurate quantification of $C_6H_6O^+$ isomers formed from phenyl alkyl ethers. The main products of neutral dissociations are $C_5H_5^+$, $C_5H_3^+$, $C_4H_2^+$, $C_3H_2^+$, CO and $C_2H_2^+$ for both isomers (Figure 1). The differences are preserved in $^+NCR^+$ spectra, suggesting that phenol and cyclohexa-2,4-dienone neutrals are separated by a significant energy barrier.

Loss of acetaldehyde from benzyl ethyl ether cation radical (1) has been re-examined [3,4]. A distonic-ion intermediate (2) pertinent to a step-wise route is generated independently by dissociative ionization of 3-methyl-2,4-dioxaspiro[5.5]undeca-8,10-diene (3) and by reaction of the methylenecyclohexa-2,4-diene cation radical (4) with acetaldehyde. The $C_7H_8^+$ (4) produced apparently isomerizes to the more stable toluene cation radical through an ion-molecule complex with acetaldehyde via reversible hydrogen transfer involving the acetaldehyde oxygen atom.



A new pc data system has been completed which controls the spectrometer scanning functions and provides integrated intensities of precursor and daughter ions.

References:

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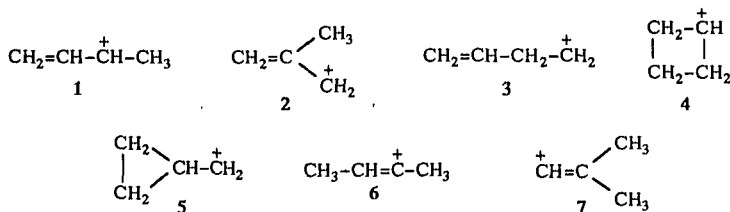
Figure 1. $^+NR^+$ (Hg(90%T)/O₂(50%)) mass spectra of A)cyclohexa-2,4-dienone and B)phenol radical cations.

A PHOTOIONIZATION STUDY OF $C_4H_7^+$ IONS IN THE GAS PHASE

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Dissociative photoionization mass spectrometry has been used to measure the $C_4H_7^+$ appearance energies for several C_4H_7Cl and C_4H_7Br precursors. The major difficulty in obtaining accurate thermochemical information from these measurements (Table 1) is the absence of any experimental data for the neutral molecule heats of formation. In most cases calculated values based on the Benson group equivalent scheme¹ were sufficiently accurate for the purposes of this study. For some molecules it was possible to use the thermochemistry associated with other fragmentation processes to modify or substantiate these estimates.



No threshold ion was observed with a heat of formation corresponding to the *trans*-1-methylallyl cation (1a).² The $C_4H_7^+$ ion formed from 3-chloro-1-butene and 1-chloro-2-butene was found to have a heat of formation close to that calculated (Table 2) for the *cis*-1-methylallyl cation (1b). This ion, which is 14 kJ mol⁻¹ less stable than its *trans* isomer, was also found from *ab initio* molecular orbital calculations to be the structure to which the homoallyl cation (3) collapsed. Photoionization of 3-chloro-2-methylpropene produced a $C_4H_7^+$ ion with a heat of formation in close agreement with experimental³ and theoretical⁴ results for the 2-methylallyl cation (2).

The $C_4H_7^+$ ion from (chloromethyl)cyclopropane had a heat of formation of 871 kJ mol⁻¹ which was assigned to the cyclopropylcarbinyl cation (5). A higher value of 886 kJ mol⁻¹ was derived for the cyclobutyl cation (4) on the basis of the chlorocyclobutane results. While the latter value is in good agreement with *ab initio* calculations, the lower value obtained here for the cyclopropylcarbinyl cation suggests that, even at the MP4/6-311G** level of theory, the relative stability is overestimated. Due to the relatively high ionization energies of the precursor molecules, the $C_4H_7^+$ ions from (bromomethyl)cyclopropane and bromocyclobutane were found to be formed with excess energy at threshold. The observation of significant molecular ion peaks in both photoionization mass spectra is evidence that the thermochemical $C_4H_7^+$ heats of formation are not significantly lower than the calculated upper limits in Table 1. The similarity between the CA spectra for these two ions⁵ is consistent with the small activation energy calculated for their interconversion.⁴

Threshold formation of 3 was not observed for 4-bromo-1-butene. Based on its CA spectrum and the calculated heat of formation, it is proposed that the $C_4H_7^+$ ion has a cyclopropylcarbinyl cation structure.

The 2-butenyl cation (6) and the isobutenyl cation (7) were calculated to have respective energies of 101 kJ mol⁻¹ and 159 kJ mol⁻¹ relative to 1a. Threshold formation was not observed for either ion. The 2-butenyl cation precursors, 2-chloro-2-butene and 2-bromo-2-butene, both had a calculated $\Delta H_f^\circ(\text{C}_4\text{H}_7^+)$ consistent with formation of 5. On the other hand, 1-chloro-2-methyl-propene and 1-bromo-2-methyl-propene, precursors for 7, had essentially the same C_4H_7^+ heat of formation, which may be interpreted as the result of formation of either 2 or 4 at threshold.

Table 1. Thermochemistry for the Gas-Phase Reaction at 298 K
 $\text{C}_4\text{H}_7\text{X} + h\nu \rightarrow \text{C}_4\text{H}_7^+ + \text{X} + \text{e}^-$

$\text{C}_4\text{H}_7\text{X}$	IE/eV	AE/eV	$\Delta H_f^\circ / \text{kJ mol}^{-1}$	
			$\text{C}_4\text{H}_7\text{X}$	C_4H_7^+
3-Chloro-1-butene	9.53	10.39	-41.2	856
1-Chloro-2-butene	9.48	10.33	-38.6	853
3-Chloro-2-methylpropene	9.52	10.65	-40.9	882
(Chloromethyl)cyclopropane	9.89	10.16	-2.6	871
Chlorocyclobutane	10.14	10.32	-2.7	886
2-Chloro-2-butene	8.91	10.62	-49.3	870
1-Chloro-2-methylpropene	8.91	10.60	-31.0	886
4-Bromo-1-butene	9.78	9.87	19.6	875
2-Bromo-2-butene	8.92	10.18	-7.3	878
1-Bromo-2-methylpropene	8.89	10.12	3.8	883
(Bromomethyl)cyclopropane	9.69	9.70	43.8	<883
Bromocyclobutane	9.87	9.88	36.8	<892

Table 2. *Ab initio* molecular orbital calculations

Ion Structure	Relative Energy (kJ mol ⁻¹)	Absolute Energy (kJ mol ⁻¹)	Level of Theory
1 a	0	845 ²	
1 b	14	859	HF/6-31G**
2	40 ^d	885	MP4/6-311G**
3	164	1009	HF/6-31G**
4	38 ^d	883	MP4/6-311G**
5	38 ^d	883	MP4/6-311G**
6	101	946	HF/6-31G**
7	159	1004	HF/6-31G**

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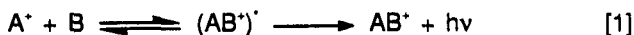
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DETERMINATION OF RADIATIVE ASSOCIATION RATE CONSTANTS BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE SPECTROMETRY.

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CANADA

The process of radiative association has been proposed to be an important mechanism in the formation of interstellar polyatomic molecules. In the experimentally achievable low vacua of the Fourier Transform Ion Cyclotron Resonance Spectrometer (FT-ICR), the products of association reactions have been observed. The stabilization of chemically activated intermediate species can occur by two mechanisms, radiative stabilization (reaction [1]) or collisional stabilization (reaction [2]).



The long trapping times achieved by the FT-ICR increase the statistical probability of observing an ion that will be stabilized by collision or the emission of photon, before it will undergo unimolecular decomposition. The low pressures which can be obtained the FT-ICR spectrometer will reduce the process of collisional stabilization and allow the radiative association process to be observed without interference from collisional stabilization.

The technique of FT-ICR has been used to determine the rate of formation of the proton-bound dimer of dimethyl ether, diethyl ether and acetonitrile from protonated species (reaction [3]) in the pressure range 5×10^{-6} mbar to 3×10^{-6} mbar.



By plotting the observed apparent rate constant as a function of pressure, the radiative association and the unimolecular decomposition rate constants may be extracted from the graphical analysis of the plot. Table 1 summarizes the radiative association and unimolecular decomposition rate constants and lifetimes obtained from FT-ICR experiments for dimethyl ether, diethyl ether and acetonitrile.

FT-ICR Variable Reaction Delay Experiment

Dimethyl Ether $P=5.0 \times 10^{-9}$ mbar

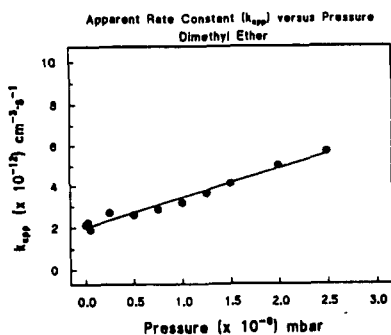
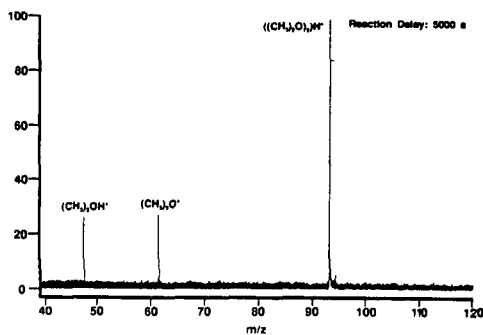
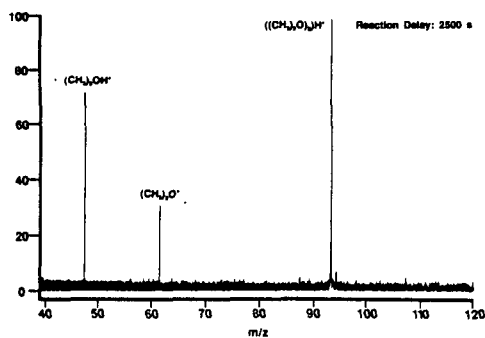
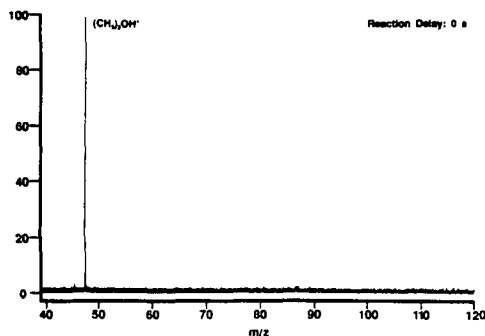


Table I

	Acetonitrile	Dimethyl Ether	Diethyl Ether
Rate Constants			
k_0 ($\times 10^4 \text{ s}^{-1}$)	17	1.1	0.13
k_{max} ($\times \text{s}^{-1}$)	41	48	20
Lifetimes			
τ_0 ($\times 10^6 \text{ s}$)	6	88	752
τ_{max} ($\times 10^6 \text{ s}$)	25	21	50

DOUBLE IONIZATION ENERGIES OF FLUOROETHANE
MOLECULES MEASURED BY DOUBLE-CHARGE-TRANSFER SPECTROSCOPY

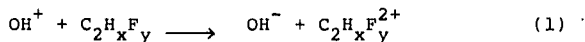
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University of the West Indies, Mona Campus,
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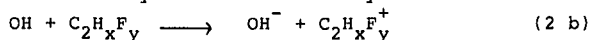
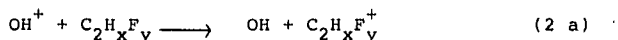
Double-charge-transfer spectroscopy [1] has been used to measure the double ionization energies of the fluoroethane molecules $\text{CH}_3\text{CH}_2\text{F}$, CH_3CHF_2 , CH_2FCHF_2 , CH_3CF_3 , CHF_2CF_3 and CF_3CF_3 .

OH^+ ions generated in the source of a ZAB spectrometer were accelerated to 6keV and underwent charge-inversion reactions in collisions with chosen fluoroethane molecules contained in the collision gas cell situated at the focal point between the magnet and electric sector of the spectrometer OH^- ions can be formed in two ways:-

- i) as a result of a single collision reaction



- ii) as a result of two sequential reactions



The reactions by which OH^- ions are formed are readily identified by observing the variation of OH^- ion signal with collision

gas pressure. In addition the translational energies of the ions formed by reactions (1) and [(2a) (2b)] are different. For reaction (1) the translational energy loss where OH^+ ions are converted to OH^- ions is given by $\Delta T_1 = \text{IE}_2(\text{C}_2\text{H}_x\text{F}_y) - \text{IE}_1(\text{OH}) - \text{EA}(\text{OH})$ (3)

while for reactions [(2a) (2b)].

$$\Delta T_2 = \text{IE}_1(\text{C}_2\text{H}_x\text{F}_y) + \text{IE}_1^1(\text{C}_2\text{H}_x\text{F}_y) - \text{IE}(\text{OH})_1 - \text{EA}(\text{OH}) \quad (4)$$

Once ΔT_1 and ΔT_2 are measured single and double ionization $\text{C}_2\text{H}_x\text{F}_y$ molecules can be obtained from Eqs (3) and (4) since $\text{IE}_1(\text{OH})$ and $\text{EA}(\text{OH})$ are known [3], [4].

Table 1 shows the values of IE_1 and IE_2 obtained for the fluoroethanes studies. This work provides the first determination of fluoroethane molecules double ionization energies and also the single ionization energies of CH_2FCHF_2 and CHF_2CF_3 have been determined for the first time.

Table 1. Double ionization energies of fluoroethane molecules.

	CH_3CF_3	CH_3CHF_2	CH_3CF_3	CH_2FCHF_2	CHF_2CF_3	CF_3CF_3
IE_2	32.5	35.0	36.4	33.3	35.8	38.7
IE_1	12.4	12.8	13.8	12.7	13.6	14.5
IE_2/IE_1	2.6	2.7	2.6	2.6	2.6	2.7

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W.J. Griffiths and F.M. Harris, Int. J. Mass Spectrom. Ion Proc., 85 (1988) 69.
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Gas-Phase Dehydrogenation of Cycloalkanes to Aromatic Hydrocarbons by Os^+ , Os_2^+ , and Os_3^+

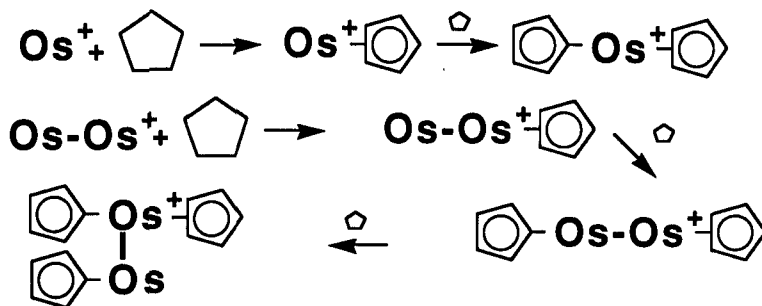
Fernando R. Tollens and Alan G. Marshall

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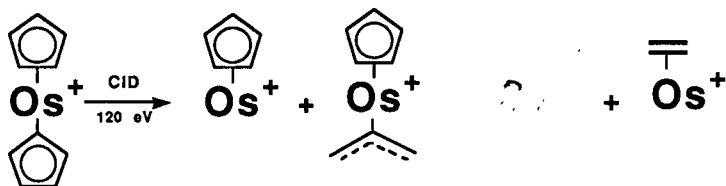
Transition metals have previously been reported to react with hydrocarbons to produce alkane elimination and dehydrogenation. Both C-H and C-C insertion mechanisms have been observed. For Fe^+ and Co^+ , for example, C-C insertion is the predominant mechanism for reaction with straight-chain hydrocarbons, whereas C-H insertion is preferred for cyclic hydrocarbons.¹ From its location below Fe^+ in the periodic table, the reaction of Os^+ with cycloalkanes should occur even more exclusively as C-H insertion; the absence of competing C-C insertion should facilitate the analysis of the stereoselectivity of the dehydrogenation reaction.

By a combination of collision-induced removal of ligand and selective ejection of all other ions, we have succeeded in isolating Os^+ , Os_2^+ , or Os_3^+ in high abundance from an electron-ionized sample of $Os_3(CO)_{12}$. Cycloalkanes exposed to Os_n^+ then undergo dehydrogenation until the 18-electron rule is satisfied for the corresponding neutral complex with Os_n . In contrast to the homologous reactions with other transition metals (e.g., Fe^+ or Co^+), cleavage of the carbon skeleton by Os_n^+ was seldom observed and need not occur by C-C insertion. Because the carbon skeleton is left intact, it is possible to devise tests for the stereochemistry of dehydrogenation. The structures and formation mechanisms of the various reaction products will now be discussed.

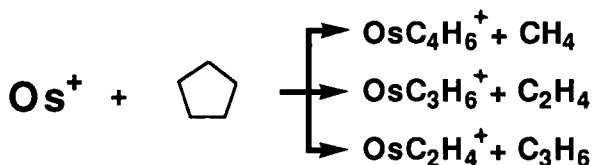
We find that Os_n^+ reacts with cycloalkanes in a concerted process to produce principally aromatic derivatives. Once the aromatic adduct has been formed, no more dehydrogenation occurs in that ring. If additional ligands are added to the metal center(s), dehydrogenation continues until the 18-electron limit is reached. For example, the reactions of Os^+ and Os_2^+ with cyclopentane give the results shown below:



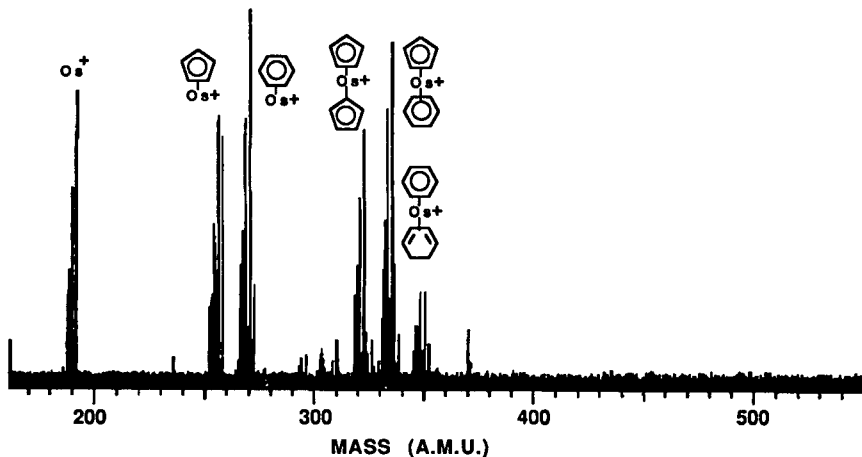
In the absence of rf excitation, no fragmentation products were observed. Low-energy CID (Os_n^+ excited to ~50 eV) shows nothing but $C_5H_5Os^+$, but high-energy CID (~120-140 eV) produced the following products in minor yield (~10%):



Although these fragmentations occur from the high-energy collision-induced processes, we were able to identify the following products from the corresponding products of thermal reactions of Os^+ with cyclopentene and methylenecyclopentane, by the same reasoning previously demonstrated for the homologous Fe^+ and Co^+ reactions.¹



Finally, the quality of our experimental data is illustrated by the FT/ICR mass spectrum (see below) of isolated Os^+ ions which have been incubated with a mixture of C_5H_{10} and C_6H_{12} .



This work was supported by N.S.F. (CHE-8721498) and The Ohio State University.

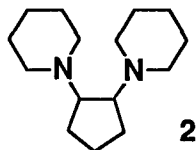
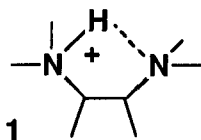
1. Jacobson, D. B.; Freiser, B. S. *J. Amer. Chem. Soc.* **1983**, *105*, 7492.

Diamine Basicities: A New Maximum Gas-Phase Basicity

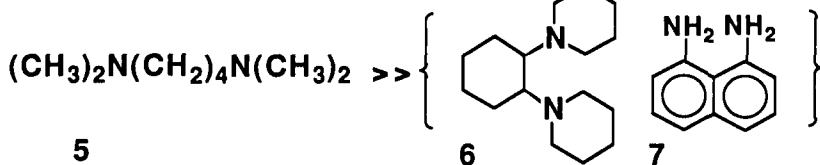
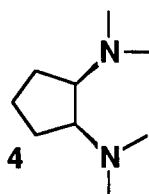
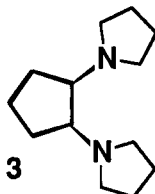
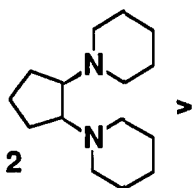
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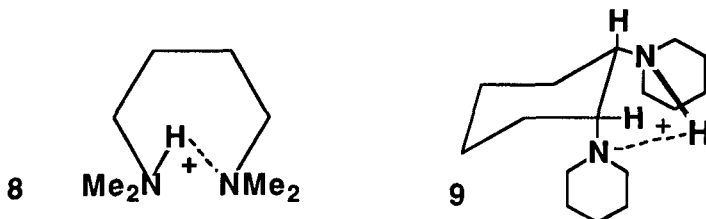
cis-vicinal diamines, a functionality hitherto neglected due to the absence of efficient methods for making them, are potentially important ligands for Li^+ , Mg^{2+} , Zn^{2+} , and Pt^{2+} . These diamines catalyze the reactions of organolithium compounds. Of significant interest is the high basicity of such diamines in H_2O -ethanol: pK_a 's of ~10 and 3, due to intramolecular H-bonding within the monoprotonated species, **1**. We have measured gas-phase basicities of several such diamines (including compounds **2**, **3**, **4**, and **6** synthesized by us) in an attempt to discover the structural determinants (e.g., cis-trans geometry, axial-equatorial conformation) of basicity and cation affinity. In the course of those experiments, we have discovered that compound **2** has the highest gas-phase basicity yet reported.



Starting from the previous maximum gas-phase basicity as a reference, we have extended the scale of gas-phase basicities. The high basicities of the diamines can be explained by intramolecular H-bonding in the monoprotonated amine. We can now report some experimental evidence as to the effect of structure on gas-phase basicity.



H-bonding is favored in compounds **2**, **3**, and **4**, for two reasons: the short distance between the two nitrogens and restricted internal rotation. The high basicity for compound **5** can also be explained by an intramolecular H-bond, because the two nitrogen groups can achieve an entropy-favored (because of the high probability that the two ends of the molecule can come together) six-membered ring conformation, **8**, which is essentially strain-free. In compound **6**, the two nitrogens are axial and equatorial (see **9**) and are thus too far apart to form a favorable intramolecular H-bond. Compound **7**, also known as "proton sponge" due to its high basicity, is in fact much *less* basic than the above-mentioned diamines.



Experiments were then performed to investigate attachment of Li^+ to these amines. Lithium ions were generated by laser desorption from LiCl . Compounds **2**, **3**, and **4** form mono-lithium complexes (e.g., **10**), whereas compound **6** forms a di-lithium complex, **11**. (The corresponding diprotonated complex of **6** has also been observed.) These experiments offer an unparalleled opportunity to compare the electrophilicities of H^+ and Li^+ . For reaction with these amines, Li^+ is found to be more electrophilic than H^+ . The formation of lithium adducts further corroborates the proposed intramolecular hydrogen bond as the basis for the high gas-phase basicities of diamines.



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THE MEASUREMENT OF ONSET POTENTIALS FOR EXOTHERMIC AS WELL AS ENDOTHERMIC
PROTON TRANSFER REACTIONS WITH A TRIPLE QUADRUPOLE INSTRUMENT

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Slightly exothermic and endothermic proton transfer reactions were investigated with a triple quadrupole mass spectrometer in an effort:

- 1) to develop a new method for determining onset potentials for exothermic reactions and
- 2) to determine optimum conditions for measuring onset potentials for both exothermic and endothermic reactions.

Both daughter ion (D^+) and parent ion (P^+) intensities were monitored with respect to instrument P^+ ion kinetic energy settings (IKE(lab eV)). Inherent problems which could bias results and which could easily be overlooked were discovered:

- 1) P^+ (and D^+) intensity changes with time. Monitoring P^+ intensity with time revealed that the intensity could decrease by an order of magnitude during the first 15 minutes after initiation of source ionization. As our data showed, a plot of D^+ vs. IKE(lab eV) is greatly influenced by such a long term drift. Monitoring P^+ intensity before collecting data is recommended. A plot of D^+/P^+ vs. IKE(lab eV) is preferred--the D^+/P^+ ratio is less subject to long term drift since both D^+ and P^+ intensities are expected to change together.
- 2) P^+ (and D^+) intensity changes with IKE(lab eV). P^+ intensity increases with increasing IKE(lab eV) from 0 at 0 eV to high values around 10 eV. At low IKE(lab eV) P^+ intensity is low; D^+ intensity must therefore also be low, even for slightly exothermic and slightly endothermic reactions. Plots of D^+ vs. IKE(lab eV) for slightly exothermic reactions should erroneously give onset potentials at a slightly positive IKE(lab eV). But poor P^+ energy resolution might allow the more energetic P^+ 's to create D^+ 's at even negative IKE(lab eV) values, giving the misleading appearance of "good" results. A plot of D^+/P^+ vs. IKE(lab eV) is preferred; using extrapolation, such a plot can be used to measure onset potentials for slightly exothermic as well as endothermic reactions.
- 3) D^+/P^+ intensity tends to increase with decreasing IKE(lab eV) at low values of IKE(lab eV), thus distorting the D^+/P^+ plot in this important region. At low IKE(lab eV) values the intensities of D^+ 's (and P^+ 's) for slightly endothermic and exothermic reactions are low; using the ratio of such small imprecise values is questionable. The trend of increasing D^+/P^+ with decreasing IKE(lab eV) might be explained with the hypothesis that at low IKE(lab eV) P^+ 's having higher IKE's are more likely to be extracted leading to a greater % of D^+ 's and to a higher D^+/P^+ ratio than expected. To measure onset potentials ignore points having small D^+ relative intensities (e.g., < 0.1) and extrapolate the remaining points on the rising portion of the D^+/P^+ curve to 0 intensity. Evidently the D^+/P^+ curve will rise before the accepted onset potential, the amount perhaps depending on the energy distribution of P^+ .

Using plots of D^+/P^+ vs. IKE(lab eV), reasonable estimates for onset potentials for slightly exothermic as well as endothermic proton transfer reactions were obtained, generally in good agreement with values expected from data obtained by other methods.

MOLECULAR ORBITAL STUDY OF REACTION PROCESSES IN THE THERMOSPRAY MASS SPECTRA OF DEUTERATED PENICILLINS

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When beta-lactam containing penicillin compounds were studied by thermospray mass spectrometry in a deuterium enriched solvent system, a number of characteristic ions were observed in which greater than 95% of all acidic hydrogen atoms were exchanged for deuterium atoms. These results are summarized in Table I. The abundant ions observed include the deuterium-adduct molecular ion (I) and the cycloreversion reaction products, namely, a deuterium-adduct substituted ketene product (III) and a deuterium-adduct substituted thiazoline product (IV). An unexpected reaction product was ion II, corresponding to ion III but with an additional exchange of a hydrogen atom for a deuterium atom.

Semi-empirical restricted Hartree-Fock molecular orbital (MO) calculations were performed in order to gain an understanding of the structures and energetics necessary for the formation of ions I-IV. All calculations were for the ground state so as to mimic thermal decomposition and chemical ionization processes. Ampicillin and penicillin V were used as the model compounds in the calculations of beta-lactam containing antibiotics.

BINDING SITE OF DEUTERON-ADDUCT IN IONS I-IV

From the computed proton affinities for ions I-IV, listed in Table II, the most likely sites for the deuterium-adducts can be deduced, viz.,

Ion I - amide oxygen O_p , beta-lactam carbonyl oxygen O_α or (if present, as in ampicillin) the amino group nitrogen. The beta-lactam nitrogen N_α is a very likely deuterium site but deuterium initiates the cycloreversion reaction.

Ions II and III - ketene carbon C_α (cyclic isomer), ketene oxygen O_α (cyclic isomer), amide carbonyl oxygen O_p .

Ion IV - beta-lactam nitrogen N_α .

ENERGETICS OF THE BETA-LACTAM CYCLOREVERSION REACTION

The results of the MO calculations for the concerted cleavage of the $C_\beta-N_\alpha$ and $C_\alpha-C_\gamma$ bonds in the beta-lactam ring, when the two bonds were stretched equally and the molecule deuterated at different sites, indicated that the reaction with the lowest activation energy (31.4 kcal/mol) occurred when nitrogen N_α was deuterated. (The same process for the neutral molecule was 61.5 kcal/mol.) Consecutive bond cleavages of the beta-lactam ring when nitrogen N_α is protonated was also investigated. The beta-lactam $C_\beta-N_\alpha$ bond cleaves first with an activation energy of only 6.6 kcal/mol. The remaining $C_\alpha-C_\gamma$ bond is then cleaved with an activation energy of 31.2 kcal/mol. These calculations show that when the beta-lactam nitrogen N_α is protonated, the substituted ketene and thiazoline products can form either in a concerted or consecutive manner with nearly the same total energy requirements. These results tend to indicate that ion I is probably less likely to be deuterated at nitrogen N_α because when this species is formed, the penicillin molecule cleaves into smaller fragments.

CH-CD HYDROGEN-DEUTERIUM EXCHANGE IN ION II

Only the exchange of a CH hydrogen atom, not normally considered labile, for a deuterium atom, can explain the additional H to D exchange in ion II. MO calculations were performed with ampicillin for the following cases:

- Tautomerization of the side-chain amide group: $CHCOND$ to $C=C(OH)ND$ followed by H to D exchange of the vinyl alcohol hydrogen.
- Exchange of aromatic CH's for CD's.
- Addition of D_2O to ketene group followed by HDO dehydration.
- Direct deuterium of ketene carbon C_α followed by deprotonation.
- Deuterium of a site followed by CH hydrogen bonding with D_2O and the release of HDO.

The most likely process was found to be "e" where the ketene carbon C_α was deuterated producing a $C_\alpha HD^+$ group. The exchange process took place as illustrated in the Figure with an activation energy of 47 kcal/mol.

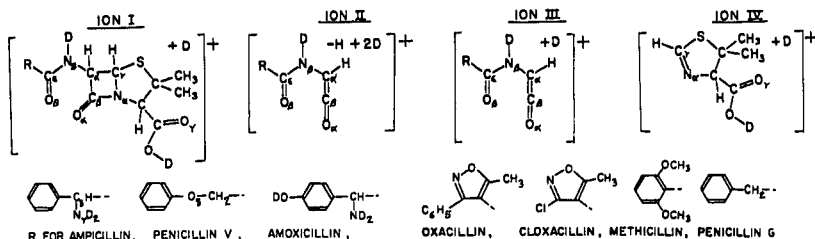


Table I

Selected Ions from Thermospray Mass Spectra of Deuterium Enriched Penicillins

Penicillin Molecule	Ion I (Penicillin+D) ⁺		Ion II (Substituted Ketene+2D+H) ⁺		Ion III (Substituted Ketene+D) ⁺		Ion IV (Substituted Thiazolidine+D) ⁺		Ratio of % Ion III to % Ion II	Ratio-d _n to Ratio-d ₀
	m/z	%	m/z	%	m/z	%	m/z	%		
Ampicillin-d ₄	355	17	196	20	195	53	162	100	0.38	
-d ₀	350	4	192	9	191	59	160	100	0.15	2.53
Penicillin V-d ₂	354	11	195	8	194	26	162	100	0.36	
-d ₀	351	7	193	22**	192	79	160	100	0.15	2.38
			211	9*	210	59*				
Amoxicillin-d ₅	372	9	213	13	212	19	162	100	0.68	
-d ₀	366	35	208	5	207	18	160	100	0.28	2.43
Oxacillin-d ₂	405	5	246	25	245	46	162	88	0.54	
-d ₀	402	4	244	15	243	15*	160	100	0.20	2.70
Cloxacillin-d ₂	439	2	280	20	279	40	162	100	0.50	
-d ₀	436	4	278	13	277	50	160	100	0.26	1.92
Methicillin-d ₂	384	0.5	225	30	224	21	162	60	1.43	
-d ₀	381	1	245	67*	244	100*	160	42	0.67	(431)
			223	8	222	23			0.35	(670)
			241	11*	240	100*			0.11	
Penicillin G-d ₂	338	2	179	6**	178	7**	162	100	0.29	
-d ₀	335	8	177	13**	176	8**	160	73	0.14	2.13
			195	6*	194	44*				
Average									2.78	

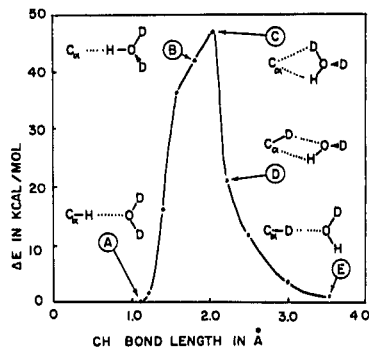
* Intense peaks corresponding to either ion II + D₂O or ion III + D₂O

** Overlapping components probably present.

Table II

Calculated Proton Affinities for Various Sites in Ions I-IV

Calculated Proton Affinity (kcal/mol)			Calculated Proton Affinity (kcal/mol)		
Deuteration Site	Ampicillin	Penicillin V	Deuteration Site	Ampicillin	Penicillin V
Ion I					
Amino N _γ	217.0		Ions II and III		
Beta lactam N _α	212.7	210.7	Ketene C _α (cyclic isomer)	224.4	216.9
Side chain amide O _β	212.6	208.1	Amino N _γ	218.5	-
Beta lactam carbonyl O _α	209.4	198.3	Amide O _β	217.4	214.8
Carboxyl carbonyl O _γ	201.3	200.1	Ketene O _α (cyclic isomer)	216.6	207.7
Side chain amide N _β	197.9	190.3	Ketene C _β	210.8	202.6
Thiazolidine S	192.8	190.5	Amide N _β	203.4	191.8
Ether O _δ		182.3	Ether O _δ	-	188.9
Para-aromatic C	181.6	193.1	Para-aromatic C	186.6	198.9
Ion IV					
Thiazolidine N _α	208.3	208.3			
Carbonyl O _γ	192.4	192.3			
Thiazolidine S	164.7	164.7			



RAPID DETECTION AND CHARACTERIZATION OF FOSSIL-DERIVED
FUELS IN WATER AND SOIL USING DIRECT PURGE ION TRAP
MASS SPECTROMETRY*

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We have recently developed a method for the rapid detection and characterization of fossil-derived fuels in environmental samples. This method uses direct purge of the volatile materials from the fuels into an ion trap mass spectrometer (ITMS), with little or no sample preparation. The entire analysis requires less than five minutes and detection limits are as good or lower than conventional methods (extraction and analysis by GC), which require much longer times.

Several types of fossil-derived fuels, including a jet fuel, an unleaded gasoline, and a diesel fuel, have been detected and characterized in water at the low ppb level using direct purge ion trap mass spectrometry. The sample (either 15 mL of water or 5 g of soil with 15 mL of distilled water added) is contained in a sealed 40 mL VOA vial. Helium is used to purge the volatiles from the sample at flow rates between 20 and 100 mL/minute. The purged materials pass through an open/split interface into the ITMS. Water is used as a proton transfer reagent, and a 50 msec reaction time is used with the ITMS. Detection limits of about 1 ppm are achieved using these conditions.

Figure 1 shows the spectrum of unleaded gasoline in water at a concentration of 200 ppb. The molecular weight distribution from m/z 79 to m/z 163 and the relative abundance of aromatic to aliphatic components is useful for characterizing fuel type. For example, the aromatic content in the unleaded gasoline is greater than that of the jet fuel, which is greater than the diesel fuel.

In addition to the water samples studied, a set of 4 soil samples contaminated with jet fuel were analyzed using direct purge ITMS. Previous analysis using conventional GC methods determined that the concentrations of the jet fuel in the soils ranged <10 ppm $\mu\text{g/g}$ to 5700 $\mu\text{g/g}$ (total organics). The conventional method included ultrasonic extraction with solvents (3 hours), followed by gas chromatography (1 hour). For the direct purge ITMS approach, the soil samples (with 15 mL of water added, see above) were slowly stirred in capped VOA vials for 2 minutes. The samples were then purged into the ITMS, requiring less than 5 minutes per sample for the entire analysis. It was demonstrated that the direct purge ITMS method has detection limits below the 10 $\mu\text{g/g}$ limit obtainable by conventional GC analysis (Figure 2).

Direct purge ITMS can rapidly detect and quantitatively determine fuels in soils and water, requiring 5 minutes or less for total analysis time, compared to 3 or more hours using conventional GC methods. Further, this technique has potential for being a field portable device.

*Research sponsored by the U.S. Army Toxic and Hazardous Materials Agency under Interagency Agreement 1769-A073-A1 with Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

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Figure 1

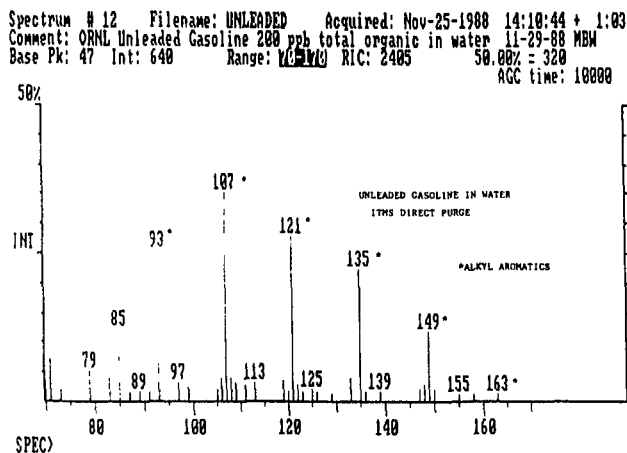
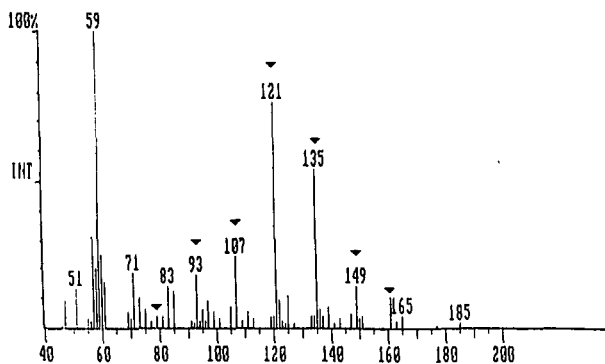


Figure 2

**JET FUEL IN SOIL (10 ug/g)
ITMS DIRECT PURGE**



**PARALLEL COLUMN CHROMATOGRAPHY-MASS SPECTROMETRY
FOR THE ANALYSIS OF COMPLEX MIXTURES***

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The analysis of polycyclic aromatic compounds (PAC) in the environment is of considerable importance due to the established mutagenicity and carcinogenicity of many of these compounds. The main difficulty associated with PAC determinations is the extreme complexity of environmental matrices. Even after extensive fractionation, the PAC fraction may contain several hundred compounds. In order to detect, identify and quantify compounds in such mixtures, methods that offer combinations of good chromatographic resolving power and detection selectivity are required. We have found that the mass spectrometer in conjunction with high resolution chromatographic separation methods (GC-MS, HPLC-MS or SFC-MS) provides the best selectivity and sensitivity for the provision of reliable quantitative data.^{1,2} One of the objectives of our work was to determine as many compounds as possible in environmental reference materials (i.e., "broad spectrum" analysis). It quickly became clear to us that no single chromatographic technique, even one as powerful as capillary column GC, could provide sufficient resolution of the complex mixtures. Therefore, we began to investigate complementary methods of separation, either in series or in parallel, followed by mass spectrometry.

The acquisition and multivariate analysis of data produced with parallel chromatographic columns (of different separation selectivity) coupled with multi-channel detectors has been termed "third order chromatography".³ Data collected as a function of time, column and detector channel allow the enhanced resolution of complex mixtures. This paper reports on the application of parallel column GC-MS and LC-MS methods to the analysis of PAC in environmental samples.

Extremely complex mixtures of PAC extracted from various samples (marine sediments, urban airborne particulates and steel foundry dusts) have been analyzed by GC/MS using DB-1 and DB-5 capillary columns and by reversed phase LC/MS (moving belt interface) using Vydac-201TP and Zorbax-ODS packed columns. For each sample, the four separate data sets have been examined by a preliminary semi-automated data processing procedure. The key steps are: (a) detection and measurement of chromatographic peaks in all mass chromatograms; (b) organization of the data with a "spreadsheet" program (Lotus 123); (c) identification of molecular ions; and (d) calculation of retention indices. Over 350 compounds have been detected in the NRCC HS-3 marine sediment reference material with 53 identified positively from retention indices and mass spectra. The most complementary data sets are provided by the DB-5 GC-MS and the Vydac-201TP LC-MS analyses, due to the very different separation selectivities. An interesting variation possible with LC-MS is to perform two analyses with the same column, but with a change in either column temperature or type of mobile phase organic modifier. Both of these parameters cause sufficient change in separation selectivity to be useful. The major problem that we faced in interpreting the data was the lack of standard compounds for matching spectra and retention indices.

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* NRCC No. 30280

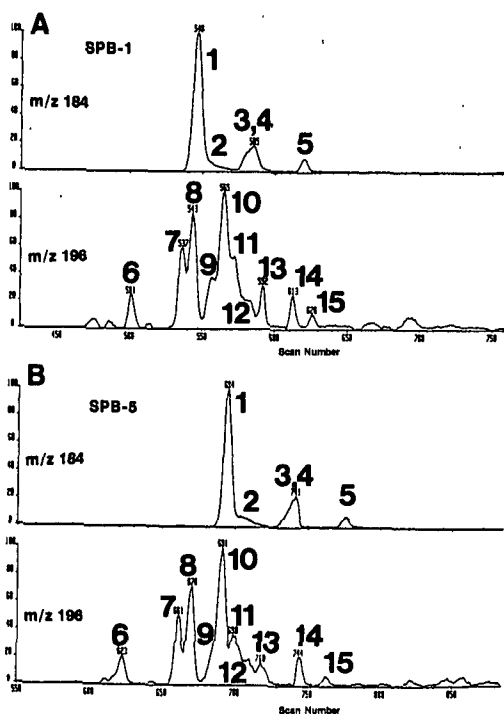
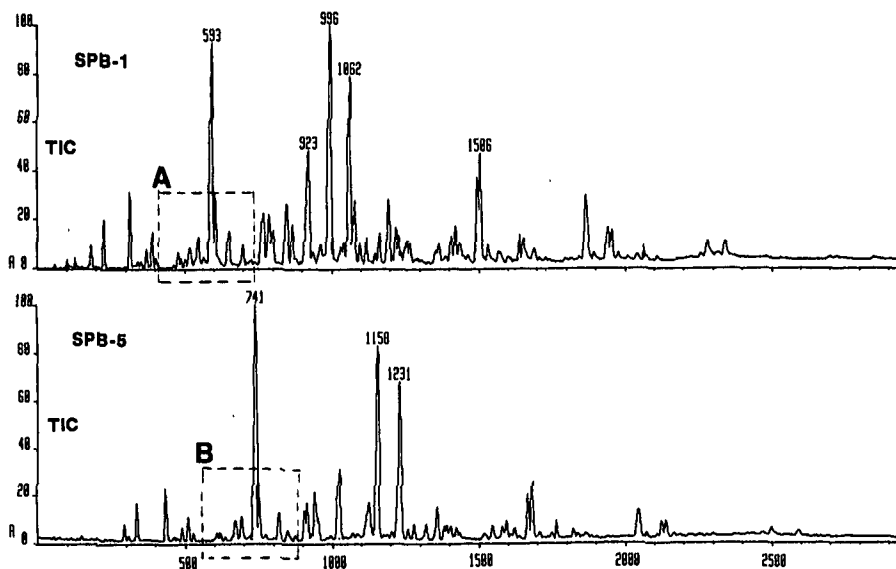
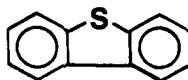


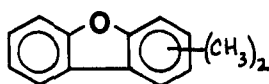
Figure 1: Effect of the stationary phase type (SPB-1 vs. SPB-5) on separation selectivity in GC-MS as shown by total ion chromatograms (TIC) and reconstructed mass chromatograms (m/z 184 and 196) for sediment PAC.



1

Isomers of 1

2-5



6-15

THE ROLE OF MASS SPECTROMETRY IN THE CLEAN-UP OF THE SYDNEY TAR PONDS

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The Sydney Tar Ponds are the product of over 80 years of pollution from the Sydney (Nova Scotia) steel mill coke ovens. The production of metallurgical coke has as byproducts, coal tar, coal dust and coke fines, the latter liberated when the hot coke is quenched with water at the end of the process. The resultant sludge has been allowed to collect in an arm of Sydney harbour, which has formed the area known as the Sydney Tar Pond. Leachate from this lagoon has led to widespread contamination of the rest of the harbour and its biota. The local lobster fishery has been terminated as a consequence of the pollution.

A multi-million dollar clean-up of this grossly contaminated site is now starting. The progress of such work depends upon reliable analytical data for polycyclic aromatic compounds (PAC) in dredged sediment samples. Major engineering decisions on the materials to be disposed of rest solely on the results of chemical analyses. The main difficulty in the analytical work is the extreme complexity of the samples. The PAC fraction of the organic burden of the material contains a large proportion of alkylated or otherwise substituted PAC. A great number of heterocyclic compounds are also present.

The use of simple, non-selective detectors, such as GC or HPLC with flame ionization and ultraviolet absorption or fluorescence detectors does not provide sufficient specificity for reliable determinations and provides inadequate information on compound structures. We have belaboured this point elsewhere (1-4). Our thesis, now as then, is that only the mass spectrometer has the right combination of sensitivity, selectivity and wide dynamic range needed for reliable analysis of such complex mixtures.

This paper will report on our use of mass spectrometry combined with various chromatographic techniques to provide detailed analyses on sediment samples. The techniques applied to date include capillary column GC and SFC and packed column HPLC and SFC. The first two were directly interfaced to the mass spectrometer, while the packed columns were interfaced through the moving belt interface. All of these methods provide complementary data leading to more reliable determination of hundreds of compounds. The availability of UV absorption spectra generated by a diode array detector during HPLC-MS analyses helps to distinguish the various isomeric ring structures present in the sample (5). Several mass spectral techniques such as negative ion chemical ionization with carbon dioxide as reagent gas (6) and selected ion monitoring at half masses (7,8), which help to increase detection selectivity, will also be applied to this problem.

To date, we have applied a modification of the technique outlined in a previous paper (9). In that work, Quilliam and co-workers, considering the analytical challenge posed by the determination of PAC in complex environmental matrices, were led to the development of a multi-dimensional chromatographic/mass spectrometric technique. In this approach, data are acquired as a function of time, column and detector channel. In the work of the present project, their approach has been modified. The detector channels have been restricted to the mass spectrometer (with the diode array as an adjunct), but the column variable has been further expanded, with the incorporation of SFC separations (both capillary and packed column). The variable selectivity of the differing separations employed is essential for complete characterisation of the PAC mixture in these samples. For example, the limited dynamic range of the capillary techniques is complemented by the relatively high column loadings possible in HPLC and packed column SFC; whereas the lower efficiency of the latter is compensated by the higher resolution capillary techniques.

At the present time, the presence of more than 500 compounds has been demonstrated in the PAC extracts from various tar pond samples. Work is proceeding along two fronts: the identification of the suite of

compounds currently found and the search for further compounds. Identification is hampered by the relative paucity of authentic standards and spectroscopic techniques are being used as an aid. Both optical (diode array) and mass (particularly chemical ionization) spectral approaches are being used. The identification of the minor components, particularly the substituted species, is heavily dependent upon the application of such data.

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NRCC No. 30281

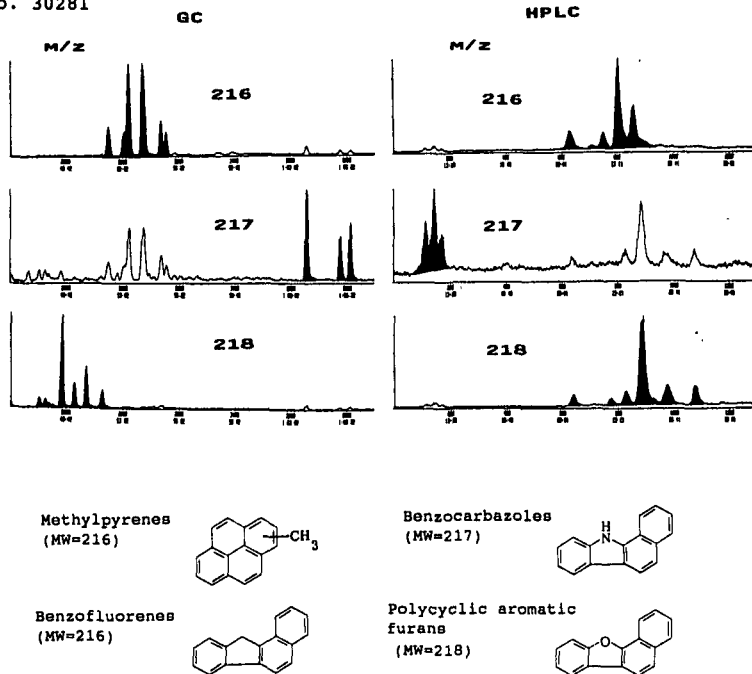


Figure 1: GC/MS and HPLC/MS reconstructed mass chromatograms (m/z 216, 217 and 218) showing the complementary separation selectivity of the two methods for different classes of polycyclic aromatic compounds. Shaded peaks correspond to those due to molecular ion species. Some example structures are given above.

CAPILLARY GC/MS CHARACTERIZATION OF CREOSOTE CONTAMINATION WITH
COMPARISON TO GC/FTIR AND GC/MIP-AED RESULTS, A MULTISPECTRAL
APPROACH TO SITE CHARACTERIZATION:

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Of the presently identified Superfund sites, a significant number are the result of contamination by wood preserving processes. Creosote constitutes one of the principal complex environmental contaminants at many of these sites. This abstract describes current efforts to characterize the environmental occurrence of creosote contamination using samples obtained from Eagle Harbor, Puget Sound. Full scan EI MS has been accomplished using a VG70EQ with two internal standards (d_8 -naphthalene and d_{10} -phenanthrene). Capillary chromatography is carried out with an oncolumn injector using a 30 m SPB5 column. In addition to polynuclear aromatic hydrocarbons (PNA's) such as phenanthrene, pyrene, and chrysene, nitrogen containing aromatic compounds (NCAC's) are also of interest. Initially, 9 NCAC's including carbazole, acridine and benzonitrile and 12 PNA's were targeted for identification and quantitation by GC/MS total ion current (Figure 1). Rapid screening of samples has made use of GC/NPD and element specific GC/microwave induced plasma emission spectroscopy which can be an important adjunct to GC/MS (Figure 2). GC/FTIR data has also been obtained to aid in identifications (Figure 3). The use of MS/MS daughter ions is currently being investigated to locate compounds of interest (Figure 4).

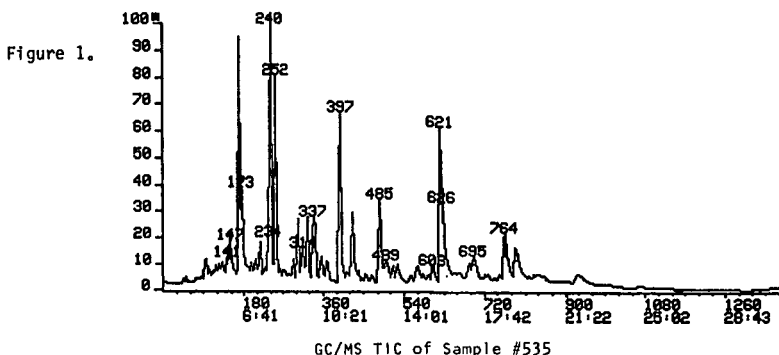


Figure 2.

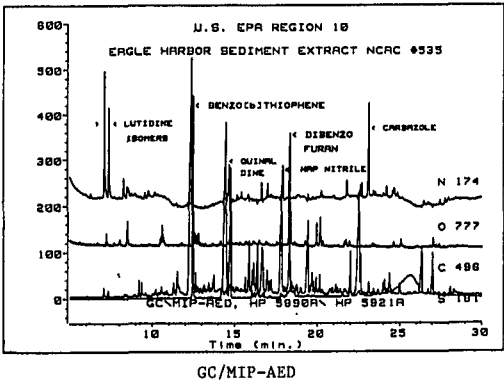


Figure 3.

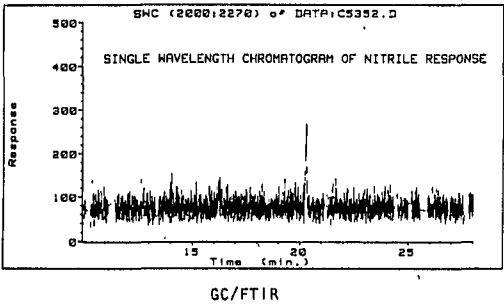
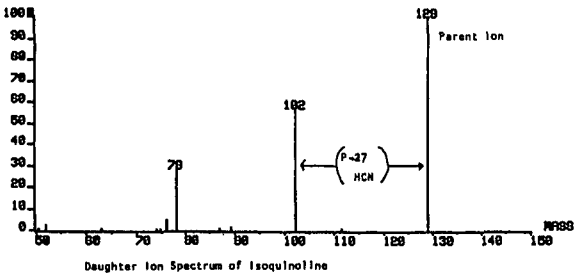


Figure 4.



Although the research described in this report has been funded by the US Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**ANALYSIS OF FIVE- AND SIX-RING NITRO PAHS: A COMPARISON OF EI, API
AND METHANE NICI MASS SPECTROMETRY**

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INTRODUCTION

Nitro-polycyclic aromatic hydrocarbons (Nitro-PAHs) are a class of genotoxic contaminants. They have been found in diesel exhaust, urban air, carbon blacks, aluminum smelters, and coal fly ash (1-5). Many techniques such as thin layer chromatography, high performance liquid chromatography, and gas chromatography have been used for the separation and identification of this class of compounds (1,6,4). Mass spectrometry (MS) has also proven to be a valuable analytical technique for the analysis of nitro-PAHs found in environmental samples (6). Since the mutagenic potency of these compounds varies considerably, even among isomers, the ability to differentiate isomers by mass spectral techniques will help for the analysis of environmental samples which might contain these compounds. This work reports the EI, API and methane NICI mass spectra of a series of five- and six-ring nitro-PAHs, including 5-nitropicene, 7-hydroxy-6-nitro-7,8,9,10-tetrahydrobenzo[a]pyrene and nitrotetrahydrobenzo[a]pyrene isomers.

EXPERIMENTAL

Nitro-PAHs

5-Nitropicene (5-NPN) was synthesized by Chemsyn Science Laboratories (Lenexa, Kansas) for N.C.T.R. The 1,3,6-nitro-7,8,9,10-tetrahydrobenzo[a]pyrene (1,3,6-NHBaP), 1,3-nitro-8,9,10,11-tetrahydrobenzo[e]pyrene (1,3-NHBaP) and 7-hydroxy-6-nitro-7,8,9,10-tetrahydrobenzo[a]pyrene (7-HY-6-NBaP) were synthesized by P.P. Fu (N.C.T.R.). All compounds were diluted in toluene.

Atmospheric Pressure Ionization MS

API was performed using a DB-5 fused silica capillary column connected directly to the APIMS source. All analyses were conducted in the negative ion mode. The source was maintained at 350°C and purged continuously with 40-60 cm³ per min of ultra high purity nitrogen. The mass spectrometer was scanned from m/z 50 to m/z 500 with a scan time of 1.5 s.

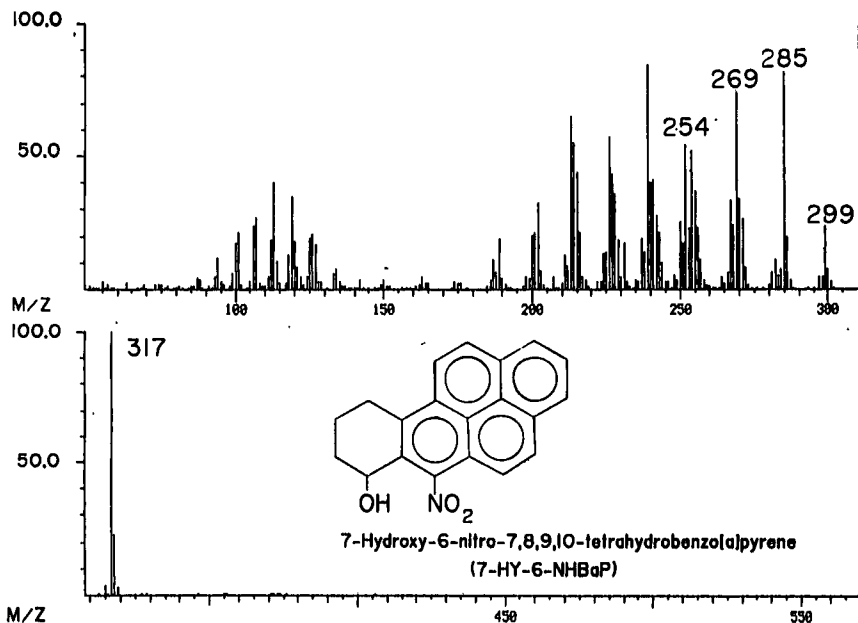
Electron Impact and Negative Ion Chemical Ionization MS

NICI and EI mass spectra were obtained on a Finnigan 4023 Automated Gas Chromatograph/EI-CI mass spectrometer. Ions were monitored using a 1.5 s scan from m/z 50 to m/z 550. The electron energy and electron multiplier were set at 70 eV and 1300V, respectively. For NICIMS, methane was adjusted to a source pressure of 0.25 torr. The source temperature was set at 250°C while for EIMS it was 270°C.

RESULTS AND DISCUSSION

The API mass spectrum showed the M⁻ for 3-NHBaP whereas the M⁻ and [M-O]⁻ were observed under NICI conditions. The EI fragmentation pattern was composed of ions at m/z 301, m/z 271 and m/z 255 for 1-NHBaP. These ions represent the M⁻, [M-NO]⁻ and [M-NO₂]⁻. For 7-HY-6-NBaP, the M⁻ was observed under API conditions. Ions at m/z 317, m/z 301 and m/z 287 observed in the NICI spectrum represent the M⁻, [M-O]⁻ and [M-NO]⁻. Also, a low-counts ion was observed at m/z 257. Under EI conditions (figure 1), ions at m/z 317, m/z 301, m/z 299, m/z 285, m/z 269, m/z 254 and m/z 252 were monitored. 5-NPN showed only the M⁻ ion under API, whereas the M⁻, [M-O]⁻ and [M-O₂]⁻ ions were observed under NICI conditions. The EI mass spectrum showed the M⁻, [M-NO]⁻, [M-CN₂O]⁻, and [M-HNO₂]⁻. Overall, the most sensitive technique was NICI. For example, a limit of detection of 80 pg was observed for 5-NPN via NICI compared to 1.4 ng with EI.

Figure 1. EI mass spectrum of 7-HY-6-NHBaP.



CONCLUSIONS

The three ionization methods provided different results for the individual compounds. Even though isomer differentiation was not possible for the nitrotetrahydrobenzo[a]pyrene and nitrotetrahydrobenzo[e]pyrene isomers, mass spectral characterization will help for further identification of these compounds that might be found in real samples.

ACKNOWLEDGEMENTS

This research was supported by the American Cancer Society Grant NP 593 and the Commissioned Officer Student Training and Exchange Program, U.S. Public Health Service. Special thanks to J. Freeman, M. Bryant, R. Hall, T. Getek, D. Miller, and L. Rushing for the technical assistance.

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ENVIRONMENTALLY SIGNIFICANT HALOGENATED COMPOUNDS IN FLUFF

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INTRODUCTION

Fluff is the ground-up material remaining after bulk metals have been extracted from shredded vehicles and consumer appliances. In the US alone, a total of 3 million tons of fluff is created annually at over 200 shredding sites. Fluff is not presently classified as hazardous waste and is sometimes used as landfill at construction sites. Polychlorobiphenyls (PCB) in fluff from several states have been reported at concentrations of up to 1600 ppm. The purpose of this study was to determine if other classes of environmentally significant organics were present.

EXTRACTION

To facilitate identification of halogenated, neutral, aromatic classes of compounds, 10 g of fluff was extracted by a modified RCRA 8280 method. This method is used to prepare environmental samples for halogenated dioxin and furan analyses.

GC/MS ANALYSIS - FULL SCAN DATA

A total ion current chromatogram of the fluff extract is presented in Figure 1. Numerous compounds identified are labeled, including the 3 classes of compounds that were most abundant: polychlorinated naphthalenes (PCN), PCB, and polychlorinated terphenyls (PCT).

SELECTED ION RECORDING DATA

To provide greater sensitivity, selected ion recording (SIR) was used to search for polychlorodibenzodioxins (PCDD), polychlorodibenzofurans (PCDF), and the polybrominated analogs (PBDD and PBDF).

CONFIRMATION CRITERIA

Several confirmation criteria were met before a peak was attributed to one of these types of compound:

- 1) The retention time was within the time window established by standards.
- 2) A signal-to-noise ratio 2.5 was obtained for the molecular ions.
- 3) A prominent and characteristic fragment ion was observed.
- 4) The ratio of the molecular ion abundances was within 20% of the theoretical value.
- 5) For furans, no coeluting ether peak was observed containing 1 or 2 additional halogen atoms relative to the target compound.
- 6) The peak did not appear in the method blank.

Displayed in Figure 2a-c are the ion chromatograms used to identify HpCDD, HpCDF, and TBDF respectively.

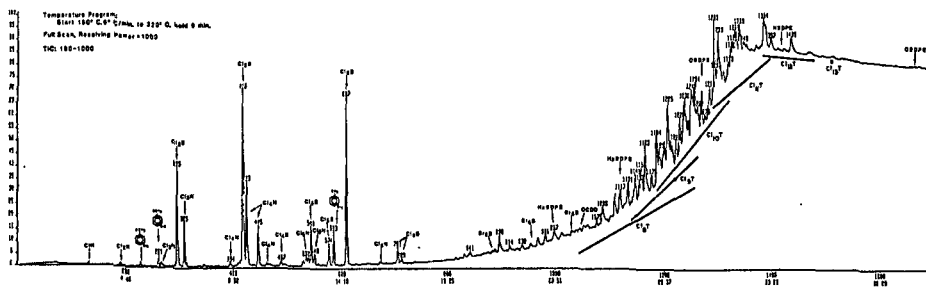
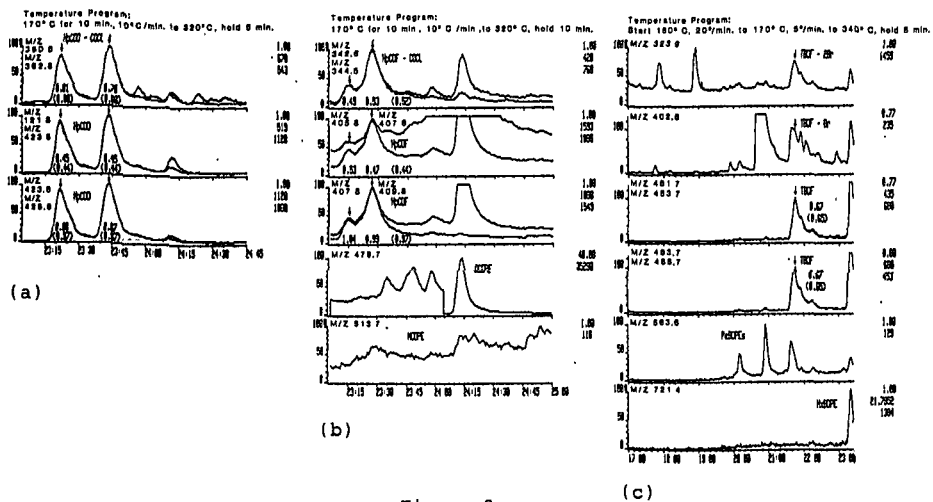


Figure 1



COMPOUNDS IDENTIFIED

- | | | |
|-----|----------------------------|------------|
| 1) | Polychloronaphthalenes: | C1 - C16 |
| 2) | Polychlorobiphenyls: | C12 - C16 |
| 3) | Polychloroterphenyls: | C18 - C113 |
| 4) | Polychlorodibenzodioxins: | C16 - C18 |
| 5) | Polychlorodibenzofurans: | C16 - C18 |
| 6) | Polychloromethoxybenzenes: | C14 - C15 |
| 7) | Polybromobiphenyls: | Br5 - Br6 |
| 8) | Polybromodiphenylethers: | Br6 - Br10 |
| 9) | Polybromomdibenzofurans: | Br4 |
| 10) | Polybromomethylbenzenes: | Br5 |

COMPARISON OF MS/MS AND SELECTED ION RECORDINGS (SIR) IN
ANALYSIS OF TCDD AND TCDF

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INTRODUCTION

High resolution gas chromatography/high resolution mass spectrometry is the traditional method for TCDD and TCDF analysis. The high resolution (10000) is necessary to resolve the TCDD and TCDF from interfering ions in the molecular ion region. An alternative approach is to use the MS/MS facilities afforded by a HRGC coupled to a hybrid mass spectrometer.

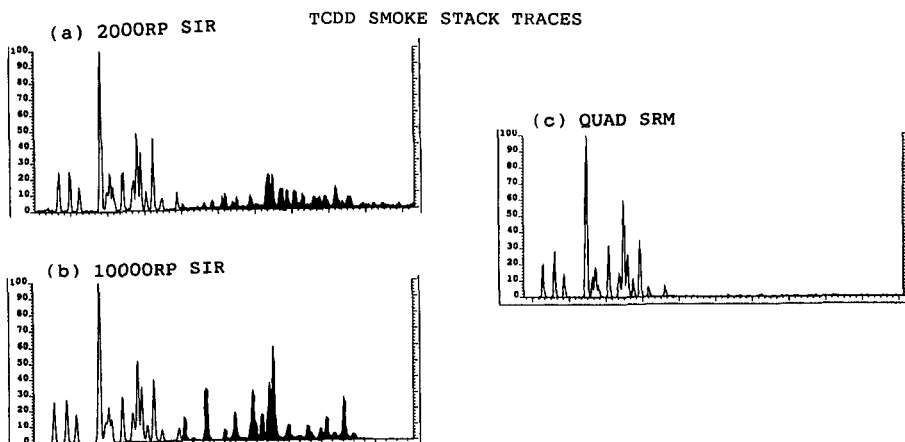
The objective of this work is to compare the linearity, sensitivity, selectivity and reproducibility of HRGC/MS/MS with HRGC/LRMS and HRGC/HRMS.

EXPERIMENTAL

HP5890 GC's were interfaced to the different mass spectrometers. A Grob type split/splitless injector was heated to 280°C. The column used was a 50m DB5. This was held at 150°C for 1 minute, then was heated at 30°C to 190°C and at 3°C to 300°C. The GC/MS interface was heated to 250°C.

The LRMS selected ion recording (SIR) and the MS/MS were performed on a VG TRIBRID. LRMS was 2000 RP. HRMS SIR was carried out on a VG 70E at 10000RP. MS/MS was achieved by monitoring the transitions between the m/z 320 and m/z 322 peaks to m/z 257. This transition corresponds to the loss of CO^{35}Cl and CO^{35}Cl respectively from TCDD. The parent ion was selected by the magnetic sector at 500 RP.

Selectivity of the three techniques was compared using a smoke stack sample containing TCDD's and TCDF's. The dioxin and furan traces exhibited interferences at 2000 and 10,000RP which were removed by MS/MS. The furan traces show the improved selectivity of HRMS over LRMS SIR.

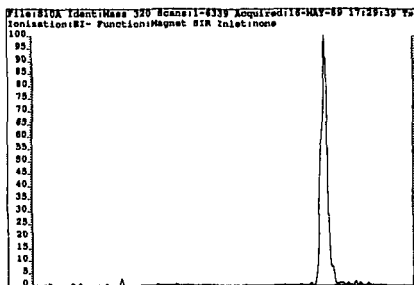
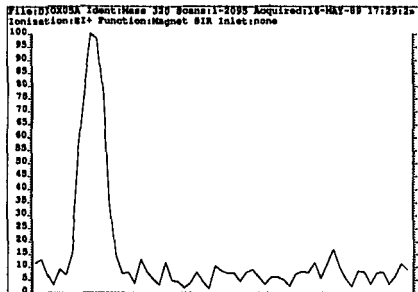


Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

Sensitivity was derived from the S/N of m/z 320 molecular ion of TCDD. S/N of 500 fg TCDD at 2000RP was 10:1 S/N on 1 pg at 10,000RP was 20:1 S/N on the transition peak (10 pg) was 6:1.

500fg TCDD 2000RP SIR

10pg TCDD QUAD SRM



Linearity was established by acquiring two replicate injections of 5, 50, 100, 1000 pg TCDD and TCDF standards. The molecular ions of TCDF and TCDD were ratioed to their ¹³C internal standards. Calibration curves yield a coefficient of correlation in all cases at 2000 and 10,000RP greater than 0.999.

Reproducibility was determined from four replicate injections of TCDD standard. The ratios of the areas of the 322:320 peaks were calculated.

	2000RP	10,000RP	500RP MS/MS
mean	0.78	0.77	0.29
theoretical ratio	0.77	0.77	0.32
standard deviation	0.009	0.037	0.009
relative standard deviation	1%	1%	3%

ANALYSIS OF MONOBROMINATED PCDD AND PCDF IN MUNICIPAL INCINERATOR FLYASH

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To reduce the levels of polychlorodibenzo-*p*-dioxins and -dibenzofurans (PCDD/Fs) in flyash generated from municipal waste incinerators (MWI), it is important to understand the mechanism and identify the precursors for PCDD/F formation during the incineration process. The information released about this, so far, is fragmentary and often controversial. The formation mechanism remains unknown. Recent findings of brominated PCDD/Fs (BPCDD/Fs) in flyash by HRGC/HRMS analysis may facilitate such studies^{1,2}.

The flyash sample preparation procedure employed for BPCDD/F analysis consisted of Soxhlet extraction, acid wash, and multi-column chromatographic clean-up. The subsequent HRGC/HRMS analysis was performed on a Carlo-Erba GC/Kratos MS-50 mass spectrometer with 70 eV EI and 10,000 resolving power. Selected ions of the target analytes were monitored at a 300 ppm mass span. A 30 and 60 meter DB-5 column were used for GC separation. The details of this analytical method were reported previously³.

Identification of BPCDD/Fs in flyash was discussed elsewhere¹. Figure 1 shows the mass chromatograms for selected BPCDD/Fs and their PCDD/F counterparts found in the same sample. Some examples of mass profile data obtained for BPCDD/Fs are also illustrated in this figure. All observed isotope ratios of those BPCDD/F ions monitored are in the acceptable deviation range (± 0.10) of the theoretical values. As observed for tetra- to penta-halogenated dibenzo-*p*-dioxin (DD) and dibenzofurans (DF) previously¹, each pair of BrCl_n-DD (or DF) and Cl_{n+1}-DD (or DF) exhibits very similar isomer profiles on a 30 meter DB-5 column. However, when the GC separation power is increased by using a 60 meter column, BPCDD/F peaks are "split", namely, more BPCDD/F isomers are recognized. Some examples are illustrated in Figure 2 as a comparison with PCDD/Fs analyzed under the same conditions. When a bromine atom is placed on PCDD/F, the degree of structural symmetry is degraded and more isomers become possible. For those compounds illustrated, the theoretical number of possible isomers is given in Figure 2. Apparently, the observed number of BPCDD/F isomers is not as many as the theoretical number. In contrast, all possible isomers of PCDD/Fs are present in MWI flyash.⁴

The data presented here indicate that BPCDD/Fs and PCDD/Fs in flyash are closely related. Those data also suggest that bromination of PCDD/F, chlorination of polybromo-DD/F, or simple Br/Cl exchange are not the main pathways for BPCDD/F formation in MWI. Laboratory experiments^{5,6} reported extensive isomers of BPCDD/Fs to be obtained under those mechanisms. Thus, BPCDD/F possibly undergoes the formation mechanism similar to their PCDD/F counterparts in MWI process. This provides many advantages of using BPCDD/Fs to study the formation mechanism of PCDD/Fs. For example, rationalization of the highly characteristic patterns of BPCDD/F isomers can give additional mechanistic information such as cyclization routes, substitution preferences, and the sequence of reaction steps. More importantly, the bromine atom in BPCDD/Fs serves as a "label" element and participates in the formation mechanism under the real conditions of MWI that cannot be easily simulated in a laboratory study.

ACKNOWLEDGMENT

We thank Drs. R.E. Clement, G.W. Sovocool, R.K. Mitchum and J.R. Donnelly for supplying the flyash sample and synthetic brominated PCDD/Fs.

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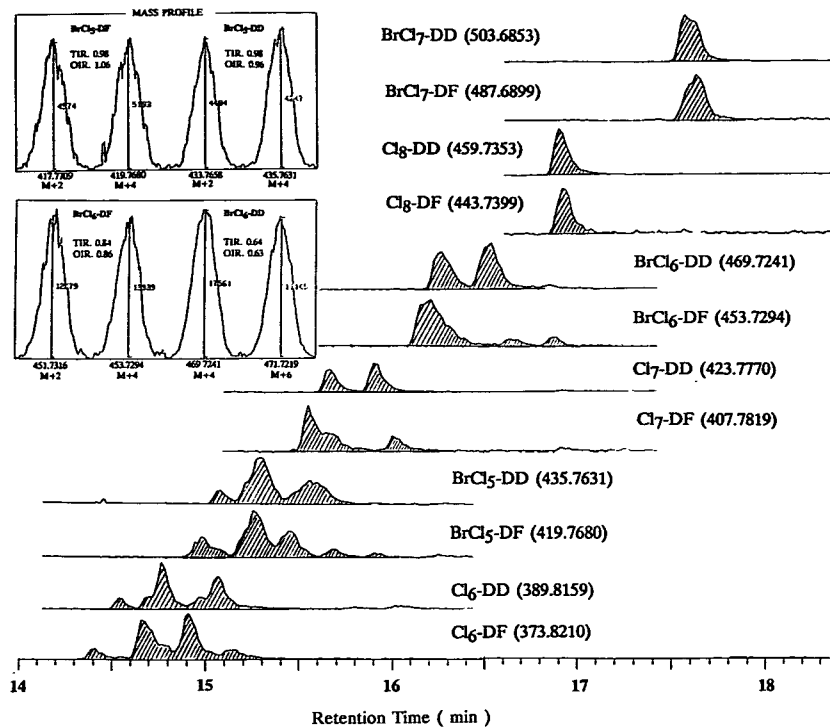


FIGURE 1: Mass (in parenthesis) chromatogram and mass profile data for selected BPCDD/Fs and PCDD/Fs found in flyash sample. A 30 meter DB-5 GC column. DF: dibenzofuran, DD: dibenzo-*p*-dioxin, TIR: theoretical isotope ratio, OIR: observed isotope ratio.

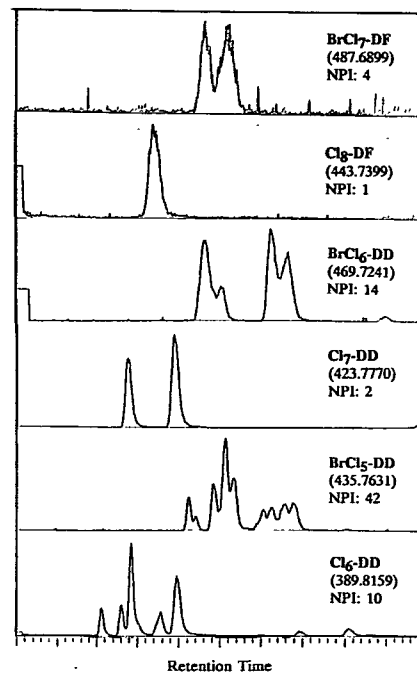


FIGURE 2: Mass (in parenthesis) chromatogram data for selected BPCDD/Fs and PCDD/Fs found in flyash. A 60 meter DB-5 GC column. NPI: No. of possible isomers; DD and DF, see Figure 1.

EI AND NEGATIVE ION CIMS OF
POLYBROMINATED FURANS IN PLASTICS

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Polybrominated dibenzofurans (PBDF) have been identified and quantitated in flame retardant concentrate pellets and in plastic resins impregnated with the flame retardant materials. The flame retardant employed was usually decabromodiphenyl ether (DBDPE). Mass spectrometric techniques used in the analysis of cleaned up extracts for tetra through octa BDF congeners included EIMS in the selected ion recording mode (SIR) at both low resolution (LR) (1,000, baseline) and high resolution (HR) (10,000, 10 % valley). In addition, negative ion chemical ionization mass spectrometry (NICIMS) has also been explored as a complementary tool. Clean up of extracts followed RCRA method 8080 (SW846) after dissolution of the sample in toluene or trichloroethane/phenol.

Table 1 gives a brief tabulation of some results obtained based on two internal standards, $^{13}\text{C}_{12}$ - 2,3,7,8-TBDF and $^{13}\text{C}_{12}$ - 1,2,3,7,8-PBDF uncorrected for response factors. Reasonable agreement is obtained between the various instruments and between LR and HR SIR. By far, the pyrolysate materials contained the highest levels of PBDF.

Confirmation of identity was based on the three most abundant ions of both the M^+ cluster and the $(\text{M}-\text{COBr})^+$ cluster. In addition, one ion from each M^+ cluster of the brominated DPE one and two bromines higher than the corresponding furan was also monitored. Chromatographic separation was achieved using either an SPB5 or DB5 column of 30m (.25 mm ID, .25micron film) programmed from 150-320 at 5°C/min. On column injection used a 1 m retention gap uncoated column (.53 mm ID) and provided an independent assessment of the role of hot injections in producing PBDF during analysis.

NICIMS of PBDF revealed that M^- and $(\text{M}-\text{Br})^-$ ions were usually base peaks. SIR in the NI mode was also pursued at LR and HR employing a lock mass. Brominated diphenyl ether responses were noted by monitoring phenoxide ions derived from the corresponding DPE.

An example chromatogram of the M^+ cluster HpBDF is shown in Fig. 1 for a PBT resin treated under extreme extrusion conditions.

An example of NI responses for a similar chromatographic window is shown in Fig. 2

Table 1. Quantitations of PBDF in PBT Resins

	PBT Resin Extrusion Conditions	Total TBDF ppb	Total PaBDF ppb	Total HaBDF ppb	Total HpBDF ppb	Total OBDF ppb
#1	70SE LR	1020	68200	27200	72300	—
	70EQ LR	2590	82800	347000	81100	—
	70EQ LR	1500	68500	708000	108000	—
#2	70SE LR	122	6380	24800	22400	17
	70EQ LR	178	8770	23200	20200	99
	70EQ LR	151	7320	32000	33100	248
(Less Extrusion)						
#3	70EQ LR	22	51	323	344	27
	70EQ HR	24	89	323	353	11

Fig. 1

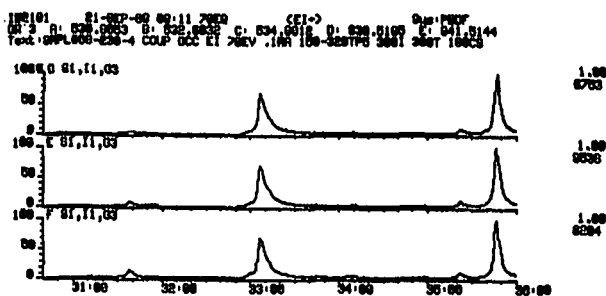
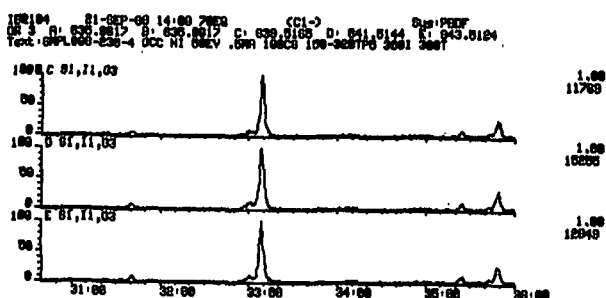


Fig. 2



Although the research described in this report has been funded in part by the US Environmental Protection Agency, it has not been subjected to Agency review and, therefore, does not necessarily reflect the views of the Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

GC/MS METHODS UTILIZED IN A SURVEY OF SELECTED XENOBIOTIC
CONTAMINANTS IN FISH FROM SITES ACROSS THE UNITED STATES.

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Concern that bioaccumulation in the aquatic food chain may represent a major route of human exposure to a number of toxic environmental contaminants prompted the Environmental Protection Agency's Office of Water Regulations and Standards to initiate the Bioaccumulation Study in 1986. The objective of the study was to broaden the base of information on anthropogenic chemical residues found in fish. Specifically, the study measured the levels of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), and 36 other pesticides and industrial chemicals. Residues of selected samples were further characterized via spectral library matching, and additional analytical effort. The data has been entered into a data base (the BIOACC data base) at EPA's National Computer Center at Research Triangle Park, NC, and is accessible by modem from any location in the nation. It will be used to identify potential risks to human health or aquatic life, identify correlations with contaminant sources, and initiate further investigations or remedial actions.

The study focused on fish because they provide a good indicator of contamination in aquatic systems, and represent a direct route of exposure to humans through consumption. EPA regional offices, and state and local agencies cooperated in collecting samples from over 400 sites. Sampling was done in both heavily polluted areas, and areas relatively unaffected by the activities of man. Target analytes were selected from among known contaminants of aquatic environments on the basis of their bioaccumulation potential, potential for human health effects, and feasibility of analysis.

The analytical methods utilized ¹³C isotope dilution techniques for quantitation of PCDDs and PCDFs. Other analytes were quantified by measuring relative responses to deuterated surrogate internal standards. Sample preparation involved a number of contaminant isolation and enrichment steps including soxhlet extraction, gel permeation chromatography, and partitioning on silica gel, potassium silicate, florisil, and carbon particulates. High resolution GC, scanning MS, and high resolution/multiple ion selection MS were utilized. Quality assurance criteria were developed for all aspects of the analyses including GC column performance, MS response, method efficiency, accuracy, and precision.

While a detailed analysis of the data is not yet complete some general observations can be made. The data indicate that PCBs continue to be a widespread contaminant in fish. Nearly 90% of the samples had PCB levels at or above the limit of detection of 1 ppb, with mean concentrations in some regions as high as 5 ppm. Over 90% of the samples contained measurable amounts of p,p'-DDE, some reaching part per million levels. Other chemicals found frequently include nonachlor, oxychlordane, chlordane, dieldrin, pentachloroanisole, hexachlorobenzene, and lindane, generally in the range of 1 to 100 ppb. PCDDs and PCDFs were detected in 3 out of 4 samples. The mean 2,3,7,8-TCDD toxic equivalency concentration (TEC) of PCDDs and PCDFs was 6.8 ppt. In 95% of the positive samples, over 70% of the TEC is attributable to 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 1,2,3,7,8-PCDD.

GC/MS Analysis of Halogenated Organic Compounds in Water, Fish, and
Sediment Samples Collected Near an Industrial Outfall in the
Calcasieu River Estuary

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Studies conducted in the vicinity of an industrial outfall near the Calcasieu River estuary, Louisiana indicate that the area is impacted by environmental contamination. The study to date has shown that water, fish, and sediment samples collected at the outfall, above and below the outfall are contaminated with a variety of chlorinated, brominated, and mixed chlorinated and brominated organic compounds.

The analytical methodology that was used to extract and analyze the samples was matrix dependent. The one liter water samples were extracted with methylene chloride (3x50 ml) using conventional liquid/liquid extraction. The extract was dried over sodium sulfate and concentrated via Kuderna-Danish (K-D) to a volume of 1.0 ml and further concentrated under a gentle stream of nitrogen to 100 μ l and analyzed by GC/MS.

The 10 gram wet weight sediment samples were extracted with methanol (100 ml), and methylene chloride (2x75 ml). The actual extraction was accomplished with a tissuemizer. After extraction, the extracts were partitioned with 250 ml of 2% Na_2SO_4 . The aqueous phase was reextracted with methylene chloride (2x25 ml). The extract was concentrated to 1.0 ml by K-D. After solvent exchange into hexane, the extract was purified by passage through a microflorisil column. The purified extract was concentrated to 5 ml by K-D and further concentrated to 100 μ l under a gentle stream of nitrogen and analyzed by GC/MS.

The 10 gram wet weight fish samples were extracted with 20% acetone-hexane (3x75 ml). The extract was concentrated to 1.0 ml by K-D, and purified by passage of the extract through a 5 gram florisil column. The purified extract was concentrated to 5.0 ml by K-D and further concentrated to 100 μ l under a gently stream of nitrogen and analyzed by GC/MS.

The extracts were analyzed by GC/MS under the following conditions. An HP-5890 gas chromatograph was directly interfaced to a VG-7035 double-focussing mass spectrometer. The gas chromatographic separations were achieved on a 30m x 0.2mm i.d. DB-5 fused silica capillary column. The oven temperature was held at 50°C for 3 min. and then temperature programmed at 7°C/min up to a final temperature of 310°C. Splitless injections of 1 µl were made with the injection port temperature at 275°C. Transfer line temperature was 280°C. The mass spectral analysis were conducted under the following conditions. Low resolution mass analysis was conducted at a resolution of 1000 over a mass range of 50-500 daltons. The magnet was scanned at rate of 1 sec/decade, and the accelerating potential was 4Kv. High resolution mass spectral analysis was conducted at a resolution of 5000 over a mass range of 200-500 daltons. The magnet was scanned at a rate of 2 sec/decade, and the accelerating potential was 4Kv. All analysis were conducted at an electron-ionization energy of 70 eV.

Quantitation was achieved through the use of response factors for standards that were readily available. Semi-quantitation of compounds without authentic standards was achieved through an average response factor value as determined from the response factors of authentic standards from each class of compound reported. Confirmation of structures was achieved by comparison of authentic standards when available or by accurate mass measurement mass spectrometry when authentic standards were not available.

The results of this study to date indicate that the estuary is contaminated with a series of chlorinated, brominated, and mixed chlorinated and brominated organic compounds. The analyte concentrations in the water samples ranged from non-detectable up to 6 µg/l. The analyte concentrations in the sediment samples ranged from non-detectable up to 25 µg/g dry wet. The analyte concentrations in fish ranged non-detectable up to 300 µg/g lipid wet. The data suggests that there is a wide distribution of the contaminants in the study area. The contaminants detected in the fish tissue at Buoy 130 (control area), Lake Charles indicates that contamination is not limited to the area in the immediate vicinity of the industrial outfall.

GC/MS IDENTIFICATION OF TRICHLOROPHENYL ETHANOL IN IMPORTED PEPPERS:
POTENTIAL MISIDENTIFICATION AS A TRICHLORO METHYL METHOXY BENZENE.

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The FDA routinely monitors various domestic and imported foods for insecticide residues. GC/MS is used to confirm the presence of those insecticides or industrial chemicals found and to identify unknown analytical responses (UAR's) in these products. A UAR was encountered during analyses of a number of red and yellow peppers imported from the Netherlands. The UAR appeared in both the Luke extract and in the 15% eluate (1). The GC RRT's relative to Chlorpyrifos on three columns were: 5% OV-101, 0.34; 3% OV-17, 0.25; and 4% SE-30/6% SP2401, 0.35. GC/MS indicated the UAR had a molecular weight of 224 contained three chlorine atoms and produced a good match using the NBS library for a trichloromethyl methoxy benzene (Anisole) compound (Fig.1). The fragment ions m/z 209, [M-15] and 181, [M-43] were consistent with an anisole compound representing $[M-CH_3]^+$ and $[M-COCH_3]^+$, respectively.

PICI confirmed the molecular weight (224) but also produced a three chlorine cluster at m/z 207, indicating loss of H_2O from the protonated molecular ion suggesting the presence of an [OH] on the molecule.

Deuterium studies confirmed one exchangeable proton on the molecule by the shift in the molecular ion cluster to 225. In addition, m/z 209 and 181 each shifted up one mass unit indicating that the deuterium was retained on these fragment ions (Fig.2).

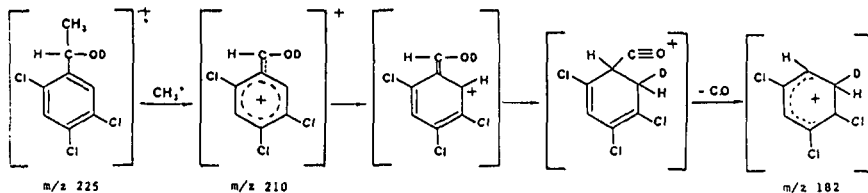
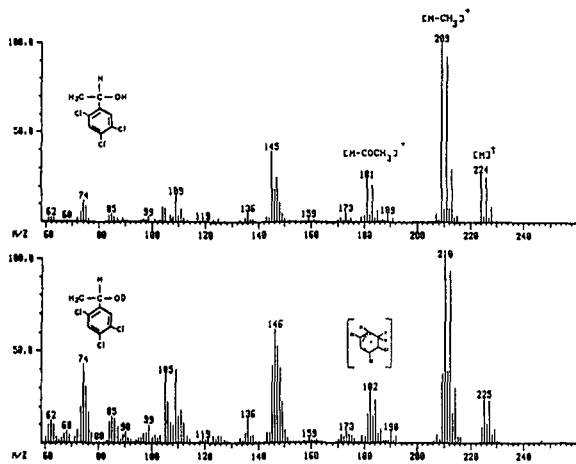
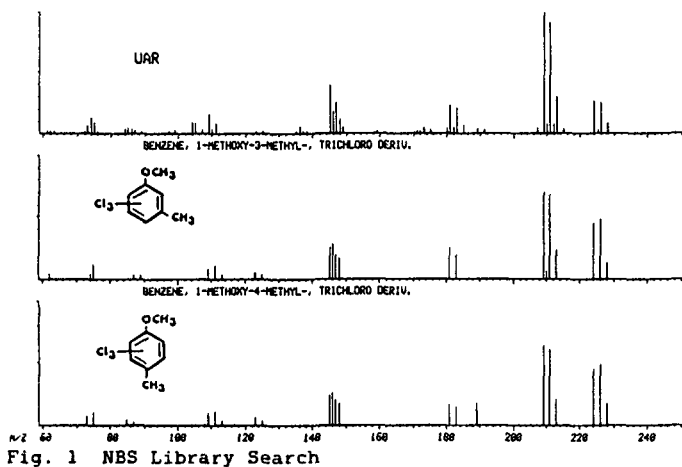
The FDA Mass Spectral Compilation of Pesticides and Industrial Chemicals (2) listed Trichlorophenyl Ethanol (TCPE) as the only other compound meeting all the criteria. TCPE is both an animal and environmental metabolite of the insecticide Tetrachlorovinphos(3,4). Reference material was obtained and the UAR was positively identified as TCPE.

Therefore, m/z 181 or the [M-43] ion in the EI spectrum represents a trichloro benzenium ion derived by loss of CH_3 and CO from the molecular ion with a two hydrogen rearrangement. This type of rearrangement was reported for benzyl alcohol and related compounds based on deuterium labeling and meta stable data (5).

A similar fragmentation mechanism is proposed for TCPE and is supported by CAD MS/MS analyses. The CAD daughter ion spectrum of the molecular ion m/z 224, shows that it dissociates to m/z 209 $[M-CH_3]^+$, m/z 181 $[M-COCH_3]^+$, 145 $[M-COCH_2Cl]^+$, and m/z 109 $[M-COCH_2Cl_2]^+$. The parent ion spectrum of m/z 181 indicates that it is derived from m/z 209 by loss of CO and from m/z 224 by loss of CO and CH_3 with a two hydrogen rearrangement (Fig.3). The deuteration experiment also shows that one of the hydrogens which rearrange onto the ring is from the [OH] moiety.

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DIRECT ON-SITE VAPOR MONITORING BY TRANSFER LINE GC/MS

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A direct atmospheric vapor sampling inlet has been used for on-line monitoring of vapors in laboratory scale reactors. The inlet (Fig. 1) consists of three concentric tubes with appropriate flow control plumbing and electronics. This inlet provides for direct introduction of a pulse of air (.5 to 2 s) into a short (1 m) coated fused silica capillary column enclosed in the heated transfer line housing of a Finnigan-MAT ITD. Effluent from the column emerges directly into the ion trap.

Data presented here were obtained by sampling from a bed-characterization reactor (for waste disposal studies) using the MINITMASS, a modified mobile Ion Trap Mass Spectrometer [1] (Fig. 2). (Alternate sampling environments have included a coal combustor, an open air chemical release, a micro-scale laser pyrolysis chamber [2], and in-building ambient atmospheres.) Vapor samples were obtained repetitively at 60 s intervals, allowing for an isothermal GC separation of various components (Figs. 3 & 4). A ~1.5 s vapor sample allowed up to 100 μ l (@STP) of reactor atmosphere to enter the transfer line for chromatographic separation. A .18 mm ID transfer line with a .4 μ m DB-5 coating (J+W Scientific) was operated isothermally at either 25, 80 or 125 C. Carrier gas (helium) velocity was ~2.7 m/s (at column temp=25 C and 4400 ft elevation). All analyses were performed using electron impact ionization.

Detection limits have been established near the 1 ppb level for various substituted aromatic compounds using this (MINITMASS) system and taking advantage of the Axial Modulation* and Selective Mass Storage* features to improve sensitivity [3]. Estimations of concentration in this presentation are provided from the calibration data obtained on toluene, ethylbenzene, o-, m-, and p-xylene [4] and general sensitivity characteristics of ion trap mass spectrometers for the compounds of interest.

In the present analyses, halogen- and oxygen-substituted aromatics have been detected at < 100 ppb (Fig. 5). Various column temperatures allowed for analysis of compounds with atmospheric boiling points from 80 (benzene) to 350 C (anthracene) present in reactor vapors (Fig. 6). To date, test analyses of high boiling compounds than these have required the reactor be run under nitrogen to prevent rapid column degradation, though compounds as involatile as dibenzanthracenes (bp 550 C) have been detected under test conditions.

ACKNOWLEDGEMENTS

The authors are indebted to JoAnn Lighty, Geoff Silcox, Eric Eddings and David Pershing for providing the soil desorber samples. Financial support of the work reported here was provided by the Advanced Combustion Engineering Research Center (funded by NSF, the State of Utah, 23 industrial participants, and US DOE), the DOD Chemical Research Development Engineering Center (CRDEC) and Finnigan-MAT Corporation.

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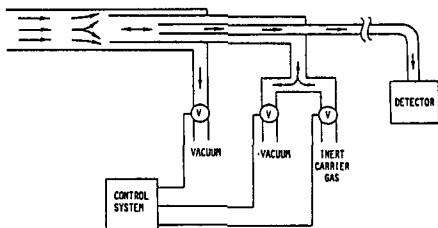


Fig. 1 Schematic drawing of vapor sampling inlet operation.

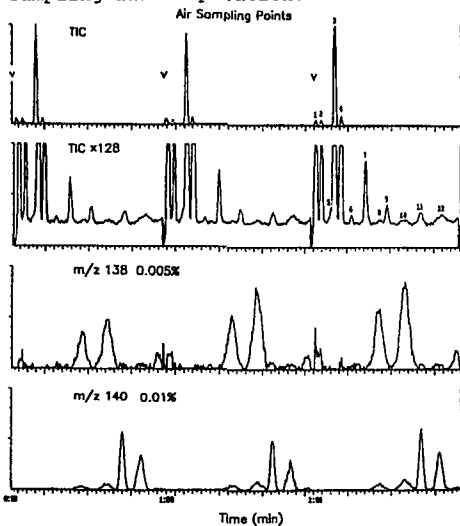


Fig. 3. Repetitive air samples and chromatographic separation of products from 0.5 wt. % ethylbenzene on 600 g of test soil. The m/z 138 and m/z 140 traces correspond to chlorostyrene and chloroethylbenzene, respectively. Products illustrated are: 1) benzene, 2) toluene, 3) et-benzene, 4) styrene, 5) Cl-benzene, 6) C_9H_{12} , 7) benzaldehyde, 8) Cl-styrene, 9) benzofuran, 10) Cl-styrene, 11) Cl-et-benzene, 12) Cl-et-benzene.

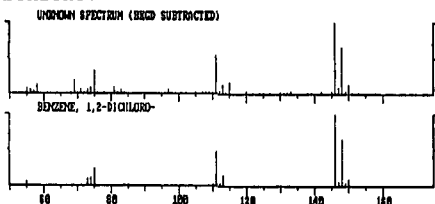


Fig. 5 Example library search for previously unknown dichlorobenzene peak, detected at approx. 10 ppb.

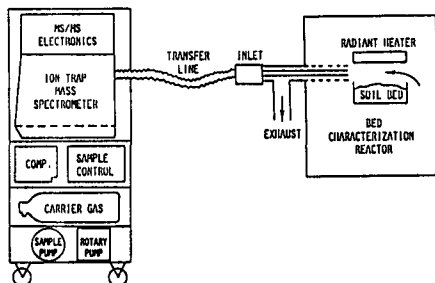


Fig. 2 Illustration of bed char-acterization sampling configuration.

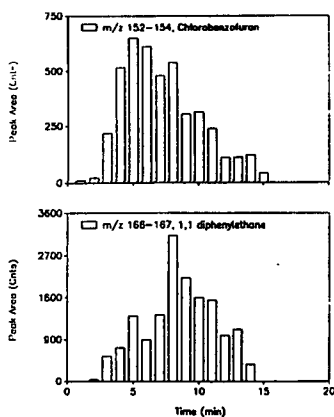


Fig. 4 Time evolution profiles of 2 desorption products.

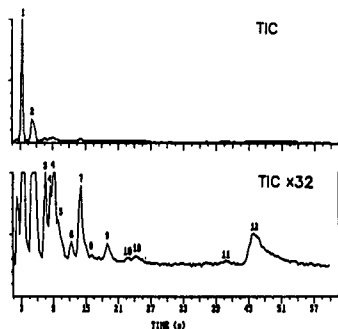


Fig. 6 Total ion trace for desorption of products from a coal tar contaminated soil. Products are naphthalenes (1,2,4&5), biphenyls (3,6,7&10) and other PNA's.

INLET SYSTEM FOR AUTOMATED GC/MS ANALYSIS OF COMPOUNDS IN
AMBIENT AIR AS COLLECTED IN STEEL CANISTERS

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The use of SUMMA Passivated stainless steel canisters in ambient air monitoring is becoming increasingly popular because of the relatively good compound stability and recovery it affords. Presently, however, methodology for the quantitative analysis of canister samples by GC/MS has not been fully developed.

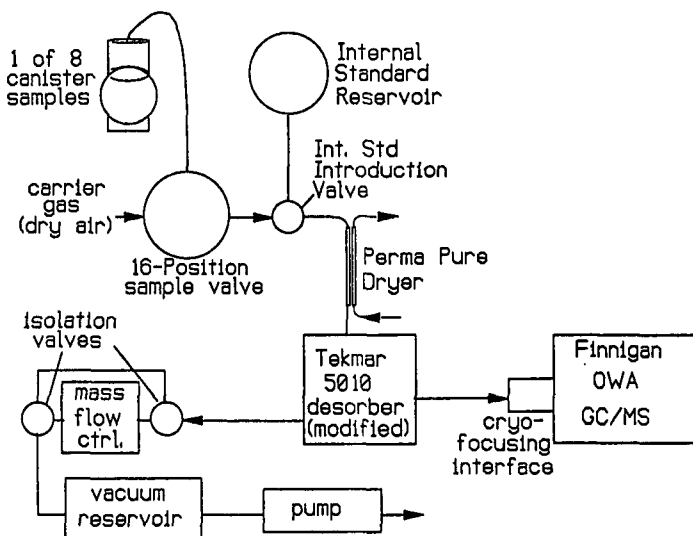
An automated inlet system has been developed which has been demonstrated to provide quantitative transfer of sample to a cryogenic trapping system at pressures above 0 psig (atmospheric pressure). The system incorporates a 16-position sampling valve where odd positions are plumbed to a zero air source to allow system purging. Sample is drawn into a modified Tekmar 5010 cryotrap/desorber using a vacuum system located downstream. Sample volumes are obtained by integrating the signal from a precision dual sensor mass flow controller also located downstream of the cryotrap. Internal standard is introduced via a 3-way solenoid valve located next to the 16-port valve.

The system is controlled using a PC/XT computer. External control is accomplished using an interface card developed in-house which contains a 12-bit A/D to digitize data received from the mass flow controller and 4 thermocouples. A D/A is also used to control a valve internal to the mass flow controller to obtain optimum sample flow rates at variable canister pressures. A separate relay board allows the operation of up to 7 valve solenoids and 3 heaters for closed loop temperature control. Additional relays allow the sample canisters to be heated individually if necessary to boost recovery of polar compounds.

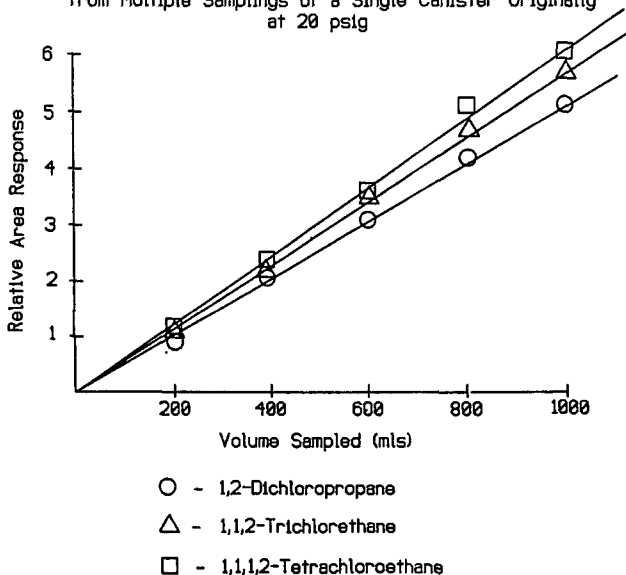
The system operates under the control of "descriptor files" which control the sequence of events during analysis. Descriptor files can also be generated to perform multiple canister background testing in a single analytical run using a combined effluent technique, or to perform automated leak checking. The control software saves all of the runtime parameters at the end of each sequence as well as sequence and run termination events to increase the level of QA/QC and decrease the occurrences of the "what happened?" scenario.

Preliminary data shown on the following page indicates that the system does provide quantitative sampling of canisters with differing pressures. The first aliquot of 200 mls was taken while the canister pressure was 20 psig. After the final 1000 ml aliquot was removed, the pressure was about 13.3 psig and yet the last data point was within $\pm 5\%$ of theoretical for a linear curve. Data obtained for subatmospheric canister sampling yielded a non-linear curve after multiple samplings. This curve differs for different manifold positions so it is assumed that our 16-port valve leaks under vacuum. A solution to this problem is being investigated.

FLOW DIAGRAM OF AUTOMATED INTERFACE



GC/MS Response of Selected Chlorinated Hydrocarbons from Multiple Samplings of a Single Canister Originally at 20 psig



**REAL-TIME, IN-SITU POINT MONITORING
BY ION TRAP MASS SPECTROMETRY**

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A transportable GC/ion trap mass spectrometer has been developed at Los Alamos National Laboratory to (1) identify and quantify hazardous organic compounds at chemical waste sites, (2) determine site boundaries, (3) document remedial activities, and (4) monitor the site after remediation. The instrument analyzes air, water, or soil samples for volatile organic compounds (VOCs) from part-per-billion (ppb) to part-per-million (ppm) levels and has been designed to mount in a 4x4 vehicle.

The transportable instrument consists of specialized sampling modules for air, soil, or water samples; a separations module containing sorbent traps and a megabore capillary column; and a detection module -- the Finnigan Ion Trap Detector. Different compound classes are accessed by selection of the appropriate separations module. Two sampling loops, which include a field interchangeable sampling module and sorbent trap, minimize dead-time between analyses by providing continuous sampling. Soil or water samples are purged with helium and the evolved organics are concentrated on a sorbent resin. A sampling pump is incorporated for air samples. After sample collection, the sorbent trap is ballistically heated and backflushed with helium to inject the organics onto the capillary column. The GC column is contained within a temperature programmed oven; carrier gas velocity is regulated with a mass flow controller. The sampling module, separations module, and associated hardware occupy a 18" x 18" x 5" box mounted on the top of ITD cover. All components are easily accessed for field maintenance. Heaters, valves, oven cooling fan, and mass flow controller are controlled through the ITD data system from an auxiliary microprocessor using FORTH and machine language.

A Finnigan MAT Ion Trap Detector was modified by removing the teflon RF ring spacers and replacing the standard 50 L/s turbomolecular pump with a 240 L/s turbomolecular pump. The fused silica capillary column was directly coupled to the ITD. Electron impact ionization was used with Automatic Gain Control (AGC). The Finnigan software was modified through the addition of key-stroke sequences and other FORTH routines. Instrument tuning, calibration, data acquisition and analysis are automated. The turn-key operating system allows the instrument to be used by minimally trained personnel.

The 24 priority pollutants in Purgeable Standards A and B can be correctly identified, quantified, and reported in a 10 minute automated sequence. A total ion chromatogram from 5 mLs water containing 50 ppb by weight A&B purgeables is shown in Figure 1. The mass chromatogram of trichloroethylene (m/z 130) obtained from 5 mLs water containing 10 parts-per-trillion A&B purgeables is shown in Figure 2. The corresponding sample mass spectrum and the library reference spectrum for trichloroethylene are shown in Figure 3.

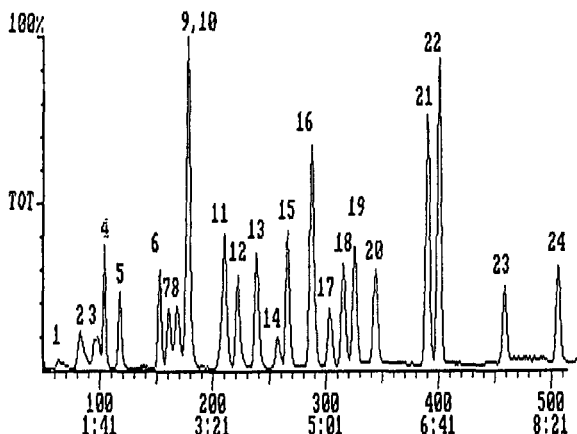
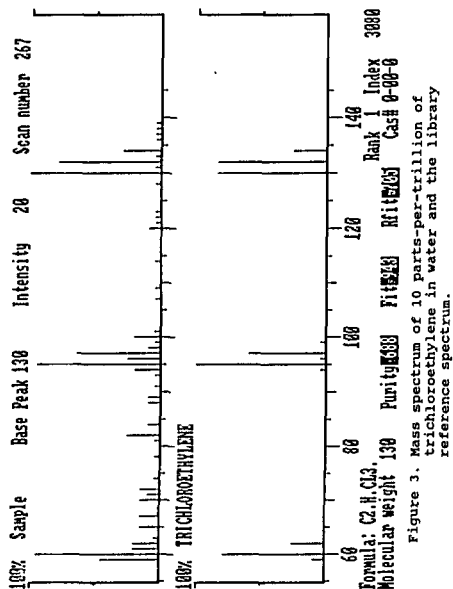
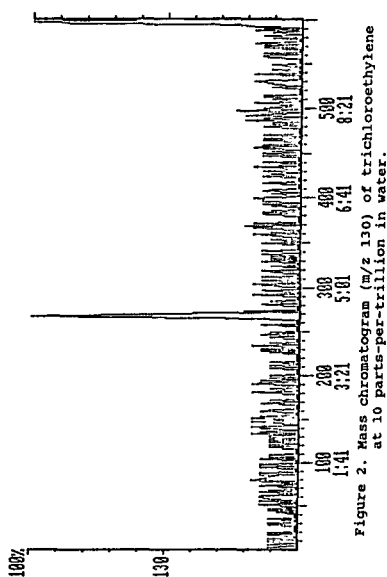


Figure 1. Total Ion Chromatogram of 50 ppb of Purgeable Standards A & B in Water.

- | | |
|-------------------------------------|---------------------------------------|
| 1. Fluorotrichloromethane | 13. Bromodichloromethane |
| 2. 1,1-Dichloroethylene | 14. 2-Chlorovinyl ether |
| 3. Methylene chloride | 15. <i>trans</i> -1,3-dichloropropane |
| 4. <i>trans</i> -1,2-dichloroethene | 16. Toluene |
| 5. 1,1-Dichloroethane | 17. <i>cis</i> -1,3-dichloropropene |
| 6. Chloroform | 18. 1,1,2-Trichloroethane |
| 7. 1,1,1-Trichloroethane | 19. Tetrachloroethene |
| 8. Carbon tetrachloride | 20. Dibromochloromethane |
| 9. Benzene | 21. Chlorobenzene |
| 10. 1,2-Dichloroethane | 22. Ethylbenzene |
| 11. Trichloroethene | 23. Bromoform |
| 12. 1,2-Dichloropropane | 24. 1,1,2,2-Tetrachloroethane |



APPLICATION OF MASS SPECTROMETRY TO
INDUSTRIAL CENTRAL ATMOSPHERIC MONITORING

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INTRODUCTION

Several techniques are available for near real-time detection of ppm and sub-ppm levels of hazardous vapors in air. However, mass spectrometry provides nearly universal detection capability, high sensitivity and wide dynamic range in one instrument. Mass spectrometry is utilized in the recently developed Industrial Central Atmosphere Monitoring System (ICAMS). This paper describes two areas important to its development: sample acquisition and sample introduction and ionization.

SAMPLE ACQUISITION SYSTEM (SAS)

Mass spectrometric instrumentation is typically used in central analyzer applications not only for technical reasons but also for economic reasons. A central analyzer requires sample transport from the multiple sampling locations. The process of providing a cost effective detection system begins with the development of a sample acquisition system that can: 1) sample many locations, 2) minimize sample transport time to the central analyzer by providing continuous flow on all sample lines, and 3) minimize the probability of interaction between sample molecules and sample lines.

A rotary valve with a novel sampling arrangement has been developed. It permits continuous flow on 50 sample lines and minimizes interactions between the gas sample and valve components. The rotary valve provides several advantages over manifolded solenoid valves: 1) reduced hardware and labor costs, 2) reduced maintenance costs, 3) improved reliability, 4) decreased space requirements, and 5) decreased system response time. Figure 1 is an illustration of the 50 port rotary valve and Figure 2 is a detailed illustration of the rotary valve/sample line interface. Only the central portion of the sample stream is selected for analysis. This minimizes channel cross-talk.

The use of teflon sample lines and filters minimizes sample molecule - sample line interactions throughout the system.

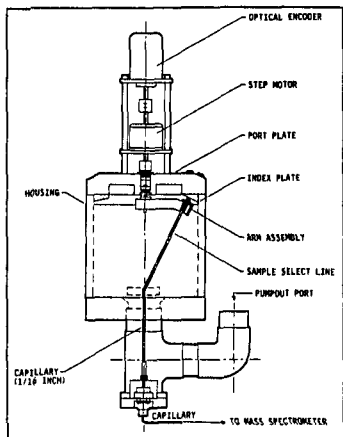


Figure 1. Rotary Valve

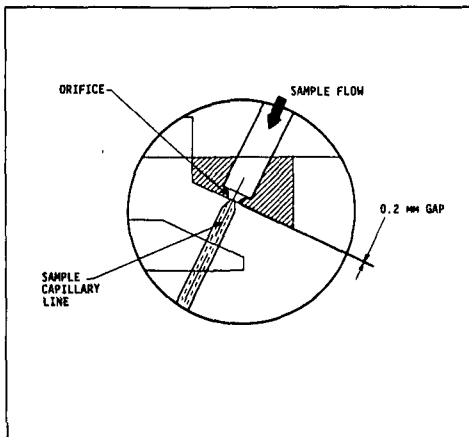


Figure 2. Rotary Valve Interface

SAMPLE INTRODUCTION and IONIZATION

Sample conversion, decomposition, and absorption can also occur in the mass spectrometer inlet and ion source. The probability of these processes occurring is decreased by minimizing the opportunity for gas molecule surface reactions in the analyzer. Increased mean free path and decreased surface area reduces these interactions.

The dual capillary inlet, Figure 3, ensures that the gas molecules "see" only teflon until within the ion source. The first capillary provides a pressure reduction from atmospheric to between 4 and 100 Torr and maintains a high gas flow rate for rapid equilibration of adsorbed species. The second capillary reduces the pressure and admits gas directly into the ionization region.

The "open" ion source configuration, Figure 4, eliminates the effects of surface interactions. Gas leaving the dual capillary inlet enters in a forward directed beam with high molecular density compensates for the loss of sensitivity associated with open or not differentially pumped source configurations. The probability of a gas molecule reacting with part of the ion source structure and returning to the ionization region is virtually eliminated. Thus, surface reaction products are minimized.

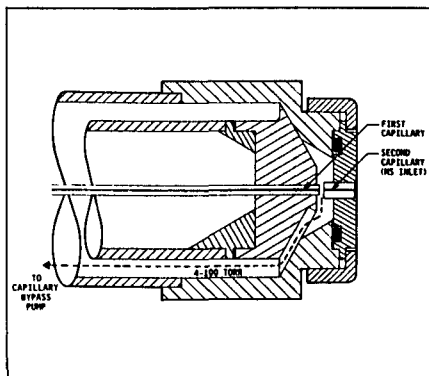


Figure 3. Dual Capillary Inlet

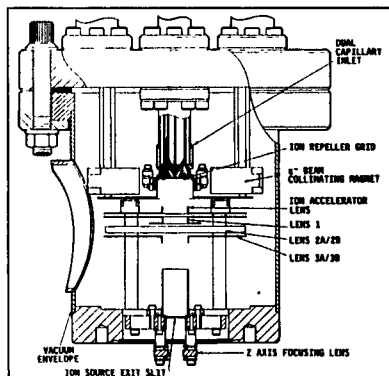


Figure 4. Open Ion Source

CONCLUSION

The rotary valve, dual capillary inlet and an open ion source in a high performance mass spectrometer based system enables the creation of a central atmospheric monitor for a variety of industrial environments. Over 200 chemicals are detectable by ICAMS. The system is configured to monitor 26 chemicals for any one application. Table I illustrates some of those chemicals.

Table I - Selected Chemicals

CHEMICAL	LEVEL TESTED	TLV	CHEMICAL	LEVEL TESTED	TLV
ACROLEIN	0.5 ppm	0.1	FORMALDYHYDE	1 ppm	1
ARSINE	0.05	0.05	HYDROGEN	98	---
BENZENE	0.13	10	METHYL ACRYLATE	2.5	10
CHLORINE	0.5	1	OXYGEN	19.6%	---
CRESOL	13	5	SILANE	1.2	5
DIBORANE	0.5	0.1	VINYL ACETATE	2.5	10
DIESEL FUEL	10	---	XYLENE	4	100
DIGLYME	1.6	---			

MASS SPECTROMETRIC ANALYSIS OF
SOME CHLOROALKYL PHOSPHATE
FLAME RETARDANTS

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During a study of the lower Mississippi River and its major tributaries, several chloroalkyl phosphate flame retardants were identified in surface water samples using gas chromatography (GC) electron impact (EI) ion trap mass spectrometry. The identifications were confirmed by matching the GC retention time (on a 30 meter, 0.25 mm i.d., 0.25 μ m film DB-5 J & W capillary column with helium at 35 cm/sec as the carrier gas) and the EI mass spectra (with electron energy, 70 eV and emission current, 0.25 μ A) with authentic standards analyzed under identical conditions on both the ion trap detector and TSQ-46 mass spectrometer. The selected compounds included tris(2-chloroethyl) phosphate, tris (chloroisopropyl) phosphate, and tris (dichloropropyl) phosphate.

The standards were reanalyzed in order to develop a rapid analysis utilizing tandem mass spectrometry. When analyzed by the traditional technique of electron ionization, no molecular ion was observed. Except tris(2-chloroethyl) phosphate, listed in the NBS Library as "ethanol, 2-chloro-, phosphate (3:1)", the spectra of these selected compounds were not present in the NBS Library of EI reference spectra. The selected compounds were ionized by positive ion chemical ionization with methane as the reagent gas at 0.25 torr. Although a protonated molecule was produced, some fragmentation occurred. For tandem mass spectrometry analysis based on the molecular ion, maximum sensitivity requires maximum production of the molecular ion. The compounds were then ionized by positive ion chemical ionization by using isobutane as the reagent gas. Under these conditions, only the protonated molecule was produced, ideal for tandem mass spectrometry analysis.

The protonated molecules, containing the ^{35}Cl isotope only, were subjected to collision activated decomposition by using argon as the collision gas. The resulting daughter spectra were produced at 15 eV collision energy and 1.5 mTorr collision gas pressure. The daughter ion spectra included sequential losses of the intact constituents from the phosphate ion. For example, the protonated molecule of tris (chloroisopropyl) phosphate, at mass 327, lost sequential chloroisopropyl units of 76 daltons, to produce strong daughter ions at masses 251, 175, and finally 99.

Variations of collision energy from 15 to 30 eV and collision gas pressure from 1.0 to 2.5 mTorr were not effective in producing one prevalent neutral loss from each protonated molecule, which would have enabled a very sensitive, specific analysis utilizing simple neutral loss scans. It was also not possible to maximize the generation of the final daughter ion at mass 99, which would have been an excellent analysis utilizing parent scans for this compound class of chloroalkyl phosphates. Therefore, a technique for the analysis of these flame retardant compounds by using isobutane positive ion chemical ionization followed by tandem mass spectrometry utilizing a neutral loss analysis was not sensitive.

DETECTION OF TRACE ORGANICS IN AIR USING THERMAL DESORPTION ITMS

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Introduction

A thermal desorption apparatus has been interfaced directly with a Finnigan ion trap mass spectrometer (ITMS) for the rapid detection and quantification of trace organics in air. No chromatographic separation of components is performed prior to introduction into the mass spectrometer. Instead, target compounds are ionized by chemical ionization (CI) and selective mass storage is used to isolate the $(M+H)^+$ ion for subsequent fragmentation by collision induced dissociation. Quantification is performed by integration of the thermal desorption profile using the characteristic daughter ions for the compound of interest. Using this technique, as little as 50 pg of a constituent desorbed from a resin trap can be identified and quantitated with a sample turn around time of only 2-3 minutes. Some applications thus far have included the detection of part-per-trillion levels of organo-phosphonates in air and the quantification of nicotine in environmental tobacco smoke (ETS).

Experimental

The thermal desorption apparatus used for these experiments was designed and constructed at Oak Ridge National Laboratory. It is designed to accept resin traps 3 inches long and 0.25 inches diameter with a 1-1.5 inch bed of resin. Thermal desorptions are performed by heating the resin traps with 450 watts of power for 10 seconds while purging with helium at a flow rate of 20-25 ml per minute. Effluent from the desorber is admitted into the ITMS through an open-split interface with a split ratio of approximately 100:1 as shown in Figure 1. A 16 unit automated version of the thermal desorption apparatus has recently been completed. In addition to the ability to perform direct thermal desorptions into the ITMS, this system also features hardware necessary for cryofocussing or capillary column dynamic focussing of desorbed constituents.

For the experiments described here, the ITMS was used in the selective mass storage mode and isobutane was used as the CI reagent. Ionization times were typically 10 msec and CI reaction times ranged from 50-100 msec. MS/MS spectra were generated for the target compounds and comparative spectra were acquired in each case using authentic standards.

Results and Discussion

Typical thermal desorption profiles are shown in Figures 2a and b. The top profile corresponds to the total ion current for the dissociation of m/z 163 from a sample of ETS. The bottom trace shows the actual profile for nicotine generated by plotting the extracted ion current for m/z 84, which is a characteristic daughter ion of protonated nicotine (m/z 163). In this case, no sample focussing was used, yet the peak shape was excellent and the width at half height was only 15 seconds. A response curve ranging from 300 pg - 50 ng of desorbed nicotine is shown in Figure 3. It was generated by plotting the sum of the integrated ion currents for characteristic daughter ions at m/z 84, 106, 120, and 132. No internal standards were used for the quantitative determinations yet the correlation coefficient for the calibration curve was 0.9996 and reproducibility for replicate samples was approximately $\pm 15\%$. Because of the low detection limits that can be achieved for nicotine, air samples can be collected on resin cartridges by sampling approximately 1 liter of air and then analyzed in less than 5 minutes. A comparative study of this method with a standard gas chromatographic method for determining nicotine in ETS has shown that the ITMS method has better detection limits and is considerably faster.

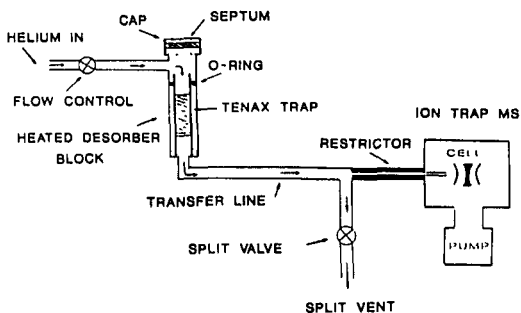
*Research sponsored jointly by the Office of the Program Executive Office - Program Manager for Chemical Demilitarization under Interagency Agreement 1769-1757-A2 and the National Cancer Institute under Interagency Agreement 0485-0485-A1 with Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

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Results for organo-phosphonate compounds are very similar to those for nicotine. Detection limits as low as 50 pg of either di-isopropylmethyl phosphonate (DIMP) or dimethyl-methyl phosphonate (DMMP) have been demonstrated and linear calibration curves have been generated from 50 pg-5 ng of desorbed material. Sample turn around of less than 2 minutes has also been accomplished for these compounds.

Direct Thermal Desorption ITMS

Figure 1



Chromatogram C:A1367 Acquired: Jan-21-1989 13:27:43
Comment: Daughter ion MS/MS scans of nicotine Isobutane CI TD sample a136.7
Scan Range: 1 - 125 Scan: 35 Int = 665 @ 1:34 100% = 17280
100%

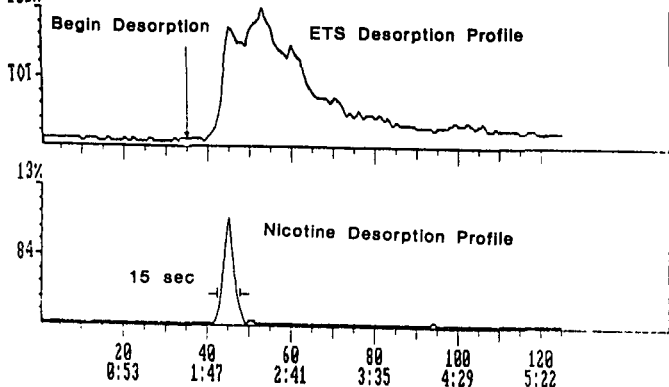
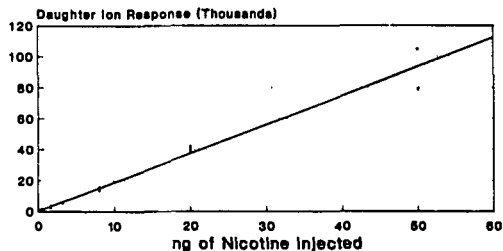


Figure 2a

Figure 2b

Nicotine in Air Direct Thermal Desorption ITMS

Figure 3



IDENTIFICATION OF O-ETHYL O'-(2-DIISOPROPYLAMINOETHYL) METHYLPHOSPHONITE
DEGRADATION PRODUCTS BY CHEMICAL IONIZATION MASS SPECTROMETRY
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The objective of this study was to develop a method for the detection and identification of products from the degradation of O-ethyl O'-(2-diisopropylaminoethyl) methylphosphonite (EDMP), which is one of the compounds used in the production of the nerve agent O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate (VX). This information is of interest from both an environmental and safety standpoint. Identification of phosphonites and other organophosphorus compounds containing the 2-diisopropylaminoethyl group is difficult on the basis of EI fragmentation alone because extensive fragmentation results in little or no molecular ion information. In this study we apply methane CI capillary column GC/MS to identify ten EDMP degradation products produced during storage.

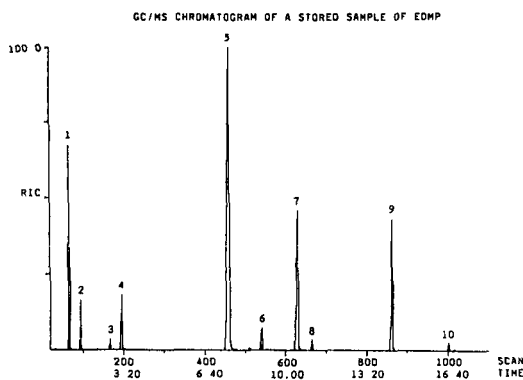
Spectra were obtained on a Finnigan 5100 GC/MS equipped with a fused silica 15m x 0.25mm ID SE-54 capillary column. Injection temp was 220°C, GC/MS interface temp was 220°C and the oven was programmed from 60°C to 220°C at 10°C/min. The CI reagent gas was methane with an internal source pressure of 0.6 Torr. The mass range was scanned at a rate of one scan per second (0.95 sec up scale with a 0.05 sec hold at the bottom) from 60-450 amu.

Figure 1 illustrates the total ion chromatogram for the stored sample of EDMP. The identification of each of the ten compounds detected is listed in Table 1. These products result from moisture-catalyzed disproportionation (1 and 9), hydrolysis (2, 4 and 6), oxidation (3 and 7) and Michaelis-Arbuzov rearrangement (8 and 10) reactions.

Table 1. Compounds Identified in a Stored Sample of EDMP

R.T. (min)	Mol. Wt.	Peak Number	Name	Structure
1.1	136	1	O,O'-Diethyl Methylphosphonite	$\text{Me-P}(\text{OEt})_2$
1.6	108	2	O-Ethyl Methylphosphinate	$\text{Me-P}(=\text{O})(\text{H})(\text{OEt})$
2.8	152	3	O,O'-Diethyl Methylphosphonate	$\text{Me-P}(=\text{O})(\text{OEt})_2$
3.3	145	4	2-Diisopropylaminoethanol	$\text{HOCH}_2\text{CH}_2\text{N}(\text{iPr})_2$
7.7	235	5	O-(2-Diisopropylaminoethyl) O'-Ethyl Methylphosphonite	$\text{Me-P}-\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ OEt (EDMP)
9.0	207	6	O-(2-Diisopropylaminoethyl) Methylphosphinate	$\text{Me-P}(=\text{O})\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ H
10.5	251	7	O-Ethyl O'-(2-Diisopropylamino- ethyl) Methylphosphonate	$\text{Me-P}(=\text{O})\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ OEt
11.1	235	8	O-Ethyl 2-Diisopropylamino- ethylmethylphosphinate	$\text{Me-P}(=\text{O})\text{CH}_2\text{CH}_2\text{N}(\text{iPr})_2$ OEt
			O-(2-Diisopropylaminoethyl) Ethylmethylphosphinate	$\text{Me-P}(=\text{O})\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ Et
14.4	334	9	O,O'-Bis(2-Diisopropylamino- ethyl) Methylphosphonite	$\text{Me-P}\{\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2\}_2$
16.7	334	10	O-(2-Diisopropylaminoethyl) 2-Diisopropylaminoethyl- methylphosphinate	$\text{Me-P}(=\text{O})\text{CH}_2\text{CH}_2\text{N}(\text{iPr})_2$ $\text{OCH}_2\text{CH}_2\text{N}(\text{iPr})_2$

Figure 1



Methane chemical ionization provides the valuable molecular and fragment ion information necessary to identify these compounds. Table 2 summarizes the characteristic CI fragmentation ions observed. The phosphonites containing the 2-diisopropyl-aminoethyl (R) group are unusual in that formation of (M-1)⁺ ions were observed rather than (M+H)⁺ ions. EDMP and compounds 6,7,9 and 10 have as their base peak the ion at m/z 128. The absence of this ion for peak 8 suggests that the Michaelis-Arbusov rearrangement forms MeP(O)(OEt)(R) rather than MeP(O)(OR)(Et). The P-R bond cleaves much less readily than the O-R bond.

Table 2. CI Fragmentation Observed for EDMP Decomposition Products

M/Z	Proposed Structure	Relative Intensity (% of Base Peak)									
		Peak Number									
		1	2	3	4	5	6	7	8	9	10
M+1	(M+H) ⁺	100	100	100	100	1	62	63	100		10
M-1	(M-H) ⁺	11			7	13	3	4	8	2	2
M+29	(M+C ₂ H ₅) ⁺	6	4	10	9	1		1	19	1	
M+41	(M+C ₃ H ₅) ⁺	12	4	4	3				6		
M-15	(M-CH ₃) ⁺	51		1	8	23	7	8	14		4
81	MeP[OH ₂] ⁺ [OH] <-> MeP[OH] ⁺ [OH]H	35	28								
91	MeP=OEt ⁺	92									
97	MeP[OH] ⁺ [OH][OH]				2						
109	MeP[OH ₂] ⁺ [OEt] <-> MeP[OH] ⁺ [OEt]H	66				7					
114	(iPr) ₂ N=CH ₂ ⁺				5	8	4	4	12	3	1
125	MeP[OH] ⁺ [OEt][OH]			9							
128	(iPr) ₂ N ⁺ (CH ₂) ₂				13	100	100	100		100	100
146	(iPr) ₂ NHCH ₂ CH ₂ OH ⁺		(100)				8			2	1
190	MeP=OCH ₂ CH ₂ N(iPr) ₂ ⁺					33				9	
192	MeP[O] ⁺ [CH ₂ CH ₂ NH=C(CH ₃) ₂][OEt]									17	

ANALYSIS OF CHEMICAL WARFARE AGENTS IN A COMPLEX MATRIX BY GC-MS AND GC-MS/MS

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Chemical weapons, although prohibited by the 1925 Geneva Protocol, have been used recently in several armed conflicts, including the Iran/Iraq war (1). Verification of chemical agent use has often been difficult, due principally to inadequate sampling and identification procedures. Gas mask canisters from protected troops at the scene of a chemical attack may be used as a sampling device. Recent international interlaboratory exercises indicated chemical warfare agent detection difficulties associated with the complexity of the battlefield volatiles. This background matrix contained numerous hydrocarbons characteristic of diesel exhaust emissions (Table I). A capillary column GC-FID, GC-MS and GC-MS/MS study was initiated with the principal objective being the development of methods for the trace detection of sarin (GB), soman (GB) and mustard (H) in the presence of numerous interfering diesel exhaust components.

Air from a diesel exhaust environment was sampled through a Canadian C2 charcoal canister for four hours at the typical working respiratory rate of 20 L/min. The canister charcoal (108 g) was Soxhlet extracted for six hours with 250 mL of dichloromethane and concentrated to 10 mL under a gentle stream of nitrogen. Extraction efficiency was estimated to be in the 10-15% range for GB and GD and 70% for H based on the extraction of 100 ug of agent spiked charcoal (10 g). Capillary column GC analyses were performed on Varian 3700 or HP 5890 gas chromatographs equipped with an on-column injector and a 15 m x 0.32 mm ID J&W DB-5 column under the following temperature program: 40 or 50°C (2 min) 10°C/min 280°C (5 min). GC-MS analyses (full scanning 400-35 u; 1 sec/decade) were performed on a VG 70/70E under the following conditions: a) EI (70 eV, 100 uA, 200°C, 6 kV) and b) ammonia CI (50 eV, 500 uA, 120°C, 9×10^{-5} Torr, 6 kV). GC-MS/MS analyses (source: 8 kV, 70 eV, 100 uA, 250°C) were performed on a VG 70S under the following reaction ion monitoring (RIM) conditions: a) H analysis (m/z 158 to 109 and m/z 158 to 96, CAD cell (58 eV, 5×10^{-7} Torr air)) and b) GB and GD analysis (m/z 99 to 79, CAD cell (50 eV, 5×10^{-7} Torr air)).

Diesel exhaust extracts were spiked at the 50 ng/mL, 5 ng/mL and 500 pg/mL level with GB, GD and H and analysed by GC-FID, GC-MS (EI and ammonia CI) and by GC-MS/MS under reaction ion monitoring (RIM) conditions. At the 20 ng level matrix interferences masked both GD and H during GC-FID. Figure 1 illustrates GC-MS (EI) chromatograms obtained for the charcoal extract and this extract spiked with 20 ng and 2 ng of GB, GD and H. All spiked chemical warfare agents, detected at the 20 ng level, were effectively masked by the matrix at the 2 ng level. The chemical noise associated with this matrix negated the use of selected ion monitoring. The specificity of GC-MS (ammonia CI), a technique routinely used for chemical warfare agent analysis (2-4), improved this full scanning detection limit for GB and GD to about 5 ng (ammonia CI not amenable to H analysis). GC-MS/MS under RIM conditions was the most specific and sensitive method for the confirmation of these chemical warfare agents in the presence of interferences levels two to three orders of magnitude greater than the spiked agents. Figures 2 and 3 illustrate the chromatograms obtained for H, and GB and GD respectively spiked into the extract at the 500 pg level. Sample detection limits (S/N=5:1) of 70 pg, 60 pg and 30 pg were estimated for GB, GD and H based on this data.

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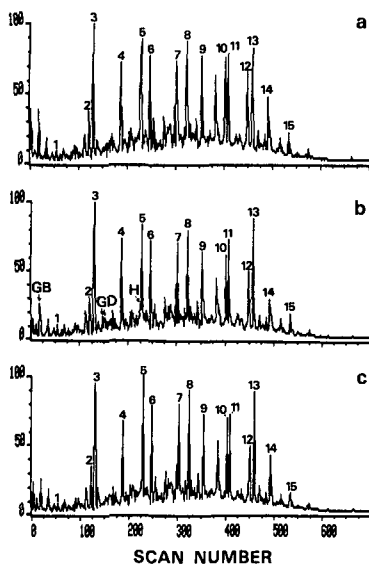


FIGURE 1

Capillary column GC-MS(EI) chromatograms of a) dichloromethane extract of the equivalent of 1.9×10^4 m³ of air sampled onto the charcoal of a C2 canister, b) the sample extract spiked with 20 ng of sarin (GB), soman (GD) and mustard (H) and c) the sample extract spiked with 2 ng of GB, GD and H. Principle sample components are identified in Table I. (15 m x 0.32 mm ID J&W DB-5; 40°C (2 min) 10°C/min 280°C (5 min)).

TABLE I

Major components identified in airborne diesel exhaust environment sampled onto C2 Canadian charcoal canister

Chromatogram Peak No.	Molecular Weight	Compound
1	128	n-C ₉ alkane
2	142	n-C ₁₀ alkane
3,5,8,11,13		charcoal impurity
4	156	n-C ₁₁ alkane
6	170	n-C ₁₂ alkane
7	184	n-C ₁₃ alkane
9	198	n-C ₁₄ alkane
10	212	n-C ₁₅ alkane
12	226	n-C ₁₆ alkane
14	240	n-C ₁₇ alkane
15	254	n-C ₁₈ alkane

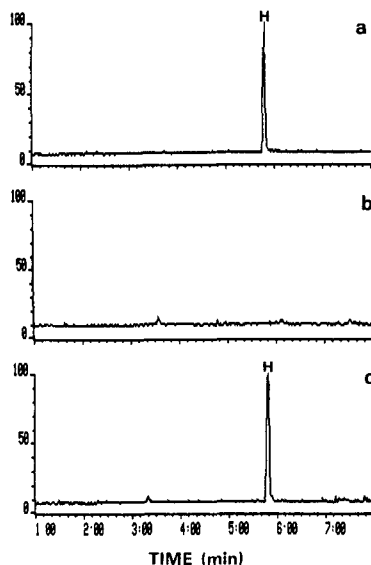


FIGURE 2

Reaction ion monitoring chromatogram for m/z 158 to 109 obtained during GC-MS/MS analysis of a) 500 pg of mustard (H), b) dichloromethane extract of the equivalent of 4.8×10^4 m³ of air sampled onto the charcoal of a C2 canister and c) the previous sample spiked with 500 pg of mustard (H) (15 m x 0.32 mm ID J+W DB-5; 50°C (2 min) 10°C/min 280°C (5 min)).

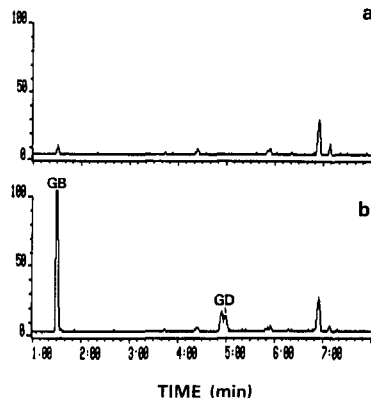


FIGURE 3

Reaction ion monitoring chromatogram for m/z 99 to 79 obtained during GC-MS/MS analysis of a) dichloromethane extract of the equivalent of 4.8×10^4 m³ of air sampled onto the charcoal of a C2 canister and b) the previous sample spiked with 500 pg of sarin (GB) and soman (GD) (15 m x 0.32 mm ID J+W DB-5; 40°C (2 min) 10°C/min 280°C (5 min)).

Use Of Benzene Charge Exchange At Atmospheric Pressure
For Low Level Detection Of Pollutants In Air*

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Analysis of trace quantities of compounds is becoming increasingly important in air pollution studies where it is often necessary to detect impurities in the concentration range of parts per billion or less. Atmospheric pressure ionization has been shown to be an extremely sensitive real time method to achieve sensitivities in the range of few tens of parts per trillion. In atmospheric pressure ionization use is made of the proton transfer reaction between the hydronium ion (H_3O^+) and the analyte of interest (M) to produce the protonated molecular ion MH^+ . This reaction depends critically on the proton affinity of the analyte of interest. Analytes with low proton affinities, like the organo-sulfur compounds, can not be detected by the atmospheric pressure ionization technique. The analytes with low proton affinity can be detected using atmospheric pressure ionization technique if one uses a charge exchange reaction with a reagent ion rather than the conventional proton transfer reaction with the hydronium ion.

We report here the use of benzene charge exchange, at atmospheric pressures, for the low level detection of 2-chloroethyl ethyl sulfide. All experiments were performed using an EXTREL triple quadrupole mass spectrometer system with an atmospheric pressure ionization source. A vapor generator based on a heated nebulizer was used to generate low levels of 2-chloroethyl ethyl sulfide in air. Concentrations as low as a part per trillion were accurately generated using such a vapor generator.

Tests were performed to measure the response of the system to varying concentration of 2 chloroethyl ethyl sulfide in air, in the range of 40 ppt to 600 ppt. Fig. 1 shows the response of the system to varying concentrations of 2-chloroethyl ethyl sulfide in air. Fig. 2 shows the linearity plot for the system response. Regression analysis using 95% confidence bounds was performed on the data. The

analysis indicates a statistical detection limit of 13 ppt using a corona discharge source, operating at atmospheric pressure, coupled to an EXTREL triple quadrupole mass spectrometer.

* This work was supported by The Office Of The Program Manager
For Chemical Demilitarization, Aberdeen Proving Ground, Edgewood,
MD under Contract No. DAAA15-86-C-0107

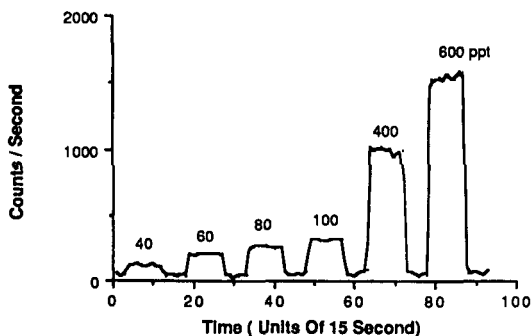


Figure 1

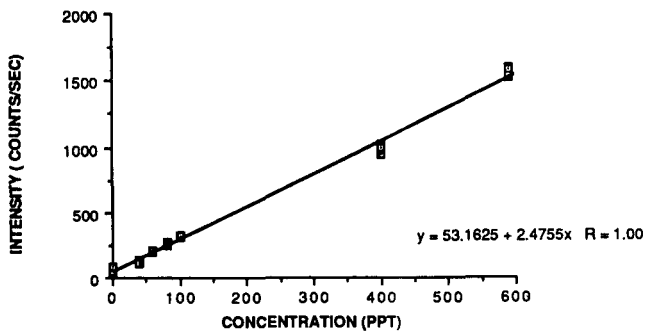


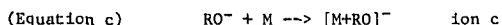
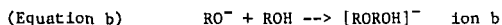
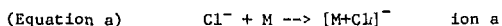
Figure 2

FORMATION OF ADDUCT IONS IN THE METHANE ENHANCED NEGATIVE ION
MASS SPECTROMETRY OF ARYL-N-METHYLCARBAMATES

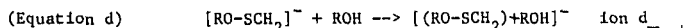
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Because carbamate pesticides are in widespread use, there is substantial interest in the development of analytical procedures for their qualitative and quantitative identification. The thermal instability of this class of compounds may be minimized by careful attention to the analytical procedure employed. The thermal instability may be eliminated by preparing the trifluoroacetyl (TFA) derivative. In our study capillary gas chromatography coupled with methane enhanced negative ion chemical ionization mass spectrometry (NICIMS) was investigated as an analytical technique for the identification of seven aryl-N-methylcarbamate pesticides (ROCONHCH_3 , where R=aryl).

Each carbamate was introduced separately through a capillary GC column into the high pressure CI source of a Hewlett Packard 5988A quadrupole mass spectrometer. The instrument was tuned in negative ion mode using perfluorotributylamine (PFTBA). Methane was used as the buffer gas and the source temperature was held at 100°C. The negative ion mass spectrum of Sevin (Carbaryl) illustrated in Figure 1a is typical of the NCI mass spectrum recorded for the aryl-N-methylcarbamates in the present study. In addition to the $[\text{M}-1]^-$ and RO^- ions, ions a, b, and c at m/z values greater than that of the parent ion, are noted. Possible ion/molecule reaction pathways responsible for formation of these adduct ions are:



Residual chloride ions are noted in our mass spectrometer source under the experimental conditions employed and result in the formation of ion a as described in Equation a. Ion/molecule reactions resulting in the formation of adduct ions similar to that proposed in Equation c have been observed previously under positive chemical ionization (PCI) conditions for heterocyclic compounds¹ and under NCI conditions for oxime carbamates.² Adduct ions of the type b (as well as of type d, see below) are observed for the first time under high pressure NCI conditions. The proposed ionization/neutralization/ionization pathway implied in Equation b for the formation of ion b is supported by the methane enhanced NCI mass spectrum of Methiocarb (Mesurol) illustrated in Figure 1b. In addition to adduct ions of the types a, b, and c, a fourth adduct ion at m/z 289 is noted in the spectrum of Methiocarb whose formation via Equation d is proposed:



As an alternative to the neutralization implied in equations b and d for the formation of ROH, fragmentation of ion c to form ion b or of ion b to form ion d, cannot be unequivocally eliminated.

It must be demonstrated that the adduct ions observed in the NCI mass spectra of these compounds are attributed solely to reactions occurring in the high pressure mass spectrometer source. EI spectra were obtained under the same GC conditions as for chemical ionization at the same retention times as for NCI mode and agreed in all respects with the published EI spectra for the compounds. The positive ion, methane enhanced chemical ionization mass spectra for these compounds also agreed with published data and again were recorded at the same RT as before.

Finally, in order to corroborate that aryl-N-methylcarbamates can readily undergo reactions in the high pressure chemical ionization source, Sevin was introduced into the source at 100°C through the GC simultaneously with the introduction of pentachlorophenol through the DIP. In the resulting negative ion spectrum shown in Figure 2 ions appear at m/z values 179, 395, and 431. These ions are not present in the NCI of either Sevin

or pentachlorophenol and so must be attributed to ion/molecule reactions between the two compounds occurring in the mass spectrometer source.

It is of interest to note that under the same experimental conditions (source temperature, source pressure, GC conditions, and sample concentration), no adduct ions are observed in the NCI of the seven trifluoroacetyl derivatives of these carbamates. The methane enhanced NCI of the Sevin-TFA derivative is reproduced in Figure 3. Apparently, the substitution of the N-H hydrogen of the carbamate with COCF_3 moiety in the derivative eliminates the ion/molecule reactions responsible for adduct ion formation.

The conclusion may be drawn that for certain classes of compounds, such as N-methylcarbamates, conditions prevailing in the high pressure chemical ionization source are favorable for ion/molecule reactions in which ion fragments derived from the substrate react with the neutral substrate. Additional ion/molecule reactions may follow the neutralization of some ionic fragments from the substrate.

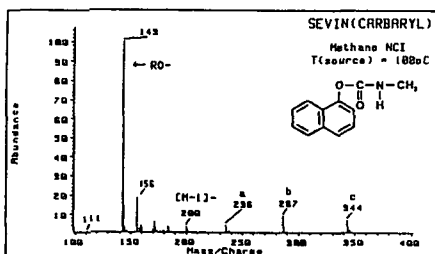


Figure 1a.

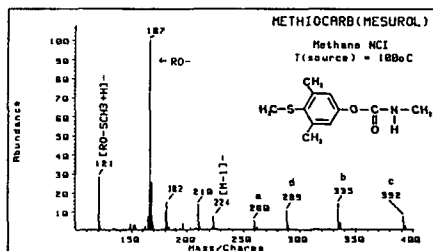


Figure 1b.

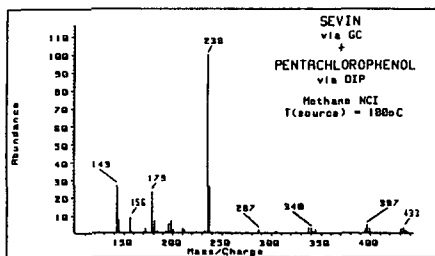


Figure 2.

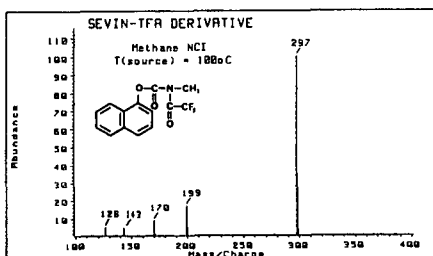


Figure 3.

1. C.A. Maryanoff, G.W. Caldwell, and S.Y. Chang, *Org. Mass Spectrom.*, **23**, 129 (1988).
2. B.C. Lynn, G.D. Marbury, and J.R. Tusshall, *Org. Mass Spectrom.*, **23**, 736 (1988).

CONFIRMATION OF ETHYLENETHIOUREA (ETU) RESIDUES IN FOODS BY CAPILLARY GC/TANDEM MASS SPECTROMETRY

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Division of Chemical Contaminants, FDA, Washington, DC 20204

Ethylenethiourea (ETU) is a degradation product of ethylenebisdi-thiocarbamate fungicides. Over 130 million pounds of these fungicides are applied annually to agricultural crops throughout the world. Concern that ETU may be an oncogen prompted the FDA to monitor the food supply for ETU.

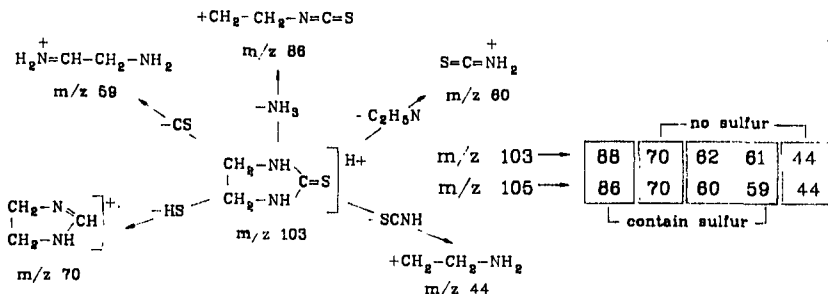
The recent introduction of a new high pressure liquid chromatographic assay employing an electrochemical detector improves FDA surveillance for ETU by circumventing many of the problems associated with previous methods. The new assay determines ETU in an aqueous system without derivatization. The electrochemical detector is sensitive and selective.(1)

In spite of the selectivity of the new assay, it was still necessary to confirm alleged ETU residues by an independent technique to eliminate any chance of false positives. Several mass spectrometry techniques were evaluated from the perspective that ETU residue confirmation could be performed with either quadrupole or magnetic instruments.

The HPLC method provides an aqueous extract containing underivatized ETU suitable for chromatographic introduction to the mass spectrometer. Nitz, et al reported the use of Carbowax 20M to gas chromatograph underivatized ETU.(2) We report that capillary gas chromatography (GC) on a cyanopropyl phenyl column (DB-1701) results in a very narrow and symmetrical GC peak that clearly augments the sensitivity of the analysis.

The EI and CI spectra of ETU are characterized by abundant species indicative of molecular weight. Additional fragmentation is of low relative abundance. Methane CI of ETU is significantly more sensitive than EI in the quadrupole instruments (Finnigan 3300, 4000, TSQ-46) that we evaluated. EI is the more sensitive mode of detection with our ZAB-2F magnetic instrument. Data are presented for the quadrupole instruments in the methane CI mode and the EI mode for the magnetic instrument.

The methane CI spectrum contains little more than molecular weight information. GC/MS/MS improves the specificity of the CI analysis by providing ETU daughter ion data at the retention time of ETU. Daughter ion isotope data suggests the elemental composition of the daughter ions and aids in the interpretation of the daughter ion spectrum:



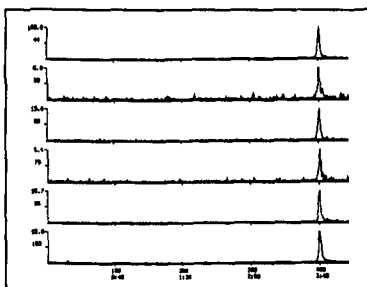
Multiple ion monitoring of the daughter ions of ETU readily confirms 10 ppb ETU in food extracts:

GC/MS/MS Conditions:

10M x 0.32mm i.d.
1u film DB-1701 J&W
inj 200C, xfer 220C
col 60-240C @ 20C/min
He carrier 140 cm/sec
methane 0.25 torr
collision cell 1.8 mtorr
collision energy -20V

MID 103 daughters:

44, 59, 60, 70, 86



Fragment ions in the EI spectrum of ETU are of low abundance relative to the molecular ion. High resolution provides the elemental compositions of the principal ions:

m/z	mass composition	error (mmu)
72	C2H2NS	0.08
73	C2H3NS	0.10
74	C2H4NS	0.09
60	CH2NS	0.07
45	CHS	0.20
43	C2H5N	0.10
43	CH3N2	0.10

Full scan low resolution EI data can provide adequate confirmation of ETU by demonstrating the absence of coeluting coextractives in a detected signal. Scan cycle time limits the sensitivity of a full scan GC/MS assay. Multiple ion monitoring is a more sensitive scan mode, but our 1979 vintage ZAB 2F is not capable of magnet stepping multiple ion monitoring under data system control. The sensitivity of the instrument for ETU in the full scan mode is 10 ng or 100 ppb.

The EI CID MIKES spectrum of the molecular ion of ETU contains broad fragment ions of low relative abundance. The majority of the detected ETU signal is carried by the parent ion. The low yield of ETU fragment ions in the EI CID-MIKES mode limits the sensitivity of this assay. The sensitivity of the instrument using multiple ion monitoring under data system control in the CID-MIKES mode is 10 ng or 100 ppb.

SUMMARY AND CONCLUSIONS

Aqueous extracts of cabbage, collards, green beans, potatoes and spinach were examined for ETU by GC/MS and GC/MS/MS. No coeluting interferences were observed at the retention time of ETU. The GC column was not affected by aqueous injections.

Capillary GC/MS/MS can provide confirmation of ETU in aqueous extracts at a concentration of 10 ppb. The instrumental conditions chosen by the analyst must consider both the capabilities of the individual instrument and the analytical behavior of ETU.

1. R. T. Krause and Y. Wang, J. Liq. Chromatogr., 11(2) 349-362 (1988)
2. S. Nitz, P. Moza and F. Korte, J. Agric. Food Chem., 30, 593-596 (1982)

MASS SPECTROMETRY OF TRIAZINES
UNDER METHANE ENHANCED NEGATIVE CHEMICAL IONIZATION CONDITIONS

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Studies on the fate of triazine herbicides in the environment have prompted the need for and the development of a sensitive and specific assay for these compounds. Because of its high sensitivity for this class of compounds and its inherent specificity, negative chemical ionization (NCI) should be useful in the screening and confirmation of triazines.

It is well documented that mass spectra obtained under electron capture, negative chemical ionization conditions are significantly dependent on a number of instrumental parameters and experimental conditions. Previous detailed NCI studies undertaken on an HP 5985B mass spectrometer (MS) have resulted in a tuning procedure and delineation of experimental parameters for this instrument. Differences in instrumental control as well as instrumental configuration between HP 5985B and HP 5988A MS made it necessary to develop a systematic tuning protocol for the HP 5988A MS operated in NCI mode under HP ChemStation control. We have demonstrated the validity of this protocol by using it to produce NCI mass spectra for decafluorotriphenylphosphine (DFTPP) with acceptable relative ion abundances for the m/z 167, 275, 365, and 442 ions. Furthermore, the instrument sensitivity was maximized by examining the variation of total negative ion current as a function of reagent gas pressure in the high pressure source of the HP 5988A MS. We are thus able to report reproducible NCI mass spectra for the *s*-triazines obtained under well defined operating conditions.

The methane enhanced NCI mass spectra of 22 triazines were acquired at ion source temperatures of 100°C and 250°C. For twenty of the compounds the M^- or $[M-1]^-$ is observed. Fragmentation patterns for $-Cl$, $-OCH_3$ and $-SCH_3$ substituted triazines are contrasted. For the $-Cl$ substituted compounds fragment ions are more intense at 250°C than at 100°C. There are, however, more fragment ions at greater intensity at 100°C than at 250°C for $-SCH_3$ substituted triazines. For the $-OCH_3$ substituted compounds most of the ion current is in the $[M-1]^-$ ion. In addition to the fragment ions observed, a number of unusual adduct ions are noted, including $[M+11]^-$, $[M+12]^-$, $[M+13]^-$, $[M+14]^-$, $[M+25]^-$, and $[M+28]^-$. An example is given in Figure 1.

The results of this study show that methane enhanced negative chemical ionization is an alternative mass spectrometric technique for qualitative analysis of *s*-triazines. The fragmentation patterns of the NCI mass spectra of these compounds were found to be a function of the substituent at the 2 position of the aromatic ring. Adduct ions were observed at abundances which could result in the misidentification of the M^- . Further investigation of the adduct ions is expected to provide their structural identities and pathways of formation in the high pressure chemical ionization source.

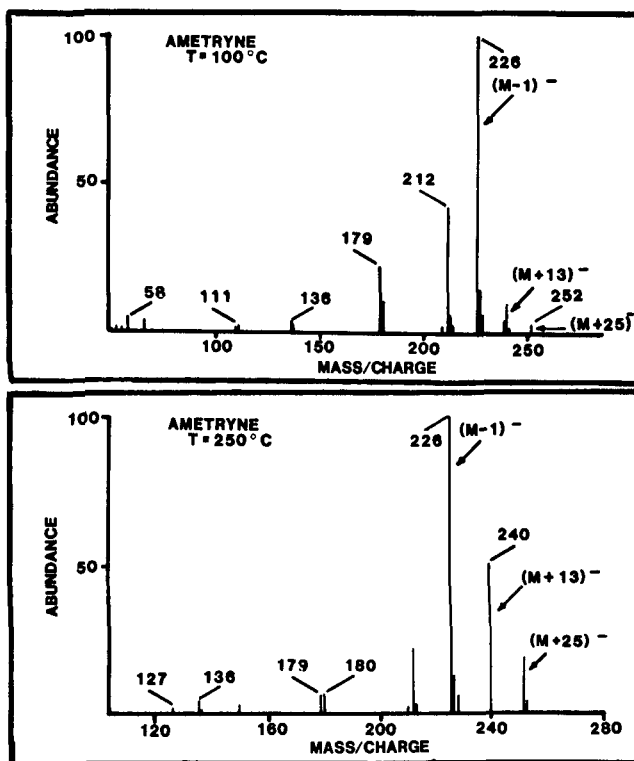


Figure 1. Methane enhanced NCI mass spectra of ametryne at source temperatures of 100°C and 250°C.

ESTIMATION OF MUTAGENIC/CARCINOGENIC POTENTIAL OF ENVIRONMENTAL
CONTAMINANTS BY ION-MOLECULE REACTIONS AND TANDEM MASS SPECTROMETRY

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Potentially mutagenic and carcinogenic chemical contaminants are recognized to be reactive electrophiles capable of modifying biological macromolecules. These represent only a small portion of the total number of contaminants in the typical environmental sample. Accurate evaluation of these chemicals to provide data for human and ecosystem risk assessment depends upon analytical procedures to isolate, identify, and quantify each chemical and to provide a measure of electrophilic reactivity. There is a substantial need for a rapid, cost-effective technique to screen complex environmental samples for contaminants. Our approach is to study ion-molecule reactions in the ion source or collision cell of a tandem quadrupole mass spectrometer using model nucleophiles to represent biological macromolecules.

The reaction of adenine with benzoylchloride has been studied in the ion source of a TSQ 45. Adenine is introduced into the ion source continuously by a solids probe and benzoylchloride by a 3m DB-1 capillary column. All species in the ion source are ionized by methane CI (0.4 Torr). Figure 1 depicts the positive and negative mass spectra obtained from the reaction of adenine and benzoylchloride. The m/z 240 is the $(P+H)^+$ of the reaction product and m/z 239 is the P^+ of the adduct. In addition to the DNA adduct ions, methane adduct ions of both reactants form in the positive mode while chloride adduct ions of the nucleophile can be observed in the negative mode. The daughter spectra of the $(P+H)^+$ and P^+ adduct ions produced in the ion source provide a means for identification of the DAN adduct. Argon CAD of 240⁺ and 239⁺ are complementary to each other; 240⁺ fragments to the benzoyl ion whereas 239⁺ fragments to the adenine ion. Further confirmation is achieved by comparison of the daughter spectra obtained from the adduct ion and the synthesized standard.

Reaction of pyridine with various electrophiles has been studied in the collision cell of a TSQ 70. Pyridine is introduced continuously into the ion source by a fine-metering valve and the electrophiles are introduced into the collision cell by a 6m DB-5 capillary column. The M^+ ion of pyridine, produced by methane CI, is mass-selected by Q1 and reacts with the neutral electrophiles in the collision cell at collision energies of 1 eV. Product ions are then mass analyzed in Q3. The mass spectrum of the allyl chloride/pyridine reaction in the collision cell is compared to one obtained from the ion source in Figure 2. The collision cell mass spectrum is simpler and less ambiguous since only one ion is present in the collision cell to react with the electrophiles eluting from the GC. In contrast, the m/z 120 ion can correspond to the $(N+C_3H_5)^+$ methane CI adduct ion of pyridine and/or the reaction product of pyridine M^+ and allyl chloride.

Figure 3 illustrates mass chromatograms obtained by alternately mass-selecting 79⁺ of pyridine and 17⁺ of methane to react with the electrophiles eluting from the GC into the collision cell. Thus, mass chromatograms are obtained which depict only the components which react with pyridine and simultaneously, the CI mass spectra of all the components in the GC sample. Chromatograms A, B, and C are a result of mass-selecting 79⁺ to react with the electrophile. Chromatogram A displays the RIC and is relatively uninformative. Chromatogram B displays the trace of 79⁺ and demonstrates the difficulty of monitoring the loss of reactant ion since loss of 79⁺ also occurs by charge exchange with the electrophile. Chromatogram C is the RIC minus 79⁺; it illustrates the capability to monitor eluents which react in any way with the reactant ion. Chromatogram D displays the RIC resulting from mass-selection of 17⁺ and subsequent reaction with the eluting electrophiles. This chromatogram illustrates the ability to detect all compounds in the GC sample whether the compounds react with 79⁺ or not.

Allyl compounds are known carcinogens which react with DNA to form adducts, whereas the analogous propyl compounds are non-carcinogenic and therefore should not react with DNA. The results of alternating between 79⁺ of pyridine and 17⁺ of methane, shown in Figure 4, were compared for equivalent amounts of the two classes of compounds. The allyl halide chromatograms C and D demonstrate the gas-phase reactivity of the carcinogenic allyl compounds with 79⁺. The propyl halide chromatograms C and D illustrate that no significant reaction with 79⁺ is observed for these non-carcinogenic compounds.

Comparison of collision cell gas-phase reactivity and mutagenicity for various carcinogenic and non-carcinogenic electrophiles displays the same order of mutagenicity, but not the same magnitude.

The ability to produce and detect products of nucleophilic/electrophilic reactions has been

demonstrated in the ion source and collision cell of a tandem mass spectrometer. Mass spectra of reactions in the ion source tend to be complicated; furthermore, it is difficult to determine which form of each species is reacting ($[M+H]^+$ or M^+). Mass spectra of reactions in the collision cell are less complicated, as only one mass-selected reactant ion is allowed to interact with the neutral electrophiles. MS/MS confirmation of the product ion by daughter mass spectra is one advantage of reactions in the ion source, since CAD of collision cell reactions cannot be performed without the use of an instrumental capable of MS/MS/MS.

Fig. 1

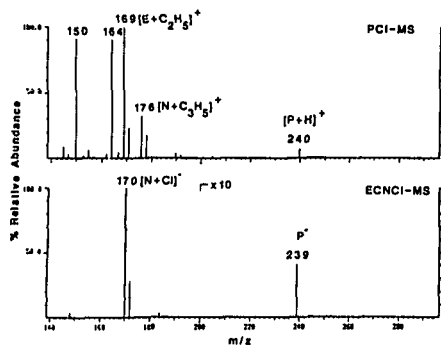
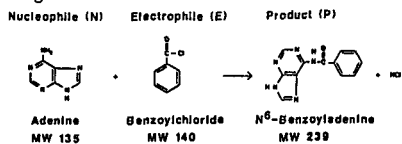
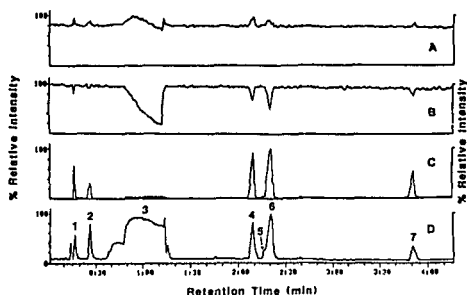


Fig. 3



1) Allyl chloride, 2) Allyl bromide, 3) Heptane [solvent], 4) Ethylbenzene, 5) Allyl isothiocyanate, 6) Styrene, 7) Hexachlorobutadiene

Fig. 2

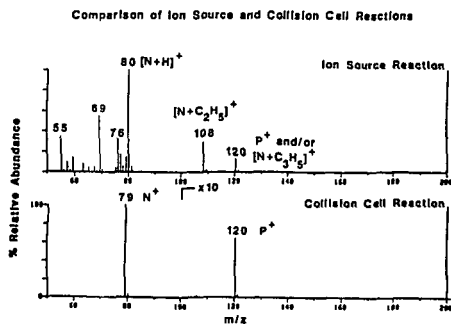
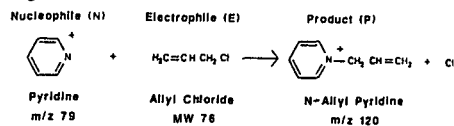
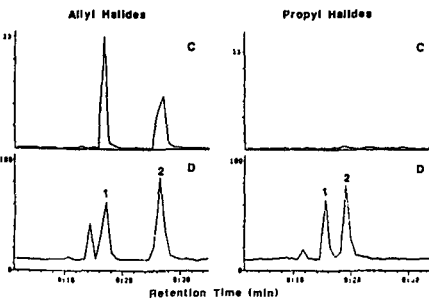


Fig. 4



1) Allyl Chloride, 2) Allyl Bromide

1) Propyl chloride, 2) Propyl bromide

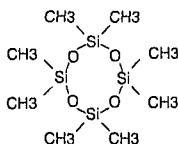
APPLICATION OF PURGE AND TRAP GC/MS FOR THE ANALYSIS OF
OCTAMETHYLCYCLOTETRASILOXANE IN WATER AT SUB-PART PER BILLION LEVELS

Edward C. Tews, Valerie J. Bujanowski, and John A. Moore
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Purge and Trap (P&T) or dynamic headspace analysis using capillary column gas chromatography is becoming more popular for the analysis of volatile organics in aqueous samples. The use of cryofocussing with capillary columns has greatly improved the resolution and analysis time over packed columns. The use of capillary columns has also allowed direct coupling between a gas chromatograph and a mass spectrometer. Using a mass spectrometer in a selected ion monitoring mode yields specific detection with high sensitivity.

The objective of this work was to develop a method for analyzing octamethylcyclotetrasiloxane (OMCTS) in aqueous samples at sub-part per billion levels

OCTAMETHYLCYCLOTETRASILOXANE



(OMCTS)

with minimal sample size. There are several problems associated with OMCTS. First, the solubility of OMCTS has been reported from 10,000 to 0.05 ppm. This vast range of values has been proposed to be due to the ease of OMCTS to form stable colloids in water, yielding high values for solubility. Second, a high Henry's Law Constant of approximately 1000 times greater than benzene has been determined for OMCTS. This high value implies that OMCTS tremendously favors the volatilization from the aqueous phase to the gas phase. Combining the above characteristics, establishing a method to detect and quantitate OMCTS will require low detection limits and careful sample handling.

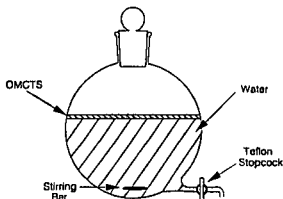
Purge and trap GC/MS was chosen to address these problems for several reasons. The purge and trap methodology has several advantages over extraction techniques. First, there is a large concentration factor, typically 500-1000 fold without involving very large sample sizes. The sample preparation is minimal which reduces the loss of volatile constituents, introduces fewer artifacts, and improves the reliability of the analysis. There are, however, associated disadvantages with purge and trap. Background chemicals in the laboratory environment are easily observed in the analysis when looking at these levels and can cause interfering problems. Many of these disadvantages can be overcome using selected ion monitoring mass spectrometry.

Advantages of selected ion monitoring (SIM) mass spectrometry are reduced interferences from other compounds (selectivity) and increased sensitivity, approximately 100 times better than full scan mode. However, the cost of the mass spectrometer as a detector can be quite high compared to other GC detectors. The reliability and selectivity of the data may overcome this disadvantage.

After loading the sample, this system of a Tekmar 4000 dynamic headspace concentrator, interfaced to a Finnigan 4600 GC/MS with a cryofocussing module was automated to collect and analyzed the data. After receiving a ready signal from the P&T unit, the mass spectrometer acquired and processed the data. During the acquisition, a procedure was utilized to change the range over which the mass data was acquired. Three ranges, first, covering a broad mass range, then SIM of two internal standard ions, and finally, SIM of two OMCTS ions were utilized. The detected peaks were checked against library entries and the OMCTS was quantitated.

This method was evaluated with six samples of OMCTS over the range of 0.75-120ppb and yielded a correlation coefficient of 0.999 comparing response versus concentration. The corresponding response factors over this range were determined to be within 5% relative standard deviation (RSD). Repeatability from five replicate samples was determined to be 5% RSD. Further evaluation determined the purge efficiency of OMCTS from water at 96% using a 5ml sample size, purged with approximately 480ml of helium. With this method defined, a solubility apparatus was assembled as shown below. This apparatus

APPARATUS FOR SOLUBILITY DETERMINATION



has a small layer of OMCTS over water which is gently being stirred to avoid vortex formation and possible colloidal dispersions. This experiment was allowed to equilibrate for at least 24 hours before sampling, generating a saturated solution of OMCTS in water. Several samples were analyzed at varying "equilibration" times and were used to determine a solubility of OMCTS at room temperature (22°C) of 77ppb.

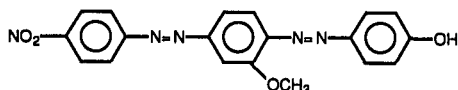
In conclusion, an automated purge and trap GC/MS method was developed for the analysis of OMCTS in aqueous samples at sub-part per billion levels using only approximately 10 milliliters of sample. The method was determined to have good repeatability and linear response from 0.75-120ppb OMCTS. This method was also used to determine the solubility of OMCTS at 77ppb.

Determination of the Photodegradation Products of Disperse Orange 29 by GC/MS and HPLC/MS

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R.D. Voyksner Research Triangle Inst. Research Triangle Pk.. NC

Azo dyes readily undergo light promoted reactions which ultimately result in fading of the color. Additionally, some dye degradation products are suspected carcinogens. Understanding the degradation process will lead to the development of more fade-resistant dyes, more environmentally safe dyes and will help in evaluating the environmental fate of existing dyes. In order to do this it is important to identify the reaction pathways involved. The use of both GC/MS and HPLC/MS will allow characterization of a wide variety of photodegradation products. In particular this study focuses on one disazo dye, Disperse Orange 29:



The fading was performed by irradiating the dye with xenon light for 1000 hrs. Both dyed fabric samples and an ethylacetate solution of the dye were faded. The solution sample was analyzed without further workup. The fabric samples were powdered and extracted with ethylacetate.

The GC/MS experiments were performed on a Hewlett-Packard 5985B quadrupole mass spectrometer. The GC separations were performed on a 15M DB5 fused silica capillary column programmed from 50°C to 300°C at 10°C/min. For EI, the scan range was 40-450 daltons, source temperature was 200°C and electron energy was 70 eV. For CI, methane reagent gas was used, the scan range was 90-500 daltons.

The HPLC/MS experiments were performed on a Finnigan MAT 4500 quadrupole mass spectrometer equipped with a Vestec thermospray interface. The separations utilized a LC-1 (25 cm x 4.6 mm) column with a 30 minute linear gradient of 25% methanol to 95% methanol containing 0.1M ammonium acetate at 1 mL/min. flowrate. UV detection was performed at 254 nm for general screening and at 410 nm, characteristic for the orange color. Thermospray spectra, generated by ion evaporation, were obtained. The vaporizer temperature was 185-190°C, source temperature was 250°C. The scan range was 140-600 daltons with both negative and positive ion detection.

The solution fading experiments proved uninformative due to the many products resulting from reaction of the dye and the solvent. For this reason, the major effort was directed towards identification of the photodegradation products from fabric fading. GC/MS analysis identified three products (Figure 1). The EI spectra showed fragmentations patterns which were used in identification. Molecular weights were confirmed by methane CI and also by direct probe high resolution MS. Thermospray HPLC/MS permitted detection of two additional photodegradation products (Figure 1). The products detected by thermospray HPLC/MS retained an orange color, evidenced by their UV-VIS adsorption at 410 nm. This indicates that the conjugation and azo linkages remain essentially intact. Confirmation of these identifications is in progress. The thermospray HPLC/MS spectra provided few fragment ions for interpretation. Also, the presence of

compounds with molecular weights greater than the parent dye suggests reaction of the photodegradation products with the solvent used for extraction.

In conclusion, the major photo-induced decomposition pathway is fragmentation adjacent to the azo bonds. Minor pathways include reduction and cleavage of the azo bonds and radical induced conversion of the nitro to an hydroxyl group.

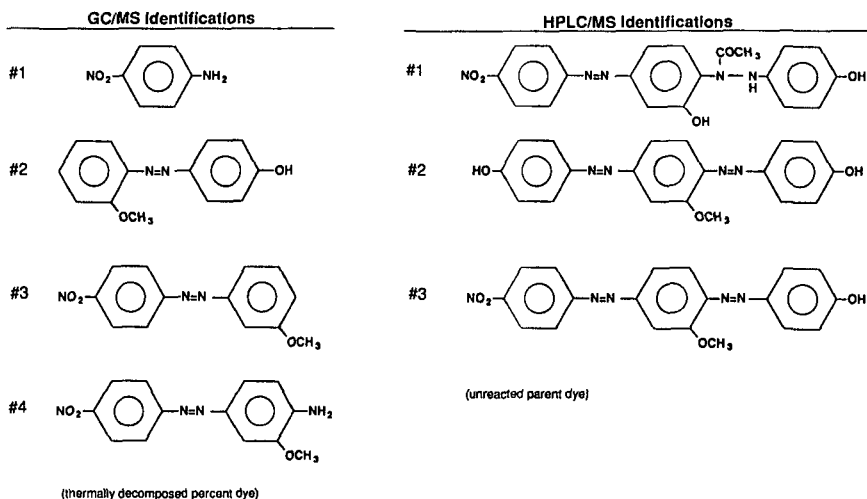


Figure 1

We wish to acknowledge the National Science Foundation for partial support of this project under grant number RII-8511734.

DEVELOPMENT AND TESTING OF ICP/MS
METHOD 6020 FOR ENVIRONMENTAL ANALYSIS

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Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) is a new technique for the analysis of complex sample matrices, such as those encountered through the US EPA Superfund Contract Laboratory Program (CLP). The proposed Method 6020 has been written specifically for use in the production environment of the CLP to allow digestion followed by analysis of complex environmental sample matrices by ICP/MS. Evaluation of comparability and acceptability of Method 6020 Inductively Coupled Plasma Optical Emission Spectrometry (ICP/OES) and Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), was the ultimate goal of a recently conducted multi-laboratory validation study.

A total of twelve Special Analytical Service (SAS) laboratories having ICP/MS capabilities participated. For comparison, a total of twelve Routine Analytical Service (RAS) laboratories, representing nearly half of these in the CLP, were utilized. The SAS laboratories followed ICP/MS 6020, whereas the RAS laboratories followed Statement of Work (SOW) 787 currently in use by CLP for ICP/OES and GFAAS analyses.

A total of sixteen different raw sample types having an historical data base were analyzed in the study. Those materials included tap water, analyte spiked waters, electroplating sludge, hazardous waste soil, river sediment, estuarine sediment, fly ash, and analyte spiked soils. Concentrations of analytes in the materials ranged from very low ppb levels to moderate ppm levels. Blind duplicates of each sample type were submitted to all SAS and RAS laboratories for digestion and analysis.

To test sample preparation precision, two of the waters and seven of the solids were digested in bulk by our laboratory following sample preparation procedures specified in Method 6020. Homogenous splits of the bulk digests were submitted in blind duplicate to each SAS and RAS laboratory. With digestion variability removed through the use of splits, comparison could be made to each laboratory's own digestion, enabling separation of sample preparation precision from instrumental analysis precision.

An alternate digestion method was also included in the study to evaluate its efficiency when compared to the currently proposed 6020 digestion. The alternate method that was used was the GFAAS preparation method from SOW 787. The GFAAS sample preparation avoids chlorides which cause interfering ions in ICP/MS. The same two waters and seven solids that were used to test the Method 6020 sample preparation precision were digested by the alternate method in bulk, and splits were submitted in blind duplicate to each laboratory.

The total number of raw and digested samples that were submitted to each laboratory was 68. This value, multiplied by 24 laboratories, gave 1632 total samples that were evaluated. Since the number of analytes totaled 22, over 35,000 analyses were received in the study. Additionally, over 50,000 Quality Assurance/Quality Control analyses were required to support the sample analyses. Participants in the study

completed and reported approximately 96% of all expected analyses. Outlier rejection by the Grubbs Test appears to be about 4% of the 35,000+ reported analysis. It is estimated that about half of the outliers may be correctable and could be reentered into the data base after modification.

DISCUSSION

Elements which are typically present at high concentrations, such as aluminum, generally showed good comparison between methods. Similar values between the soil, the bulk 6020 soil digest splits, and the bulk GFAAS soil digest splits indicate sample preparation methods are equivalent to present CLP methods. The deviations on the bulk digests were smaller than on the individual laboratory digests, indicating a significant portion of method precision is due to sample preparation. The largest factor, however, remains attributable to analytical measurement precision. Elements occurring at high concentrations presented more problems than other analytes from an operational standpoint with ICP/MS. The high levels that can and do appear in environmental samples must be diluted to get levels within the linear dynamic range.

Medium-to-low-level analytes such as cadmium also showed good comparability between the data bases. The superior sensitivity of the ICP/MS technique was clear for all analytes in this concentration range.

Low level GFAAS analytes such as arsenic generally showed good comparability. Most errors found in a historical data base using ICP/OES seemed to be the result of the poorer sensitivity with the ICP/OES technique, when analyzing for arsenic. Data for silver also demonstrated the better detection limits achieved by ICP/MS.

In conclusion, for the elements and matrices discussed, the proposed Method 6020 obtained values comparable to those obtained by ICP/OES analysis in the CLP. In some instances, the ICP/MS method is clearly superior to those presently employed in the CLP, particularly when very low levels are being analyzed or when an analyte is difficult to determine by the CLP method (e.g., selenium and arsenic).

NOTICE

Although the research described in this article has been funded wholly or in part by the Environmental Protection Agency Contract 68-03-3249 to Lockheed Engineering and Sciences Company, Inc., it has not been subject to the Agency's review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

FACTORS CONTRIBUTING TO THE EFFICIENCY AND LINEARITY OF COLD-ON-COLUMN INJECTORS
FOR USE IN THE GC/MS EPA METHOD 625

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Chemical compounds analyzed according to EPA Method 625 vary greatly in their chemical and physical properties. The compounds range from the nonpolar polycyclic aromatic hydrocarbons to the polar benzidines and phenols. To quantitatively analyze these compounds to very low concentration levels, the GC/MS system must be designed to handle these varied properties. As shown in a previous presentation,¹ large gains in response, linearity, and reproducibility were made in going from hot Grob split-splitless to cold-on-column injection and the deactivation of connectors and ionization chamber walls.

Further work on the GC/MS system has improved both overall and long-term performance. A comparison has been made between two types of on-column injectors. The first type is not gas-tight during injection, while the second type is. The non-gas-tight injector forms the pressure seal during injection between the injection needle and the metal body of the injector. The gas-tight injector employs a triple layer, prepunched septum and a domed, side-delivery syringe needle. Table 1 shows the importance of a gas-tight injection. These data show that if the compound is volatile at the injection conditions of the cold-on-column injector, approximately 20% of the compound is lost. Consequently, the gas-tight injector prevents discrimination on the basis of volatility.

After combining the gas-tight on-column injector with the other modification, good responses and linearity were obtained for the compounds of Method 625. However, these responses and linearity for the more polar compounds, especially the phenols, were not well maintained over a period of time. At this point, a new prototype injection system was developed that effectively removed the time dependence of the system. Before the new injection system was installed, a decrease of approximately 30% in the response factors of the phenols was common in the first several days of operation. Table 2 shows the results of a 14-day study with the new injection system. Except for 2-methyl-4,6-dinitrophenol, there is essentially no change in the response factors for the phenols over this period. The reason for this change is now understood and is being corrected for in the design of the injector. The design and performance of the new injector shows great promise of long-term stability for even the most highly reactive compounds of EPA Method 625.

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*Operated for the U.S. Department of Energy by Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400.

Table 1. Decrease in area with non-gas-tight injection compared to gas-tight on-column injection

Compound	$\frac{\text{Area ratio non-gas-tight}^a}{\text{Area ratio gas-tight}}$
1,4-Dichlorobenzene-D4 ^b	0.802
Naphthalene-D8 ^b	0.832
Acenaphthene-D10 ^b	0.838
Phenanthrene-D10 ^{c,d}	1.000

^aArea ratio = $\frac{\text{Area of GC peak of compound}}{\text{Area of GC peak of phenanthrene}}$

^bVolatile at the injection conditions.

^cNot volatile at the injection conditions.

^dThe area of phenanthrene-D10 did not change from non-gas-tight to gas-tight injection; therefore,

$\frac{\text{Area of phenanthrene-D10 non-gas-tight}}{\text{Area of phenanthrene-D10 gas-tight}} = 1.$

Table 2. Time effects with new system

Compound	m/z	Response Factors	
		Day 1 (100 ng)	Day 14 (100 ng)
1,4-Dichlorobenzene-D4	152	I.S.	I.S.
Phenol	66	0.182	0.226
2-Chlorophenol	130	0.338	0.367
Naphthalene-D8	137	I.S.	I.S.
2-Nitrophenol	139	1.55	1.44
2,4-Dimethylphenol	77	0.338	0.378
2,4-Dichlorophenol	164	1.40	1.36
Acenaphthene-D10	164	I.S.	I.S.
4-Chloro-3-methylphenol	77	0.071	0.084
2,4,6-Trichlorophenol	196	0.248	0.262
4-Nitrophenol	65	0.055	0.058
Phenanthrene-D10	189	I.S.	I.S.
2-Methyl-4,6-Dinitrophenol	198	0.342	0.440
Pentachlorophenol	266	0.705	0.728
2,4-Dinitrophenol	184	0.315	0.304

Conditions:

1. New prototype cold-on-column injection system.
2. New prototype interface.
3. Restek, RTX-5, 30 meter, 1.0 μ mdf, 0.32 mm IC.

THE QUANTITATIVE EFFECTS OF ION ABUNDANCE TUNING CRITERIA
ON MODERN QUADRUPOLE MASS SPECTROMETER SYSTEMS,
RESULTS FROM AN INTERLABORATORY STUDY

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As a part of an interlaboratory study to understand the effect of ion abundance tune on quantitative GC/MS analysis, approximately 20,000 GC/MS response factors (RF's) and related statistics have been compiled for 82 analytes of environmental interest. Data was acquired over five tune regimes for seven different GC/MS systems using a common EI GC/MS protocol, common analytical standards and a floppy disc based data submission protocol. This paper reports selected aspects of this study.

With the elimination of the worst performers for each tune, interlaboratory, five level RF RSD's were found to average approximately 10, 12, 15, 11 and 13 percent for tunes A, B, C, D and E, respectively. The worst laboratory performance was found when "low" mass ion current was suppressed. From a quantitative analysis perspective, this observation suggests that new ion abundance criteria should carefully consider lower limits of low mass values, as significant non-linear response can be observed, quite naturally, when ion current is suppressed. (RF RSD's for certain systems approached an average of 25.0 percent for the 82 analytes studied when m/z 51 of DFTPP was in the 5-20% range.)

The effect of ion abundance tune was directly observed in GC/MS RF values. For example, response factors varied across all tunes for all seven laboratories as expected, depending on the degree of enhancement or suppression of ion current for the mass range of interest. Within laboratory "tune caused" RF variation was reduced by an average factor of 4 when corrected for relative ion transmission. Unfortunately, however, between laboratory RF values differed significantly. (For example, for hexachlorobenzene, the 6 laboratory mean RF range was 0.187-0.297. One laboratory had an RF value which was approximately twice previously published values, and it was eliminated from this comparison.) This observation prompted attempts to derive intersystem correction factors needed to establish a practical EI RF reference set. Intersystem correction factors were derived which yielded, for hexachlorobenzene, estimated/observed RF values whose average intersystem agreement was within a narrow range compared to unaligned values, i.e., approximately 3%. These results suggest that a practical reference set of EI GC/MS RF values is attainable despite the significant design differences among the modern quadrupole GC/MS systems studied.

These and other observations, along with data presented at this meeting in our paper addressing qualitative aspects of this study (1, 2), suggest that a national reference set of EI GC/MS RF values (where $N = 82$ compounds) and related statistics can replace ion abundance criteria for DFTPP (where $N = 1$ compound). As we originally proposed at the 1983 ASMS meeting (3), such an approach would provide a more thorough characterization of the quantitative and qualitative capabilities of modern quadrupole GC/MS systems utilized in environmental applications of GC/MS.

Table 1.							
HCB Mean RF Values, As Acquired							
	<u>Laboratory</u>						
<u>Tune</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
A, Traditional	0.19	0.19	0.29	0.25	0.31	0.16	0.53
B, 442 base peak	0.19	0.28	0.34	0.45	0.39	0.21	0.65
C, per B low m/z suppressed	0.19	0.38	0.50	0.31	0.55	0.20	0.85
D, 51 base peak	0.18	0.16	0.17	0.17	0.24	0.10	0.32
E, per D high m/z suppressed	0.16	0.12	0.09	0.12	0.13	0.09	0.21

Table 2.							
HCB Mean RF Values, As Aligned							
	<u>Laboratory</u>						
<u>Tune</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
A, Traditional	0.19	0.19	0.29	0.25	0.31	0.16	0.53
B, 442 base peak	0.20	0.22	0.30	0.29	0.29	0.18	0.54
C, per B low m/z suppressed	0.20	0.23	0.32	0.28	0.26	0.19	0.52
D, 51 base peak	0.18	0.18	0.26	0.28	0.32	0.19	0.59
E, per D high m/z suppressed	0.17	0.14	0.05	0.27	0.32	0.18	0.63

References:

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2. A. D. Sauter, et al., "The Qualitative Effects of Ion Abundance Tuning," presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics.
3. A. D. Sauter, "GC/MS Accuracy Criteria," presented at the 31st ASMS Conference on Mass Spectrometry and Allied Topics, p. 908.

ANALYSIS OF HAZARDOUS SUBSTANCES IN FISH TISSUE BY GC/MS

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Many hazardous substances can be bio-accumulated through food chains in water and can remain in high concentration at the end of the biological system. Analysis of target compounds and pesticides from the hazardous substances list (HSL) in fish tissue monitors our water system as well as the environmental toxicological impact on our ecosystem.

This paper presents a GC/MS method developed in our laboratory for analysis of hazardous substances in fish tissue. The preparation method uses a hexane-methylene chloride (50/50 V/V) solution with a tissue mixer to extract fish tissue sample. (1)

The clean-up and concentration process involves the removal of lipids by gel permeation and Kuderna-Danish (K-D) concentration. By means of this simple sample preparation, the GC/MS method is applicable to the analysis of hazardous substances in fish tissue. Sixty five semi-volatile HSL compounds and twenty pesticides in fish tissue can be analyzed in a single GC/MS run. Figure 1 shows the total ion chromatogram of sixty five semi-volatile HSL compounds and six surrogate standards spiked into blank fish tissue sample. Figure 2 shows the GC/ECD chromatogram of fourteen pesticides spiked into the blank fish tissue.

Recoveries and method detection limits are shown in Table 1 and Table 2. The recoveries were in the range of 41-100% for eleven matrix spike semi-volatile compounds, 63-106% for six semi-volatile surrogate standards and 57-92% for fourteen pesticide standards, respectively. The relative standard deviations of method recoveries for these compounds are in the range of 2.6% to 9.6%. Recoveries of sixty five semi-volatile HSL compounds are within the range of 40-106% except for Benzoic Acid, 2,4-Dinitrophenol, Bis (2-Ethylhexyl) phthalate and Di-n-Octyl phthalate.

The method detection limits of eleven matrix spike compounds are in the range of 0.54 to 3.0 ppm with 95% confidence (2) by GC/MS and 0.0060 to 0.0096 ppm for thirteen pesticides and 0.12 ppm for Archlor 1254 by GC/ECD.

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Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

CHARGE EXCHANGE CI/ELECTRON ATTACHMENT NCI AS AN ALTERNATE
IONIZATION MODE FOR CHARACTERIZATION OF ENVIRONMENTAL SAMPLES:
APPLICATION TO MARINE MAMMALS

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The use of Argon charge exchange positive ion chemical ionization pulsed with electron attachment negative ion chemical ionization was investigated as an alternative ionization mode to the well established electron impact ionization mode for routine characterization of environmental samples. Although CE-CI and NCI have been shown to be of significant potential value for routine environmental sample analysis, EI remains almost exclusively the ionization mode of choice. Two primary reasons exist for this fact, 1st large EI spectral libraries are available for assistance in spectral interpretation, and 2nd the U.S. EPA Contract Laboratory Program requires EI for all GC/MS work. The argument for use of Argon PPINICI rather than EI is not simply having two ionization modes rather than one, but that the addition of negative ion information offers uniquely sensitive and selective data for highly toxic molecules¹. The success of Argon PPINICI then to replace EI depends upon the acceptance of searching EI libraries with CE spectra², and meeting quantification criteria established in QA/QC documents. We have investigated these points.

CE and EI spectra for over 100 pesticides and industrial compounds commonly found as environmental contaminants were searched against the EPA/NBS EI mass spectral library and ranking of FIT and RFIT results for the correct response were compared. It was found that the success rate for correctly identifying the analyte was equal (>95%) for both ionization modes. Also, a sensitivity comparison for 35 analytes indicated comparable levels of detection, and regression coefficients of quantification standards were comparable. Further, relative standard deviations for peak areas indicated that "sensitive" compounds were more stable during CE than EI.

Argon CE-CI/NCI was used along with both low and high resolution GC/MS in a study of contamination of Atlantic Bottle Nosed Dolphins by pesticides and industrial chemicals. This study was undertaken because of world wide concern over the deaths of numerous species of marine mammals³. Our initial analysis showed high levels of PCBs and chlorinated pesticides frequently found in freshwater biota. PCB levels, for instance, were found as high as 275 ug/g lipid in liver of one of the animals. This value is nearly 20X that measured in the liver of a human victim of PCB poisoning in Yu Cheng, Taiwan⁴.

- 2 -

Perhaps the most interesting observation was the complex distribution of bromine containing contaminants. Although marine species contain naturally brominated organics⁵, we have identified a series of polybrominated diphenyl ethers which we believe have anthropogenic origin. We observed that the distribution of tribromo- to heptabromo- compounds in the dolphins was similar to the distribution observed in a commercial flame retardant formulation. Polybrominated diphenyl ethers have also been identified in fish from Europe⁶. These studies continue.

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**"DIRECT TRACE ANALYSIS OF VOLATILE ORGANICS IN AIR AND WATER
BY MEMBRANE/MASS SPECTROMETRY"**

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Trace analysis of organics in water or air by mass spectrometry generally requires laborious and time-consuming extraction and concentration procedures. Sample work-up may be eliminated with a membrane device. Non-polar volatile organics may be continuously analyzed from air or water with ppb detection limits and a dynamic range of 10^3 (Figure 1). In this study, the membrane is a 1" long, .020" i.d., .037" o.d. silicone hollow fiber (Dow Corning Silastic Medical Tubing).

The sensitivity of membrane techniques is determined by the enrichment factor (the analyte/matrix permeability ratio) and parameters including membrane area/thickness ratio, sample linear velocity, and temperature. Permeability is the product of diffusivity and solubility. Steady state permeation is achieved within a few minutes, as governed by diffusivity. Compared with non-polar organics, air and water are quite insoluble in the silicone polymer. The membrane serves as a very good organic extraction medium as well as a barrier between the sample and the high vacuum MS.

We investigated two membrane sampling geometries reported in the literature; flow-over(1) and flow-thru(2). A sample flowing through the inside of the hollow fiber with molecules of interest permeating to the outside (flow-thru) demonstrated more than 10X the sensitivity of the alternate geometry of flowing the sample over the outside while analyzing the extract on the inside (flow-over). Figure 2 shows response vs. concentration plots for these two geometries. Flow-thru is preferred since the area/thickness ratio is maximized (the hollow fiber does not collapse) and high linear velocities are easily obtained.

Heating the membrane inlet produces desirable as well as undesirable effects: Diffusivities increase, therefore response times decrease with increasing temperature (Figure 3). However, heated membranes exhibit lower solubilities for organic compounds. In the case of air samples, this means that the membrane extracts organics from the matrix less efficiently. This decrease in solubility is not offset by the increase in diffusivity. A decrease in organic permeations result. Organics in heated aqueous samples exhibit greater permeations. However, the increase in water permeation is even greater. Decreased enrichment results (Figure 4). Proposed explanations include; a) partitioning of organics into the membrane increases at higher temperatures, and b) mixing improves at higher temperatures. In any case, enrichment is independent of membrane surface area, thus improved sensitivities are more efficiently obtained at low temperatures by using a longer hollow fiber. Table 1 shows response time and detection limit data for several compounds.

ACKNOWLEDGEMENTS

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FIGURE 1

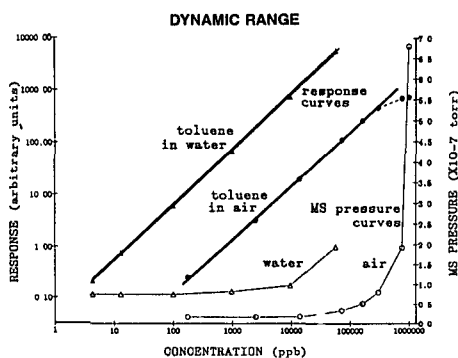


FIGURE 2

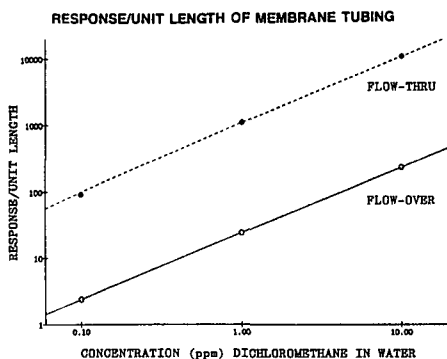


FIGURE 3

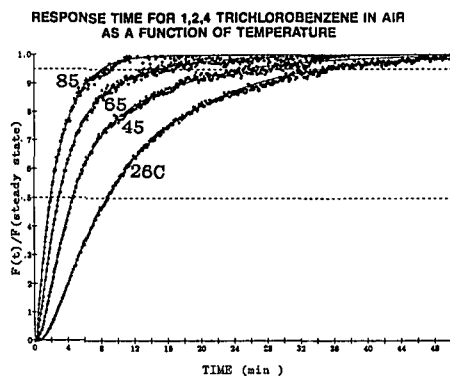


FIGURE 4

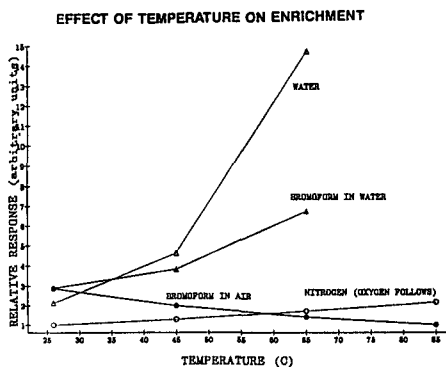


TABLE I

**SURVEY OF TEMPERATURE EFFECTS ON
RESPONSE TIME AND DETECTION LIMITS**

Detection limits are analyzer dependent. Detection limits of less than 50 ppb for dichloromethane in air at 75°C have been reported (3).

COMPOUND	$t_{50\%}$ (~ 5-10%) minutes		DETECTION LIMIT (ppb)			
	28°C	45°C	IN AIR 28°C	45°C	IN WATER 28°C	45°C
Chloromethane	0.23	0.19	325	368	-	-
Dichloromethane	0.34	0.30	669	742	6	5
Chloroform	0.42	0.36	182	233	7	5
Carbon Tetrachloride	0.52	0.38	130	163	18	13
Vinyl Chloride	0.25	0.20	269	301	-	-
Vinylidene Chloride	0.38	0.30	101	125	0	5
Trichloroethane	0.41	0.35	30	39	29	25
Tetrachloroethane	0.78	0.55	29	34	32	25
Dibromomethane	0.57	0.40	28	38	106	93
Bromoform	1.30	0.88	14	21	62	34
Benzene	0.43	0.32	42	54	3	2
Toluene	0.59	0.46	31	38	2	2
Ethylbenzene	0.86	0.60	15	19	2	2
Chlorobenzene	0.93	0.63	12	14	8	7
1,3-Dichlorobenzene	4.40	1.70	3	3	23	10
1,2,4-Trichlorobenzene	8.70	4.60	3	5	51	29

**DETERMINATION OF SELECTED PRINCIPAL ORGANIC HAZARDOUS
CONSTITUENTS FROM INCINERATORS USING
CAPILLARY COLUMN GC/MS/DS.**

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INTRODUCTION

The Resource Conservation and Recovery Act (RCRA) requires that facilities which treat hazardous waste by incineration ensure that the incinerator are operated in a manner which do not endanger human health or the environment. The incinerators must be operated so that principal organic hazardous constituents (POHC's) are destroyed and/or removed with a minimum efficiency of 99.99%. To determine the destruction and removal efficiency (DRE) an analysis to quantify POHC's in the waste feed material and stack gas effluent must be performed.

Characterization of stack emissions from hazardous waste incinerators constitutes a challenge for the analytical chemist. The use of packed columns for the characterization of POHC's in incinerator stacks have been reported. However if the sample consist of complex products from incomplete combustion it may cause high background in the analytical system and thus interfere in the identification and quantification of the compounds of interest. The modification presented here makes use of capillary columns to improve resolution. In addition, capillary columns yield better quality mass spectra, and decrease the analysis time. Information on the use of high resolution capillary column and selective ion monitoring low resolution mass spectrometry to quantitate POHC's in incineration stack in order to meet the EPA trial burn test criteria will be presented.

EXPERIMENTAL

The sampling of POHC's in the stack gas was performed using the VOST. Approximately twenty liters of gas were sampled. After sampling, VOST cartridges were sealed and stored in clean 25 x 150 mm screw-cap test tube and kept in a refrigerator until the analysis. All field samples were taken from the stack of a liquid waste incinerator above the mist eliminator at an elevation of approximately 42 feet. Cartridges were then spiked with internal and surrogate standards and thermally desorbed for 11 minutes with a flow of He at 40 ml/min at 180° C. The gas is carried into an organic free water column and the organics trapped on the analytical trap of the purge and trap concentrator. The analytical trap is then heated rapidly to 180° with the carrier gas flow reversed so that the effluent flow is directed to a cryogenic system in the inlet of the GC/MS (HP5890-MSD). The pre-column is automatically heated and the organics are directed to a GC where they are separated and detected with a mass spectrometer in the single ion monitoring mode. The GC conditions were as follows: carrier gas- He, 1 ml/min; column temperature program, hold 5 minutes at 35° C then to 110° at 4° C/minute and hold; run time 25 minutes; MS parameters: electron ionization at 70 eV, source temperature set at 280° C, capillary column connected to the ion source through an open split interface. At least three ions for each of the the target POHC's are monitored, the observation time is 50 msec. Qualitative identification of target POHC's is obtained by comparing retention time data and the intensities of the most prominent ions monitored. Concentration of the POHC's are determined from the integrated ion current for the particular POHC's and following the internal standard technique. Normal routine for analyzing a set of cartridges from each of the method evaluation runs was to analyze a blank Tenax and blank Tenax/charcoal cartridge spiked with internal and surrogate standards, a calibration standard, a VOST leak check sample, and the VOST samples.

EPA audit cylinders were obtained from Research Triangle Park, NC. Audit standards were sampled using the VOST at the same flow rate utilized in the field and analyzed following the same procedure outline above. The amounts (ng) of POHC's delivered to the Tenax cartridges were calculated from the volume of gas sampled.

RESULTS AND DISCUSSION

The POHC's (carbon tetrachloride, toluene and chlorobenzene) were selected taking into account the EPA ranking system. The relative standard deviation of the response factor for selected POHC's was less than 10 % for a particular calibration in the 50-1000 ng range, and less than 11% taking into account all calibrations made. Relative standard deviation for the response factor of surrogate standards was less than 15 %. No significant differences in response factor of surrogates and POHC's obtained at different dates was observed.

Replicate analysis of a standard check sample, at the 5 and 20 ng/L gas phase concentration, was used to assure that the analyte in question has been detected and quantified acceptably and reliable. Results for carbon tetrachloride appear to be similar with total recoveries ranging from 85 to 111% of the expected value. Although a breakthrough volume of 20 liters has been reported for carbon tetrachloride adsorbed in Tenax, percent recoveries were higher than 85% in all cases. No major deficiency in the method or the use of Tenax for quantitatively sampling carbon tetrachloride was observed. Results for toluene showed a much narrower range. The extremes varied from 88% to close to 100% of the expected value. Results for chlorobenzene are also narrow ranging from 84% to 90% of the expected value. No significant differences were observed in the average recovery and standard deviation obtained at the two concentration levels.

The method detection limit (MDL) calculated, based on sampling 20 liters, are quite reasonable although, the recoveries are consistently over 100 %. MDL ranged from .11 to .55 ng/L. No significant differences were observed in the recoveries of target POHC's obtained at this concentration range with the ones obtained at a concentration of 5 and 20 ng/L. Based on these detection limits a hazardous waste incinerator achieving a destruction removal efficiency of 99.999%, the POHC's can be present in the waste feed at a concentration as low as 500 ppm and still be detectable in the stack by the GC/MS analysis.

In the analysis of EPA audit cylinder a negative bias was observed for all cases, even for toluene. The highest negative bias was observed for chlorobenzene, although the concentration determined was well within the uncertainty level of the cylinder ($\pm 17\%$). The negative bias obtained for carbon tetrachloride, -4%, was very low considering that approximately 50% of the breakthrough volume (20L) was sampled. Our results indicate that Tenax is suitable for sampling quantitatively these compounds.

Two trial burn test involving three days of sampling were done. A minimum of three samples per day were collected. The first sample on each day was used to evaluate the occurrence of breakthrough. All other samples were analyzed in pairs. Results for carbon tetrachloride were consistent among each of the sampling days and between the two tests. The relative standard deviation was less than 25% in the 1 ng/L range for single pair analysis. Results for chlorobenzene show a higher relative standard deviation, however, the concentration at which is observed is much lower. The results are consistent among each of the sampling days and between the two tests. A difference in the concentration of toluene was observed between the two tests. However, excellent agreement exist among the concentration of toluene obtained during sampling days in each of the tests. Differences in the results can be due to operational conditions of the incinerator or differences in the synthetic waste composition. In general, the results were quite good and did not indicate major deficiencies in the sampling and analysis methods. Accuracy of three replicate cartridges analyzed were better than the $\pm 50\%$ suggested by EPA.

A formal quality control program was conducted in order to establish the acceptable precision and accuracy for the analysis of POHC's following this method. Upper and lower control limits for method performance was determined using the average area response and standard deviation for the internal standard, d_8 -benzene. The reproducibility of the internal standard area counts (m/z 84) was very good with an average variation of less than 30%. No significant difference in d_8 -benzene area counts with the type of trap; e.g. Tenax and charcoal/Tenax traps used for stack sampling (wet) and Tenax traps spiked with the calibration standards (dry) was observed. Among the surrogate standards utilized, the recoveries for fluorobenzene exhibited the best precision while BFB exhibited the worse, d_{10} -ethylbenzene fell somewhat in the middle. This behavior can be due to the difference in retention time between each of the surrogate and d_8 -benzene used as internal standard.

DETERMINATION OF ORGANICS IN PUBLICLY OWNED TREATMENT WORKS (POTWS) BY ON-COLUMN GC/MS: A COMPARISON OF THREE FACILITIES. Linda B. Clark¹, Robert T. Rosen², Thomas G. Hartman², Thomas Atherholt³, Charles Hertz⁴, and Joseph D. Rosen¹. Food Science Department¹ and Center for Advanced Food Technology², Cook College, Rutgers University, New Brunswick, NJ 08903; Coriell Institute³, Camden, NJ; Division of Science and Research, NJ DEP, Trenton, NJ.

The function of Publicly Owned Treatment Works (POTWs) is to treat household waste and municipal sewage. The Resource Conservation and Recovery Act and the Domestic Sewage Exclusion provides that when hazardous waste is mixed with domestic sewage, the subsequent waste is not legally considered hazardous. This exclusion makes it very cost-effective for many industries to discharge their waste to POTWs.¹

Much analytical data has been accumulated concerning priority pollutants from POTWs. However, little data has been published on other materials present in the treated water. On-column GC/MS using electron ionization was the technique utilized, since it is simple and applicable to environmental analysis, and allows for the determination of many polar and thermally labile organics. In addition, EI allows for library searchable data. We have identified approximately 300 components from the three POTW sites. Classes of organics found include: phenols, halogenated compounds, heterocyclics, polyaromatic hydrocarbons, nitrogen and sulfur containing (non-heterocyclic), ketones and aldehydes. A few examples of compounds identified are bromotetradecane, furobenzopyrantrione, trichlorophenol, tetramethyldecynediol, methylanthralene, and methoxybenzene acetonitrile.

The large number of non-priority pollutants found in the effluent raises many questions about the types and amounts of organic compounds being discharged from these facilities into the drinking water supply. It was observed that the two POTWs which had a 30% industrial contribution had much higher concentrations of organic compounds, including nitrogen containing and halogenated species, in their effluent.

In comparison to a previous study¹ on semi-volatile priority pollutants (+10) in POTWs, using US EPA method 625, it was observed that the on-column injection technique is far superior for the detection of organics in complex mixtures.

A companion Salmonella typhimurium assay on total effluents revealed that the facility which had the largest number of heterocyclics, nitrogen containing (non-heterocyclic), polynuclear aromatics compounds, and halogenated species exhibited the highest mutagenic activity. This facility also had the highest industrial contribution, demonstrating a need for improved methods to eliminate organics from POTW effluent.

¹Fields, T.W., Mueller, R.M. and McGeorge L. "The Occurrence and Fate of Toxic Substances in New Jersey Sewage Treatment Facilities". Office of Science and Research, NJ Department of Environmental Protection, Sept. 1986.

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USE OF THE ISOTOPE COMPOSITION OF LEAD AS A NATURAL TRACER IN
THE ENVIRONMENT

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Elemental lead is composed of four stable isotopes, with mass numbers 204, 206, 207, and 208. Isotopes Pb-206, Pb-207, and Pb-208 are formed continuously by the radioactive decay of U-235, U-238, and Th-232, respectively, while Pb-204 has no radioactive precursors. The relative abundance of the four isotopes in an ore depends on the geological time at which lead was segregated from the source rock and concentrated in a mineral deposit. The Pb-206/Pb-207 ratio in the world's most intensively mined deposits varies from 1.04 to 1.39, and in spite of the mixing and averaging processes in manufacturing, significant differences in the isotope ratios of lead in lead products are maintained.

This study explored the feasibility of using the differences in the isotope composition of lead as an indicator of the contribution of various emission sources to the lead in the environment and man in Finland, and especially in the Helsinki area.

The ratios of the stable isotopes were determined in the sources of lead in Helsinki (gasoline, incinerator and lead smelter emissions, coal), in the Helsinki air, and in the indicators of long-term deposition of lead: lichen, highway soil, and lake sediments. Isotope ratios were also determined in autopsy samples of liver, lung, and bone from individuals from Helsinki. The isotope ratios were measured mainly in samples from the Helsinki city area, with a few samples from Lapland studied for comparison.

Pb-206/Pb-207 ratios in the samples were determined with a thermal ionization mass spectrometer after chemical separation of lead by anion exchange and electrodeposition. The total lead concentrations were determined with an atomic absorption spectrophotometer using graphite furnace method.

Wide variations were found in the isotope ratios of lead in gasoline, the ratios of Pb-206 to Pb-207 in samples collected between 1968 and 1987 ranged from 1.063 to 1.1727. The mean of the isotope ratios of 33 samples was 1.124 ± 0.026 , which indicates that the lead in gasoline additives used in Finland mainly originates from geologically older deposits (Precambrian, such as in Australia, with low isotope ratios).

Significantly higher ratios (Pb-206/Pb-207 1.149-1.226) than in gasoline were obtained for other pollution sources in Helsinki -- the main waste incinerator, the two lead smelters, and coal. It can be concluded that, in general, lead used in manufacture of lead products other than lead alkyls for gasoline originates from geologically young deposits with high Pb-206/Pb-207 ratios.

Isotope ratios and lead concentrations in Helsinki air at the end of the 1960s and beginning of the 1970s were comparatively high, indicating the major influence of the incinerators, lead smelters, and coal burning at that time. Better control devices in the secondary lead smelters and shutting down of the incinerators have decreased the atmospheric lead concentrations in the Helsinki area in recent years. The influence can also be seen in the isotope ratios of lead in air samples, which have diminished from 1.156 in 1972 to about 1.110 in the 1980s. The mean of the ratios in the air samples collected between 1966 and 1984 was 1.123 ± 0.013 .

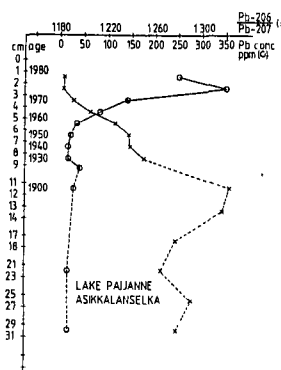
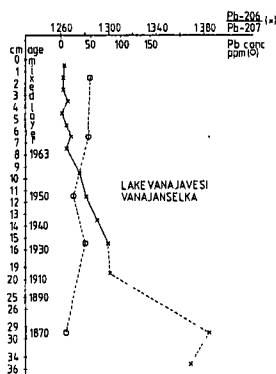
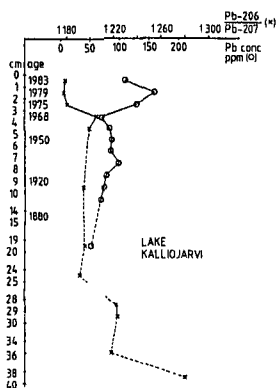
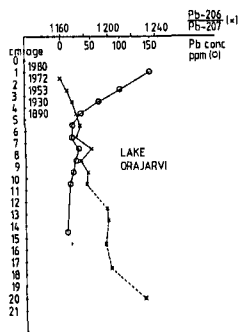
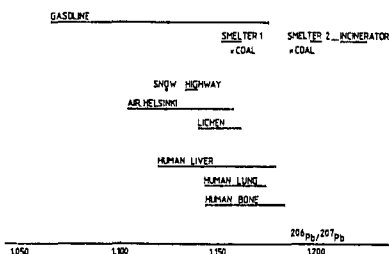
Snow represents a short-term indicator of the deposition. The Pb-206/Pb-207 ratio in snow from the center of Helsinki in 1984 was 1.123 ± 0.002 , which is the same as the mean ratios in gasoline and air samples over that period. Clearly gasoline was the source of lead in snow. The lead in soil taken from beside a highway likewise originated almost entirely from vehicle exhausts, since the mean Pb-206/Pb-207 ratio of the soil samples was equal to that in gasoline (soil 1.136 ± 0.003).

Lichen effectively absorbs lead from atmosphere and soil, and being a slow growing plant it is an excellent indicator of long-term deposition. The mean of the ratio in all the lichen samples was 1.148 ± 0.006 . This value is significantly higher than the mean ratio in gasoline or in Helsinki air. A significant difference was found between the isotope ratios of lead in lichen from Southern Finland (1.152 ± 0.006) and from Lapland (1.144 ± 0.003). This is evidently due to the industrial lead emissions in Southern Finland. The decrease in the ratios of both lichen groups from 1966 to 1985 reflects the diminished influence of the industrial sources of lead in recent years.

The mean Pb-206/Pb-207 ratio in human liver, lung, and bone were 1.142 ± 0.015 , 1.151 ± 0.011 , and 1.156 ± 0.013 , respectively. No difference was found between the tissues from Southern Finland and Lapland.

The wide range of isotope ratios of lead in Finnish gasoline complicates the evaluation of lead sources from isotope ratios. According to calculations made with a highly simplified model, about 70-80% of the lead in Finnish human tissues of individuals dying in the late 1970s originated from gasoline. In earlier years when industrial sources made a greater contribution to the atmospheric lead, an estimated 60-80% of the lead in Helsinki air was from gasoline. In the 1970s and 1980s almost 100% of the lead in air was estimated to originate from gasoline.

In the lake sediments, a correlation was found between the isotope ratios, lead contents, and depth. The lowest isotope ratios were measured at the surface, where lead concentrations were highest. Lead in the lowest parts of the cores clearly originated from non-anthropogenic sources. Deep in the sediment, Pb-206/Pb-207 ratios were about 1.2-1.3, which is equal to the ratio in bedrock. The amount of anthropogenic lead increases toward the surface and the ratios approach those of lead in air. It was estimated that in the studied lakes, about 40-60% of the total lead in the surface sediments was of atmospheric origin.



COMPARISON OF LC/MS AND CZE/MS FOR THE ANALYSIS OF ENZYMATIC DIGESTS AND RELATED BIOLOGICAL SAMPLES, Jack Henion and Eric Huang, Drug Testing and Toxicology Program, Cornell University, 925 Warren Dr., Ithaca, NY 14850.

There is growing interest in the analysis and characterization of complex biological samples. Typically the composition of intractable samples such as fermentation broths and enzymatic digests requires analytical methodology beyond the capability of "modern" capillary GC/MS and electron ionization mass spectrometry. The limits of such analytical techniques are challenged because the physical characteristics of the components in such mixtures are considerably different than those of drugs, metabolites and many environmentally important compounds. The peptides and intact proteins present in a tryptic digest, for example, are typically organic ions dissolved in aqueous solution. These ions often have molecular weights close to or beyond the mass range of our mass spectrometers, are thermally unstable, and characteristically involatile. It would appear that new approaches to characterizing these compounds are needed.

To further complicate the issue the chemical complexity of enzymatic digests and other biological mixtures requires highly efficient separation techniques in order to isolate the individual components for further characterization. Typically HPLC is the method of choice for resolving the components from each other, but sometimes even this technique lacks the necessary capability to isolate individual components. Even if HPLC does separate the components of interest preparative collection of each peak is a tedious and time consuming process.

Recent developments in capillary zone electrophoresis (CZE) suggest that separation efficiencies approaching several hundred thousand plates are possible. This capillary format of classical electrophoresis promises to compliment HPLC and has aroused considerable interest in the fields of biotechnology and biochemistry.

Unfortunately routine HPLC and CZE typically uses conventional non-specific UV or fluorescence detection. The availability of successful and routine on-line mass spectrometric detection would be of considerable value. Unfortunately, most common approaches to LC/MS do not lend themselves to handling compounds like high molecular weight peptides and proteins. Recently, however, developments in electrospray ionization in the laboratories of Fenn, Smith and Henion have shown that this alternative approach offers some unique benefits. Our adaptation is referred to as ion spray LC/MS and this system provides routine, high sensitivity on-line LC/MS and CZE/MS on an atmospheric pressure ionization mass spectrometer (APIMS).

Ion spray LC/MS provides a simple, ambient temperature interface between a liquid inlet system and an APIMS. If the effluent analytes are ions in solution and in a flowing stream of between 10-50 $\mu\text{L}/\text{min}$ the total effluent may be passed through the ion spray LC/MS interface, subjected to electrospray ionization, and the gas phase ions produced by coulombic explosion or field assisted ion evaporation are then sampled into the mass spectrometer. The benefits of this process include very mild ionization, high sensitivity, and multiple charging. The latter aspect is very useful for determining the molecular weights of peptides and proteins which lie far outside the mass range of the mass spectrometer. This results because

the denominator of "m/z" can become very large thus allowing even a quadrupole mass spectrometer to pass high molecular weight compounds.

EXPERIMENTAL

The APIMS system used in this work was a Sciex TAGA 6000E tandem triple quadrupole mass spectrometer (Sciex, Inc., Thornhill, Ontario, Canada). The ion spray LC/MS interface was constructed in-house and has been described in the literature. It is a concentric pneumatic nebulizer carrying micro HPLC effluent through a stainless steel capillary held at 3kV. The electrospray droplets are further nebulized and evaporated at ambient temperature by coaxial nitrogen gas with a measured linear velocity of over 200 m/sec. The APIMS system was operated in either the MS or MS/MS mode with a collision energy of 50 eV in the latter. The CZE system was also constructed in-house and the exit of the capillary was connected directly to the same ion spray LC/MS interface with no other changes. The anode of the CZE capillary was held at 30kV while the ion spray interface acted as the cathode. Typically, therefore, the voltage across the CZE column was 27kV. The details of this system have appeared recently in the literature.

RESULTS AND DISCUSSION

A variety of enzymatic digests have been analyzed by the LC/MS and CZE/MS systems described above. These include tryptic digests of hemoglobin, myoglobin, bovine serum albumin, and human growth hormone. On-line total ion current profiles in addition to selected ion current profiles have been obtained under gradient LC/MS conditions consisting of 1mm x 100mm C8 packed columns using gradients going from 0-100% over 30-45 min. The quality of the TIC is comparable to that obtained from UV detection, and the molecular weight of each component may be obtained even if it lies outside the mass range of the mass spectrometer. Thus multiply-charged ions of high molecular weight peptides may fall easily within the 1400 dalton mass range of our instrument.

The same digests described above for LC/MS may be subjected to on-line CZE/MS with concomitant increase in separation efficiency, but usually significantly reduced selectivity. Thus a TIC electropherogram may have most of the peptides eluting within a rather short time region of the separation, but the rest of the electropherogram may be devoid of peaks. This can be a troublesome matter which can require considerable development time to optimise the separation. In addition, the very small injection volume (5-15nL) for CZE requires at least low ppm concentrations of samples injected to have enough material to detect with the mass spectrometer. Also, a careful balance must be met between buffer concentration and type that will affect a CZE separation and those conditions desirable for ion spray mass spectrometry. Usually a compromise can be met, but it may require extra development time.

In summary, both on-line LC/MS and CZE/MS separation and identification is possible using the ion spray interface to APIMS. Mild ionization and multiple-charging provide two valuable benefits to identifying biologically important compounds. Given a choice between these two separation/inlet systems LC/MS using packed microbore or possibly packed capillaries seems to offer the best compromise between practical sample handling and adequate separation efficiencies. It is possible that improvements in CZE technologies may expand the practical utility of this potentially exciting analytical technique.

COMBINING CF-FAB WITH SEPARATIONS TECHNIQUES

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Continuous-flow fast atom bombardment (CF-FAB) mass spectrometry has been shown to be an extremely useful technique for the analysis of aqueous samples. Since a great deal of work is done with samples derived from biological sources where complex mixtures of compounds are to be analyzed, combining separations techniques with CF-FAB offers important advantages. Perhaps foremost is the capability of performing a complete analysis of molecular species directly on an aqueous sample routinely at the 2-10 pmol level without the need for sample preparation and derivatization methods.

The basic principal of operation of CF-FAB involves the balance of the flow of liquid to, and evaporation from, the sample stage in the source of a FAB mass spectrometer. For normal commercial instruments, this maximal rate of flow is approximately 20 $\mu\text{L}/\text{min}$, depending on pumping capacity. A narrow bore (e.g., 75 micron) fused silica capillary is used to deliver the liquid to the sample stage in order to isolate the high voltage present in the source of magnetic mass spectrometers. Easy use of the device is accomplished by utilizing the direct insertion lock of the instrument. Formation of gaseous ions is accomplished by bombarding the surface of the liquid sample with energetic xenon atoms.

Several significant advantages have been noted when CF-FAB is used for sample introduction as compared to the normal or standard (std) FAB method. First, the mass spectra obtained with CF-FAB are characterized by a significantly lower background than that produced by std-FAB (1), the result of the higher aqueous content of the solutions being analyzed (only 5% glycerol is used). The major effect of this is to significantly increase the sensitivity, often 100-fold or more. Second, the dynamic nature of the surface of the sample using CF-FAB leads to a decrease in the ion suppression effect often seen with samples analyzed by std-FAB. This suppression effect is produced as a result of the tendency of a more hydrophobic compound to occupy the surface of the liquid relative to a more hydrophilic compound.

The combination of mass spectrometry and high performance liquid chromatography offers a powerful method to routinely identify peptides in proteolytic digests. We have focused on the use of microbore LC with 1 x 50 mm C-18 columns operated at flow rates of about 25 $\mu\text{L}/\text{min}$, under both isocratic and gradient elution conditions, because these conditions are well suited to the CF-FAB technique (2). The general technique utilizes a pre-injector splitter to provide rapid gradient formation (200 $\mu\text{L}/\text{min}$) while allowing only 25 $\mu\text{L}/\text{min}$ to pass into the column. A second splitter is used to direct 5 $\mu\text{L}/\text{min}$ to the mass spectrometer and 20 $\mu\text{L}/\text{min}$ to the UV detector or fraction collector. Most peptide mixtures are separated with an acetonitrile gradient from 0 - 50%. Glycerol is kept at 5% in all solutions. The mass spectrometer is generally scanned from m/z 2800 - 400 in approximately 10 seconds per scan. The mass spectra show excellent signal-to noise characteristics at the 2-10 pmol level.

We have also coupled CF-FAB with capillary zone electrophoresis. This separations technique is particularly well-suited because it has very low flow rates (about 0.1 $\mu\text{L}/\text{min}$) and has the potential for achieving separations with millions of theoretical plates. An interface is required between the exit end of the CZE capillary and the entrance to the CF-FAB capillary because of the different rates of flow. The CF-FAB probe requires about 5 $\mu\text{L}/\text{min}$ to maintain stable operation whereas electro-osmotic flow only provides about 0.1 $\mu\text{L}/\text{min}$. Any greater flow in the CZE capillary would destroy the high resolution separations achieved. The interface we have used is essentially a coaxial design and is similar to that reported by Minard (3) and Tomer (4), and is

described in more detail elsewhere in this volume. Essentially, it allows the mass spectrometer to draw the 5 $\mu\text{L}/\text{min}$ volume necessary for the CF-FAB probe without disturbing the much lower flow volume of the CZE. The separation buffer is aqueous in nature, containing 40 mM ammonium citrate or acetate at pH 5. Samples are loaded pneumatically and are of the order of 0.2-20 pmol for peptide mixtures.

We have combined microdialysis with CF-FAB (5). Microdialysis is a technique which separates low molecular weight compounds from complex mixtures containing high molecular weight polymers, such as enzymes, in microliter volumes. In one study, the microdialysis probe built by Carnegie Medicin (Uppsala, Sweden), was placed in a solution containing 400 pmol of bombesin in 20 μL of buffer and carboxypeptidase added. The inner volume of the probe was continually flushed at 4 $\mu\text{L}/\text{min}$ with water containing 5% glycerol. This solution then flowed into the MS through the CF-FAB interface. The consecutive loss of five C-terminal amino acid residues could be followed through identification of the series of truncated molecular species produced.

CONCLUSIONS

CF-FABMS can be coupled with separations methods to provide a very effective analytical tool for the analysis of mixtures of compounds. The molecular specificity of mass spectrometry can be used quite effectively in these on-line analytical applications where aqueous samples are directly used. The work cited above demonstrates the viability of the techniques employed and points to the advantages which will be obtained as this methodology and technology is improved and developed.

ACKNOWLEDGEMENTS

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CARBOHYDRATE STRUCTURAL DETAIL BY SELECTIVE OXIDATION, DERIVATIZATION AND MASS SPECTROMETRY

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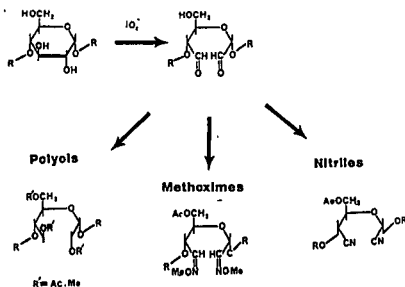
The problems of oligosaccharide separation appear to be greatly assisted by SFC. Subsequent molecular weight profiles of these components, using SFC-MS, provide further structural insight and introduce the techniques used very successfully in GC-MS, but expanded to compounds of high molecular weight, (e.g., isotope dilution, single ion monitoring). Most important of these techniques has been the opportunity to utilize signal enhancing derivatives coupled with negative-ion chemical ionization (1). This combined approach of SFC-NCIMS is demonstrated in Figure 1.

Other details of carbohydrate structure, however, are only partially resolved by mass spectrometry. Molecular weight and oligomer linear sequence can be determined by desorption MS, and branching by CID (2). Linkage composition and the determination of structural isomers require techniques that include derivatization, polymer degradation, and a chromatographic approach to resolve the complex mixtures obtained. The details of linkage position and component isomers are not available in FAB-MS spectra.

Alternatively, Angel, et.al., (3) has studied oxidized, reduced and methylated oligosaccharide samples by FAB to relate sequence to linkage position. This generalized approach appears most attractive as a technique to introduce greater molecular specificity into these isomeric samples. We have initiated studies into this oxidation-derivatization approach on polysaccharides and analyzed the products by DCI-, FAB-, and SFC-MS. Amino sugars, N-linked glycans, mono-, di-, and oligosaccharides have been tagged with pyridinyamine (PA) and/or pentafluorobenzyl aminobenzoate (PFBAB) reducing-end derivatives and the samples periodate oxidized. These materials were analyzed directly to evaluate the stability of the reducing-end tag under oxidation conditions and to maximize yield. Three derivatives were studied which were prepared by: (i) reduction and permethylation, (ii) hydroxylamination and acetylation, and, (iii) methoxylamination and acetylation, Scheme 1.

OXIDATION & DERIVATIVE FORMATION

Scheme 1



Problems were experienced with recovering small amounts of sample under conditions of reduction and permethylation, although these procedures appeared satisfactory with larger amounts of starting material. Nitrile derivatization was most interesting as a sample for subsequent CID studies, but quantitative nitrile formation has so far proven to be difficult under the conditions studied. Interestingly, the first step of hydroxylamination seemed to be more quantitative

than the subsequent step to induce nitrile formation. Of the three approaches, the methoxylamine derivative proved to be most suitable. Control studies with low molecular weight samples indicated quantitative derivatization and application of this chemistry to N-linked glycans, previously labeled with PA is progressing satisfactorily. Presented in Figure 2 is the FAB-MS of the $\text{Man}_6\text{GN-PA}$ methoxylamine peracetyl derivative. Although only partially apparent in this FAB-MS, fragmentation appears directed to glycosidic linkages providing sequence along with considerable insight into linkage, (monomer mass intervals), furan/pyran structure and branching detail. Coupling of selective chemistry, derivatization, SFC-MS with CA, and array detection could hold considerable promise for resolving the analytical complexity in this most difficult field.

References

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- 3.) Angel, A.-S., Lindh, F. and Nilsson, B. Carb Res 168:15-31 (1987)

Figure 1

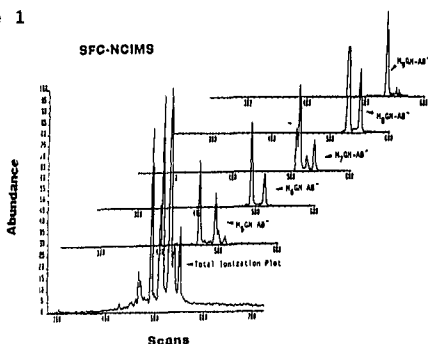
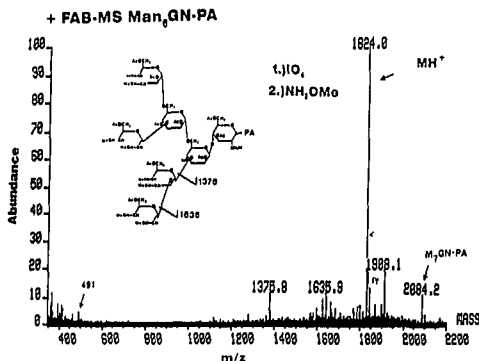


Figure 2

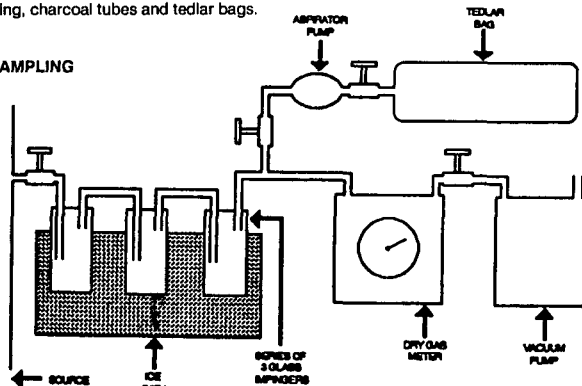


"SAMPLING AND ORGANIC ANALYSIS OF ODOURIFEROUS EMISSIONS FROM AN OIL-SANDS PLANT".
P. Thanawala, J. Roberts, and D. Kym - Suncor Inc. Oil Sands Group, P.O. Box 4001, Fort McMurray, Alberta T9H 3E3 Canada.
B. Brassard, J. Machin, D. Maxwell and J.V. Headley - Chemex Labs Alberta Inc. 2021 - 41 Avenue N.E. Calgary, Alberta T2E 6P2 Canada.

In a complex tar sands processing operation there are a number of potential sources of emissions which are perceived to effect air quality both in the plant area and downwind. As part of an odour impact assessment program to study this problem, investigations have been performed in which sensory studies have been combined with detailed chemical analyses of the composition of fugitive emissions and ambient air. Characterization of the latter pertaining to GC/MS analyses are discussed in this paper.

The broad scope of this project required the use of several sampling techniques and analytical methodologies.¹ Groups of compounds specifically targeted for analysis included alkenes, thiophenes, thiois (mercaptans), thioethers (sulphides), dithioethers (disulphides), alkyl benzenes, polycyclic aromatic hydrocarbons (PAH's) and aliphatic hydrocarbons. Other compounds studied included hydrogen sulphide and carbonyl sulphide. Samples types investigated included (a) processed gas sources, (b) liquid hydrocarbon sources, (c) and ambient air around a discharge tailings pond. Samples were collected using grab sampling, cryogenic trapping, charcoal tubes and tedlar bags.

CRYOGENIC SAMPLING



Cryogenic trapping was employed to separate wet hydrocarbon gases into three phases. Water and naphtha present in gas streams were condensed in glass impingers. The dry gas volume corresponding to the volume of liquid collected was measured using a dry test meter. At one cubic foot increments during sampling the aspirator pump was actuated to proportionally fill the tedlar bag.

Instrumental analyses were conducted using extracts from solvent desorption and automated headspace sampling. Qualitative GC/MS analyses were conducted using a Hewlett Packard 59970C MSD. Reference standards were employed to confirm GC/MS identifications as required. Quantification of the component of interest was performed using off line GC/FID and GC/FPD, utilizing external standards. The accuracy of the quantitative data is estimated to be $\pm 25\%$ based on preliminary evaluation of collection and desorption efficiencies of test mixtures.

The emissions from sources investigated are summarized in Tables 1-5.

VOLATILE SULPHUR COMPOUNDS ANALYSES OF TEDLAR BAG SAMPLES (ppm)

TABLE 1

Group of Compounds	Tailings Pond 1			
	Near Plant 4 Discharge	50 Ft. Downwind of Plant 4 Discharge	Near Plant Gates	Bird Floor
H ₂ S	ND	ND	ND	ND
COS	ND	ND	ND	ND
Methyl Mercaptan	6.0 - 7.6	9.0	ND	9
Ethyl Mercaptan	ND	ND	ND	ND
Thiophene	ND	ND	ND	2
Propyl Mercaptans	ND	ND	2.5	3.5
Butyl Mercaptans	ND	ND	ND	ND
Amyl Mercaptans	ND	ND	ND	ND

GC/MS ANALYSES OF CHARCOAL TUBE SAMPLES

TABLE 2

Group of Compounds	Tailings Pond 1		Near Plant Gates	Bird Floor
	Near Plant 4 Discharge	50 Ft. Downwind of Plant 4 Discharge		
Total Saturated and Aromatic	778-1034	735	ND	777
Total Olefinic	356-505	342	ND	393
Total Other (includes Methyl Thiophene)	45-48	ND	23	
Concentrations - mg/m ³	31			

HEADSPACE GC/MS ANALYSES OF VOLATILE COMPONENTS IN TAILINGS POND (OCTOBER 1988 SAMPLE)

TABLE 3

Group of Compounds	4G-33 (LIQUID PHASE)
Aromatic Compounds	mg/L
Organosulphur Compounds (Thiophenes)	22
PAH's	7
Hydrocarbons (C ₁₅ -C ₁₆)	ND
	234

SEMI-QUANTITATIVE RESULTS GC/FPD ANALYSES

TABLE 4

Compounds	LIQUID PHASE, ppm
Thiophene	8
C1 Thiophene	184
C2 Thiophene	75
C3 Thiophene	8
Carbonyl Sulphide	ND
Methyl Mercaptan	ND

ESTIMATED LEVELS OF ODORIFEROUS EMISSIONS FOR PROCESSED GAS SOURCES

TABLE 5

Processed Gas Sources	H ₂ S	Organosulphur Compound	THC
4E1	3.25	6.2	775
4E5	0.02	0.24	28

Units are lbs/hr.

ND = Not detected (<1 ppm)

The results indicate that (1) cryogenic sampling is an effective means of separating high dewpoint gas streams into three distinct phases to facilitate analysis, (2) automated headspace sampling is well suited to the study of airborne vapours, (3) on site analysis of reduced sulphur compounds is required to minimize artifacts and (4) reduced sulphur compounds and alkenes are the major odoriferous compounds detected and were most prevalent around the tailings discharge pond.

References

¹ NIOSH 1500, 1515, 5023, 1550 ASTM 2945, ASTM D-323, Anal. Chem. (1988) 60,216

The Gas Chromatographic Separation and
Mass Spectrometry of Neutral and Phenolic Polymeric
Chlorinated Aromatic Compounds Found in Technical Pentachlorophenol

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Technical pentachlorophenol contains a considerable amount and variety of highly chlorinated impurities. In addition to the neutral and phenolic dimeric compounds normally reported (dibenzodioxins, dibenzofurans, diphenyl ethers), a series of phenoxy polymers of those compounds also exists (Figure 1). We have 1) developed a separation scheme to remove impurities and concentrate the compounds of interest for mass spectral examination and trace level determination; 2) empirically sought a combination of injection technique and capillary column to provide optimum separation of these compounds and stay within the safe temperature limits of the column; and 3) confirmed the identity of these compounds and subsequently investigated their electron capture (E.C.) chemical ionization behavior.

The separation procedure is outlined in Figure 2. Technical Pentachlorophenol (80 gms) was dissolved in 2 liters of 0.6 N NaOH and extracted exhaustively with dichloromethane to give the neutral fraction. The pH was adjusted to 7 with H₂SO₄, and the solution was extracted again with dichloromethane to provide the phenolic fraction. Residual phenolic or neutral impurities in each fraction were removed by use of deactivated alumina; the phenolic fraction was methylated, and then each fraction further separated on supergrade basic alumina.

The Mass Spectrometry was performed using a Finnigan 4023 (with a 4500 Source) equipped with a Varian 3400 glc and a short interface heatable to 400°C. Separations were carried out on a J and W 30 Meter DB-1 capillary with 0.25 MM ID and 0.1 µm phase thickness. The column was programmed from 50°C to 330°C using a 3 step program with a 2°/min rate during elution of the major polymeric compounds. In the electron impact mode the source was operated at 190°C; In the methane E.C. mode the source was operated at 190°C and 120°C with an indicated pressure of 0.65 torr; in the hydrogen E.C. mode the source pressure was 0.55 torr and temperature 120°C.

Typical chromatograms for the polymeric region of each fraction are shown in Figure 3. The E.C. sensitivity for the polymers is high relative to the dimers with the greatest sensitivity being shown for the polyethers. The predominate electron capture fragmentation pathways can be seen in the spectra shown in Figure 4. Two or three examples were chosen for each basic structure to show the range of fragmentation observed for that species.

The polymers generally show cleavage of the ether linkages with the production of phenoxide anions (dimeric and monomeric). Loss of chlorine is also common; at 190°C loss of a single chlorine predominates while at 120°C sequential loss of additional chlorines becomes dominant. Dissociative capture is generally the rule; the molecular ion is rarely observed. Phenoxy dibenzodioxins show cleavage on both sides of the ether linkage to form phenoxide and "dioxin" anions.

The E.C. spectra provide information about the number of rings; the number of chlorines on each ring and the basic structure. The appearance of the fragmentation patterns in an observed spectra for a given core structure are extremely dependent on the position of the chlorines and phenoxy substitution. We are currently studying the relationship between the observed pattern and the structure of the molecule.

Fig. 1

HIGHER POLYMERS IDENTIFIED BY GLC-MS

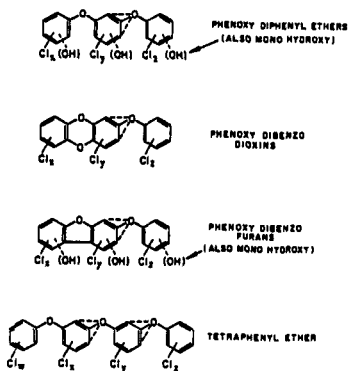


Fig. 2

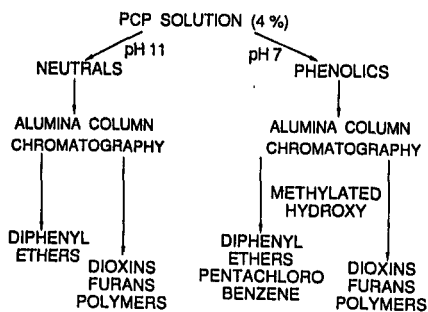


Fig. 3

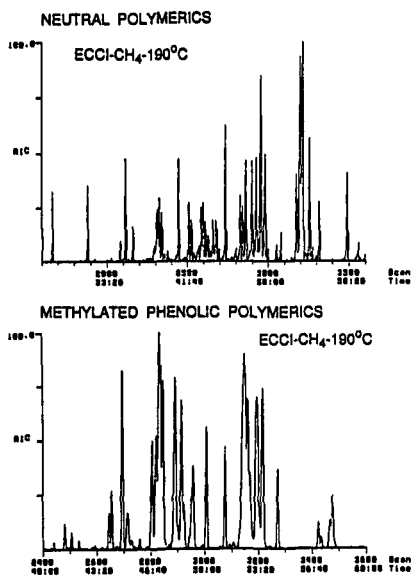
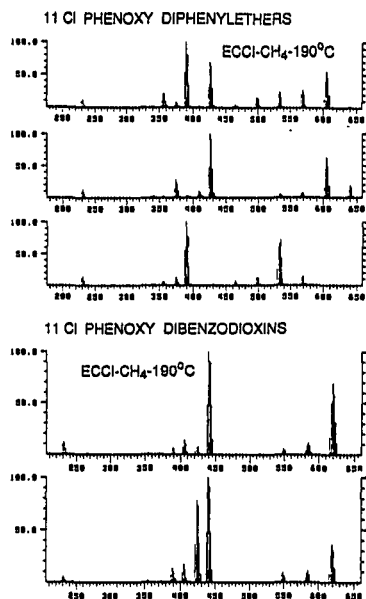


Fig. 4



CHARACTERIZATION OF POLYAROMATIC HYDROCARBONS BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY

Chhabil Dass

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This study reports on characterization of polyaromatic hydrocarbons (PAH) by fast atom bombardment (FAB)-mass spectrometry (MS). PAHs are highly mutagenic and demand sensitive methods for their characterization in environmental samples. GC and GC-MS have been used with success. The requirement of a reasonable vapor pressure limits the use of these techniques to low mass PAHs only. FAB-MS has proven to be a valuable technique for non-volatile compounds. In positive ion FAB-MS, the molecular ion is generally formed by addition of a proton to yield $(M+H)^+$ ion. PAHs have poor proton affinity, and therefore, do not yield measurable $(M+H)^+$ ion current when polar matrices are used.

Recently, we reported a FAB-MS method for characterization of a set of anthracycline antibiotics (Dass, et al.). By use of sulfolane as a FAB matrix, it was demonstrated that these antibiotics yielded abundant radical cation M^+ . The rationale for the formation of radical cations was the presence of conjugated π -electrons system. Because PAHs are also characterized having conjugated π -electrons, it is expected that they may be amenable to FAB-MS when sulfolane is used as the liquid matrix.

The experiments were performed by using a VG 7070E-HF instrument. FAB was accomplished with ca. 7 keV Xe atoms. The MS/MS spectra of the PAHs were acquired in the B/E linked-field scan technique. Collisionally-activated dissociation (CAD) of the mass selected M^+ was carried out in the first FFR with helium as the collision gas.

A typical example of the FAB mass spectra using sulfolane as the matrix is presented in Figure 1 for pyrene (m/z 202). An abundant M^+ ion is seen at m/z 202. The use of glycerol as the FAB matrix did not yield any meaningful information (Figure 2). Only a negligible ion current was obtained at m/z 203 (see inset of Figure 2). Other PAHs examined also yielded M^+ radical cations in reasonable abundance.

The use of FAB-MS was also explored for differentiation of isomeric PAHs. The ratio $(M+H)^+/M^+$ was found to be characteristic of individual PAH. As is evident from the data in Table 1, the isomeric anthracene and phenanthrene (m/z 178), pyrene and fluoranthene (m/z 202), and the four isomers of m/z 228 are all distinguishable on the basis of the $(M+H)^+/M^+$ ratio.

Although the fragment ions are not identified readily from the matrix background, the MS/MS technique can be used to provide a diagnostic mass spectrum. A B/E linked-field scan technique coupled with CAD was used to obtain the MS/MS spectrum. Figure 3 exhibits a typical B/E -CAD spectrum of fluoranthene. The B/E -CAD data can also be used to differentiate the isomeric compounds as is exemplified for anthracene-phenanthrene and pyrene-fluoranthene isomeric pairs.

Reference:

Dass, C., Sheshadio, R., Israel, M., and Desiderio, D.M. Biomed. Environ. Mass Spectrom. 17, 37 (1988).

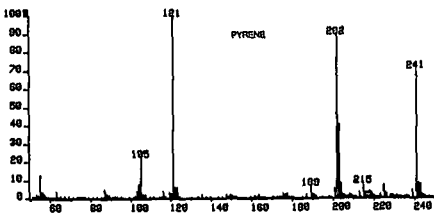


Figure 1

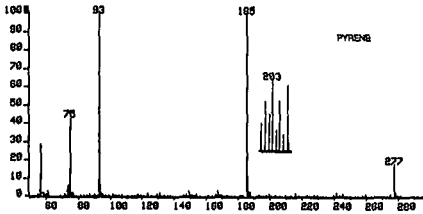


Figure 2

Table 1

PAH	(M+H) ⁺ /M ⁺	σ
Phenanthrene	0.3442	0.0149
Anthracene	0.6447	0.0304
Pyrene	0.4398	0.0071
Fluoranthene	0.5051	0.0347

PAH	(M+H) ⁺ /M ⁺	σ
1,2-Benzanthracene	0.5459	0.0185
2,3-Benzanthracene	0.7989	0.0178
Chrysene	0.5829	0.0270
Triphenylene	0.4670	0.0156

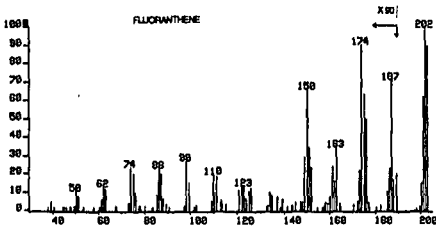
PARTIAL CAD SPECTRA

m/z		Pyrene	Fluoranthene
187	M-CH ₃	112	71
176	M-C ₂ H ₂	45	83
174	M-C ₂ H ₄	100	100
163	M-C ₃ H ₃	38	39
150	M-C ₄ H ₄	73	72
137	M-C ₅ H ₅	14	10
121	M-C ₆ H ₆	27	12
98	M-C ₈ H ₈	37	34
74	M-C ₁₀ H ₈	38	27

PARTIAL CAD SPECTRA

m/z		Anthracene	Phenanthrene
163	M-CH ₃	12	22
152	M-C ₂ H ₂	34	73
151	M-C ₂ H ₃	48	100
150	M-C ₂ H ₄	23	48
123	M-C ₄ H ₇	74	26
121	M-C ₄ H ₉	100	23
111	M-C ₆ H ₇	39	13
69	M-C ₈ H ₁₃	20	7

Figure 3



RAPID ANALYSIS OF VOLATILES ORGANICS IN WATER AND SOIL USING DIRECT PURGE INTO AN ITMS

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Analysis of volatile organics in water typically includes a trapping step which concentrates the materials prior to analysis by GC/MS. This step adds at least 15 minutes to the GC/MS analysis, which can require up to an hour. We have demonstrated that volatile organics can be purged directly from water into an ion trap mass spectrometer without an intermediate trapping (concentration) step, allowing the entire analysis to be completed in a few minutes. In addition to speed, other characteristics of direct purge ITMS approach are selectivity, sensitivity, and accuracy.

All experiments were conducted using a Finnigan ITMS with the standard open/split capillary GC interface and specially equipped with two 330 L/s air cooled turbomolecular pumps and an electropolished vacuum chamber. The open/split GC interface was used to introduce the samples directly to the ITMS. In the case of purged solutions and soil slurries, a 40 mL glass VOA vial was used as a purge vessel. As shown in Figure 1, a screw cap and teflon coated silicon cap liner were placed on either side of a stainless steel disk and were used to seal the purge vessel. Two 1/16" stainless steel tubes were welded through this disk, with the longer tube (extending to near the bottom of the purge vessel) serving as an inlet for the helium purge gas. The shorter tube (flush with the disk) serves as the exit for the purged materials and passes through a tee, with one line joined to the open split interface of the ITMS and the second line connected to a needle valve. This valve can be adjusted to change the flow into the ITMS transfer line. Helium is used as the purge gas at flows between 20 and 100 mL/min. Water from the purged sample was used as the chemical ionization reagent and a 50 msec reaction time was employed. The ITMS was used in the selective mass storage mode to obtain optimum sensitivity.

The purge profile of benzene (Fig 2) illustrates that the volatile material is efficiently purged in just a few minutes. The instrument was calibrated for quantitative measurements by plotting the sum of the integrated ion currents from the purge profiles for each concentration level. Using this approach, detection limits of 1 ppb or less have been achieved for benzene, toluene, tri- and tetrachloroethylene, methylene chloride, vinyl chloride, chloroform, chloroethane, and bromomethane in 15 mL of water. Calibration curves were linear over two orders of magnitude with a precision of about 15% without the use of an internal standard. Using the MS/MS capabilities of the ITMS, benzene has been detected in water at 500 parts per trillion (see Fig 3). Even lower detection limits are possible if the open-split ratio is increased.

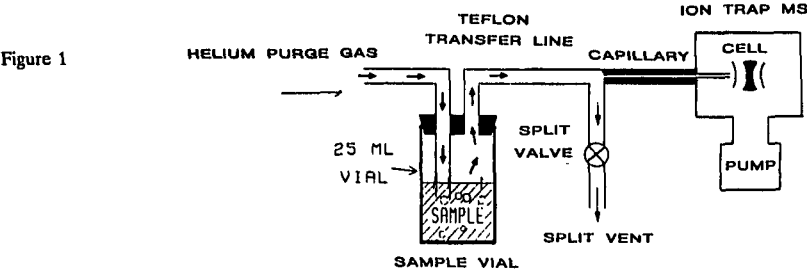
Volatiles in soils can be analyzed similarly. In this case, 5 g of soil is weighed into a 40 mL VOA vial and 15 mL of distilled water is added. The capped vial was slowly stirred with a magnetic stir bar for two minutes prior to analysis. Detection limits achieved with volatiles in soils are estimated to be in the low ppb range at this time.

The direct purge method shows considerable promise as a means of rapidly screening environmental samples for the presence of volatile organics or to quantitatively measure targeted compounds much faster (and more inexpensively) than conventional purge and trap GC/MS methods. Potential applications include use in hazardous waste site characterization, spills, and real-time monitoring, such as wells, soil, bioreactors, and process streams. We have recently used this approach for detection of targeted materials in physiological samples as well.

*Research sponsored by the U.S. Army Toxic and Hazardous Materials Agency under Interagency Agreement 1769-A073-A1 with Martin Marietta Energy Systems, Inc., under contract DE-AC05-84OR21400 with the U.S. Department of Energy.

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ITMS DIRECT SAMPLING APPARATUS



Chromatogram BENZ100P Acquired: May-21-1988 15:27:26
Comment: BENZENE IN WATER AT 100 PPB DIRECT PURGE INTO ITMS WITH HELIUM
Scan Range: 1 - 382 Scan: 139 Int = 195000 @ 1:46 100% = 227500

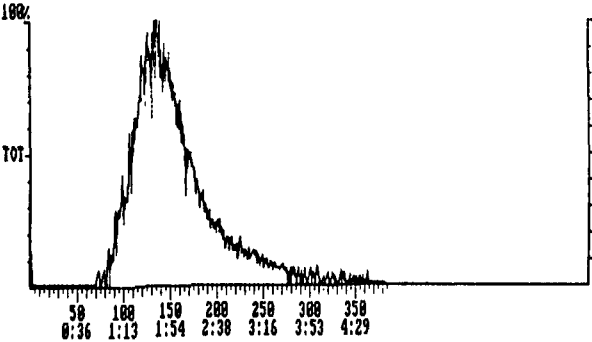
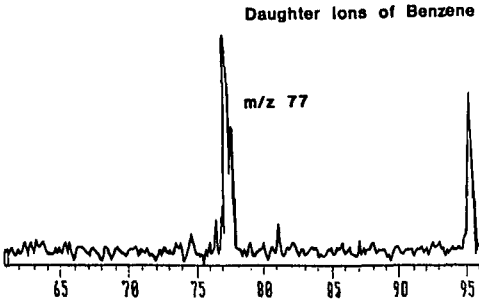


Figure 2

ITMS Direct Sampling
Benzene in Water

Concentration < 500 ppt

Figure 3



COMPARISON OF TWO MAGNETIC INSTRUMENTS IN OBTAINING NEGATIVE CHEMICAL IONIZATION MASS SPECTRA. Robert T. Rosen¹, Thomas G. Hartman¹, Joseph Lech¹ and Joseph D. Rosen², Center for Advanced Food Technology¹ and Food Science Department², New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey 08903.

Negative ion chemical ionization (NICI) is the method of choice for the quantitation of environmentally sensitive species and for the determination of metabolites and parent compounds of many pharmaceuticals in biological fluids. NICI is especially useful for compounds with good electron capturing properties, or for polar metabolites which can be derivatized with halogenated reagents.

While all manufacturers of mass spectrometers offer negative ion capability on their research instruments, it has been only recently that publications have been presented studying the effects of temperature, pressure and source parameters on the reproducibility of NICI spectra for quadrupole mass spectrometers. Oehme *et al.* compared a Finnigan 4000 MS with a HP5985B MS. They found that the Finnigan MS showed major ions characteristic of Cl^- and HCl_2^- , and while the Hewlett Packard MS did not show appreciable amounts of these ions, both systems had the same high mass spectrum. Stemmler and Hites studied instrument parameters on a HP5985B, and designed experimental criteria for duplicating spectra on their HP mass spectrometer on a day to day basis. These papers implied that it would be extremely difficult to develop a universal NICI method which would apply to different makes and models of quadrupole mass spectrometers.

Our study involved the use of two different magnetic mass spectrometers for the NICI study of macrocyclic trichothecene fungal toxins. These compounds are ideal for NICI as they contain an electron capturing butadienyl lactone moiety. One major factor suspected in obtaining reproducibility was reagent gas pressure. Spectra taken from an instrument with low CI source pressure compared to high CI source pressure were compared. The instruments had optimum source pressures of 0.3 and 0.8 Torr. It was observed that the NICI spectra on the two instruments did not differ significantly at low concentration (picogram to low nanogram range), but did differ at high concentration in the ion sources. Other source parameters, with the exception of repeller voltage and source temperature, were not important factors in reproducing spectra. In magnetic instruments the repeller, in CI, should always be set to zero volts, and when set to zero, the spectra between the two instruments were similar. Also, problems involving a time-based calibration as compared to a Hall probe or field calibration were discussed.

The conclusion of this study was that the differences in spectra were related to the total concentration of methane in the ion source and had little relation to parameters such as source voltages. The methane "electron sink" or reagent gas is actually fairly poor relative to the electron capturing properties of the sample molecules themselves. It is postulated that at higher sample concentration, the analyte itself becomes the molecule directly involved as an electron capturing agent and the NICI mass spectra start to differ because of the direct fragmentation of the analyte after capture of a high energy electron.

References:

Stemmler, E.A. and Hites, R.A. Biomed. and Environ. Mass Spectrom. 15 659-667 (1988).

Oehme, M., Stockl, D. and Knoppel, H. Anal. Chem. 58 (1986).

This is New Jersey Agricultural Experiment Station paper E10535-89-3. CAFT is an initiative of the NJ Commission on Science and Technology.

Identification of a derivative of kepone in sediment
by gas chromatography-mass spectrometry

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Kepone (chordecone, CAS #143-50-0, $C_{10}Cl_{10}O$, mol. wt. 486) is an organochlorine insecticide derived from the dimerisation and oxidation of hexachlorocyclopentadiene. It is closely related to mirex $C_{10}Cl_{12}$. In the USA it was used primarily as an anti-fireant agent, but its uses were more extensive worldwide. From 1966-1975 more than 3 million pounds of kepone were manufactured in Hopewell, Virginia, with more than half being synthesized in 1974-1975. Production was halted in 1975 with the identification of health hazards. Due to improper disposal and spillage, James River sediments from Hopewell to the mouth of the river, a distance of approximately 100 miles, became contaminated with kepone (1). As a result of the spillage, the James River was closed to commercial fishing in 1975. Although this ban has been progressively relaxed on various occasions since then, it was not lifted fully until 1988. Toxicity of kepone includes both chronic and acute effects. It is a possible carcinogen and is known to cause spinal deformities in fish.

Kepone, in common with many organochlorines, is highly persistent. To date there has been no evidence of degradation products of kepone in sediments (2) and relatively little information on biological metabolism. During a project to monitor occurrences of organochlorines in fish and sediments from Virginia, a number of unanticipated compounds were observed in samples from the James River. These samples were prepared by soxhlet extraction followed by gel permeation chromatography and analysed by fused silica capillary gas chromatography with electron capture and Hall electrolytic conductivity detection. Additional separation by high performance liquid chromatography on a semi-preparative cyano-amino column was used to fractionate the sample and reduce sample complexity prior to the GC-MS analysis. Ubiquitous pollutants such as PCB were excluded by collection of polar fractions. The resulting polar fractions in which the compounds of interest were observed were analysed by GC-MS.

The GC-MS (Extrel, Model ELQ 400-2) was used in NCI with methane as the moderator gas. A number of unknown chlorinated compounds were observed in the sediment extract. These included a compound with a molecular weight of 488, two mass units greater than kepone. The fragmentation pattern of the unknown showed losses of 2 HCL, 2 Cl and CO. The molecular ion is ~10% intensity while the base peak cluster is at m/z 346 and corresponds to the loss of 2HCL and 2Cl. This pattern, along with the GC retention time, corresponded to those of kepone alcohol ($C_{10}Cl_{10}H_2O$). In kepone alcohol, the double bonded oxygen atom has been reduced which accounts for the increase of 2 in the molecular weight. Although this compound and its glucuronide have been described as metabolites in man, it has not been identified in sediments before. The source or mechanism of formation in the sediments are unknown. Quantities of kepone alcohol vary considerably, ranging from 0-50% of the kepone concentration (up to ~100 ug/kg). The kepone alcohol is concentrated primarily in the areas closest to the original sources of the kepone. The identification of the other unknown chlorinated compounds and the distribution of the kepone alcohol in the lower James River are currently being investigated.

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Polychlorinated Dibenzo-p-dioxins (PCDD) and Dibenzofuran (PCDF) Levels in Humans: Vietnam Veterans; Occupational (Production) and Environmentally Exposed (Residential and Recreational) Individuals

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INTRODUCTION

Worldwide public concern of the toxicity of PCDDs and PCDFs has catalyzed extensive research in many toxicologically related areas. One of these compounds 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), has been called the most toxic compound synthesized by humans. 2,3,7,8-TCDD is only one of 22 TCDDs and only one of 75 (mono-octa) PCDDs--there are 135 PCDFs. Several chemical processes produce PCDDs and PCDFs as trace contaminants. Trace levels of these compounds are also produced during incineration of waste materials, combustion of leaded gasoline, and the bleaching of wood pulp. These compounds are remarkably stable and the most toxic ones, those that are substituted with chlorine at the 2,3,7,8-positions, bioaccumulate in the human foodchain. Twelve to fourteen of these 2,3,7,8-substituted PCDDs and PCDFs can generally be found at the parts-per-trillion (ppt) levels in the lipid stores of humans, particularly those living in an industrialized society. We have developed and are currently applying adipose tissue and serum methods for measuring PCDDs and PCDFs which utilize high-resolution gas chromatography/high-resolution mass spectrometry with an isotope-dilution technique for quantification. Large-scale epidemiologic studies (U.S. Air Force Ranch Hand; NIOSH Occupational Workers; Missouri residents; and Seveso, Italy residents) that have detailed medical information, as well as laboratory body burden measurements of PCDDs and PCDFs, offer the best opportunity to find any possible correlation between health effects and exposure.

RESULTS FROM VARIOUS STUDIES

VIETNAM VETERANS

From 1962 to 1970, the herbicide Agent Orange was used as a defoliant in Vietnam. The herbicide was contaminated from less than 1 to greater than 20 parts-per-million with 2,3,7,8-TCDD, which was formed as an unintentional byproduct during the manufacture of one of the compounds used in formulating Agent Orange. The veterans in studies that actually measured 2,3,7,8-TCDD body burden levels can be divided into two groups: 1) those who handled Agent Orange as part of their military duties (Operation Ranch Hand); and 2) those who served in a combat role but did not directly handle Agent Orange (ground troops).

Ground Troops. A large-scale study of U.S. Army Vietnam veterans was conducted by CDC to determine if military records could be used to identify veterans exposed to Agent Orange. No association was found between current serum TCDD levels and any indirect measure of exposure. The serum TCDD distribution in the Vietnam veterans was nearly identical to that in non-Vietnam veterans (see Table 1 and Figure 1).

Agent Orange Handlers. In 1987, the U.S. Air Force (USAF) in collaboration with CDC began a study to measure 2,3,7,8-TCDD levels in 150 members of USAF Ranch Hand Unit which sprayed Agent Orange in Vietnam and 50 matched controls. The results from this study show a large difference between the mean level in the Ranch Hand veterans and the controls (see Table 2). The distribution of levels in the two groups also differed markedly as seen in Figure 2. As the data in Figure 2 shows, however, ~38% of the Ranch Hand veterans had levels less than 20 ppt which is within the range normally found in unexposed populations. Because of this, rather large misclassification, the USAF has decided to measure TCDD levels in all 1,267 Ranch Hand members.

Table 1. TCDD Levels--Ground Troops

Place of Service	Mean + SD (ppt)
Non-Vietnam (n=97)	4.1 ± 2.3
Vietnam (n=646)	4.2 ± 2.6

Figure 1. TCDD Distribution in Vietnam/ Non-Vietnam Ground Troops

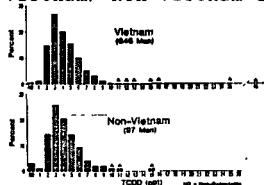
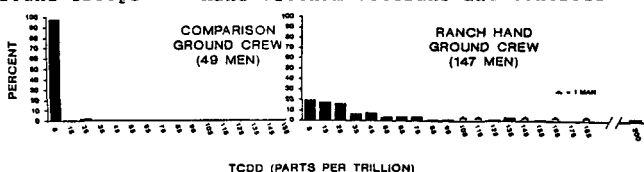


Table 2. TCDD Levels--AO Handlers

TCDD (ppt)	Ranch Hand (n=147)	Controls (n=49)
Mean	49.4	5.2
Median	26.5	4.7
Range	3.2-313	2.0-21.3

Figure 2. TCDD Distribution in Ranch Hand Vietnam Veterans and Controls



Half-Life of 2,3,7,8-TCDD in Humans. The half-life of 2,3,7,8-TCDD in serum was measured in 36 USAF Operation Ranch Hand Vietnam veterans. From serum samples taken in 1982 and 1987, the median half-life was found to be 7.1 years (95% confidence interval about the median of 5.8 to 9.6 years).

MISSOURI CASE/CONTROL STUDY

The CDC in collaboration with the state of Missouri conducted a study to compare TCDD levels in persons who had potentially been exposed to 2,3,7,8-TCDD contaminated waste oils, with Missouri residents with no known exposure. Participants from each of the four exposed groups had TCDD levels greater than the highest level measured in the control group (see Table 3). The workers involved in production of substances contaminated with TCDD had levels higher than those that worked in other departments of the same company (See Figure 3).

Table 3. Adipose Tissue TCDD Levels

in 51 Exposed Missouri Participants (ppt)

Exposure Status	n	Range	Mean	SD	Median
TCP Production	9	42-750	245	287	122
Horse Arena	16	5-577	145	202	24.1
Residential	16	5.2-59.1	26.8	18.9	19.5
Waste Hauling	10	3.7-25.8	12.4	8.3	9.0
Controls	128	ND-20.2	7.0	4.0	6.1

SEVESO, ITALY

The CDC in collaboration with Italian scientists measured TCDD in 14 serum samples that have been collected in 1976 immediately following the plant explosion in Seveso, Italy. The results of the study are shown in Figure 4 and are the highest levels ever reported in humans. The three highest levels are from children who developed the most severe types chloracne. As can be seen in Figure 4, however, no apparent threshold level for chloracne is obvious.

Figure 3. TCDD Levels in Company Employees vs. Days Employed in Production

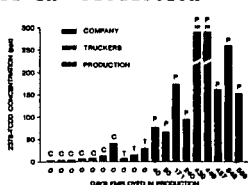
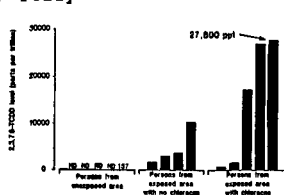


Figure 4. Serum 2,3,7,8-TCDD Levels in Zone A Residents (with and Without Chloracne) and Controls (Zone S) From Seveso, Italy



Analysis of Polychlorinated dibenzo-p-dioxins and Polychlorinated
dibenzofurans by Hybrid-Mass Spectrometry

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Problems arise in the analysis of tetrachlorodibenzo-p-dioxins (TCDDs) when samples are co-contaminated at high levels with interfering compounds. Sample-clean up procedures and mass spectrometric techniques are commonly used to enhance selectivity. Sample clean-up procedures using a combination of chromatographic materials are generally capable of removing interferences but cases exist, however in which these procedures are not effective. For example, if a sample is co-contaminated with TCDD at ppt levels and PCBS at ppb levels, PCBs can remain in the sample extract. Resolving powers of 300,000 for the hexachlorobiphenyl isomers and 48,000 for the heptachlorobiphenyl isomers are needed to resolve the TCDD confirming ions at m/z 257 and m/z 259 from interferant ions, and a resolving power of 12,500 is needed to resolve the heptachlorobiphenyl isomers from the TCDD analyte ion at m/z 322 (Tondeur *et al.* 1987). Sample clean-up procedures are also not effective in removing chlorinated xanthenes. Tetrachlorinated xanthenes remaining in fish and sediment extracts co-elute with TCDDs and interfere with the molecular ion of TCDD at m/z 320 (Kuehl *et al.* 1987). A resolving power of 18,000 is needed to resolve these ions from each other. Mass spectrometric techniques used to enhance selectivity are electron-capture negative ion chemical ionization (ECNCI), high-resolution mass spectrometry (HRMS) and mass spectrometry/mass spectrometry (MS/MS). Of these techniques, MS/MS shows the greatest promise for selective analysis of 2,3,7,8-TCDD. This isomer is not responsive in ECNCI and high-resolving powers are needed to resolve interferant ions. The power of MS/MS lies in transmitting a parent ion into the collision cell and forming characteristic daughter ions by collision-induced-dissociations. Instrumental parameters affecting the formation of daughter ions, and thus sensitivity of tandem MS/MS methods are collision gas, collision energy and collision-gas pressure. The objective of this study was to evaluate the effect of collision energy and collision-gas-pressure on the formation of PCDD and PCDF daughter ions.

The instrument used in these experiments was a VG 70-250SEQ hybrid mass spectrometer. The instrument has a EBQ₂ configuration. The first mass analyser (MS1) is a Nier-Johnson type forward geometry double-focusing configuration (EB), and is followed by a collision region formed by a RF-only quadrupole field (Q₁) and a quadrupole mass filter (Q₂).

TCDD ions monitored in HRGC/MS experiments were m/z 319.8965 (M^+) and m/z 321.8936 ($M+2^+$); ¹³C-labelled TCDD ions monitored were m/z 331.9368 (M^+), m/z 333.9339 ($M+2^+$). A PFK lock mass ion m/z 318.9792 was monitored at a resolving power of 10,000 and m/z 316.9824 was monitored at a resolving power of 18,000. Dwell times were 40ms or 60ms.

In HRGC/MS/MS experiments parent ions of PCDDs and PCDFs and ¹³C-labelled TCDD (332,334) were transmitted into the collision region using MS1 in the selected-ion-monitoring mode at a resolution about 700. Multiple-reaction-monitoring of daughter ions formed due to the loss of COCl during collision-induced-dissociations was completed by synchronously stepping the voltages on MS2. These ions were chosen because the loss of COCl is selective to PCDDs and PCDFs. Collision energies were varied between 10-50 eV and 60-100 eV in 10 eV steps with sampling times of 15ms, or maintained at 20 eV with sampling times of 40 or 60 ms.

Initial studies were conducted to determine the general trends in daughter ion formation as a function of changes in collision energy and

argon-gas pressure. Collision energies were varied between 10-100 eV across the GC peak at pressures of 1×10^{-5} mB, $.5 \times 10^{-6}$ mB and with no gas in the collision cell. Similar trends were observed for all compounds investigated - 2,3,7,8-tetrachlorinated dibenzo furan (TCDF), 1,2,3,6,7,8-hexachlorinated dibenzo-p-dioxin (HxCDD), octachlorinated dibenzofuran (OCDF) and octachlorinated dibenzodioxin (OCDD). Greater responses were observed at higher gas pressures and at collision energies of 20-40 eV. In the interest of determining whether the optimum collision energy varied between PCDD and PCDF isomers, we compared the response between 1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD. Greatest responses for both isomers were observed at the highest pressure tested - 1×10^{-5} mB and collision energies optimized at 20-40 eV.

Having determined the range of optimal collision energies to be 20-40 eV, our next experiments were conducted to further evaluate the effect of argon pressure on the formation of the M-COCl daughter ion of TCDD and TCDF. A collision energy of 20 eV was used and pressures were varied between 1×10^{-5} mB, 3×10^{-5} mB and 1×10^{-4} mB. Formation of the M-COCl daughter ion optimized at the highest pressure of 1×10^{-4} mB. Increases in response by a factor of about 2 and 3.5 were observed between 1×10^{-4} mB and 3×10^{-5} mB and 1×10^{-5} mB, respectively. The detection sensitivity using a collision energy of 20 eV and a collision-gas pressure of 1×10^{-4} mB was then determined. A S/N of 43:1 was measured on 8.6 pg and S/N=34:1 was measured on 11.6 pg 2,3,7,8 - TCDF. In our final experiments, the selectivity of the method was demonstrated through the analysis of municipal ash and pulp effluent extracts. In these cases, interferences were eliminated by HRGC/MS/MS and not by HRGC/MS at resolving powers of 10,000 and 18,000.

For the conditions used in this study, the sensitivity of HRGC/MS/MS for the analysis of PCDDs and PCDFs is enhanced at increased collision gas pressures and at lower collision energies. A collision energy optima was between 20-40 eV and enhanced sensitivities at argon pressures of 1×10^{-4} were observed. These optimal conditions do not appear to vary between isomers or congeners. The detection sensitivity for 2,3,7,8 - TCDD and 2,3,7,8 - TCDF under these conditions was about 50x less than HRGC/MS. In the cases we examined HRGC/MS/MS improves selectivity. These cases represent those in which the overriding concern is selectivity not sensitivity. In such instances HRGC/MS/MS is a powerful and useful technique.

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2. Tondeur, Y., W.N. Niederhut, J.E. Campana, S.R. Missler. 1987. Biomed. & Env. Mass Spectrometry, 14, 449-456.

A $C_{15}H_6Cl_{12}$ MOLECULE IN TECHNICAL CHLORDANE AND IN HUMAN ADIPOSE TISSUE

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Introduction. Technical chlordane, a mixture of more than 50 compounds (1, 2), is a common pesticide. Due to its toxicity, its carcinogenic potential, and its persistence in the environment, chlordane was banned from general use in 1981, and it was voluntarily removed from the market by Velsicol in 1988. Because of technical chlordane's commercial and environmental importance, there have been many efforts aimed at identifying (and quantitating) its components. Compounds with 10 carbons and up to ten chlorines have been previously reported or identified (3-6). We report here the presence of a compound with 15 carbons and 12 chlorines in technical chlordane and in human fat.

Experimental. Human breast tissues were obtained from the pathologist at Bloomington Hospital, Bloomington, IN. The fatty tissue was homogenized into a fine paste, and 10-20 g of the homogenate were mixed with pre-extracted, anhydrous Na_2SO_4 . This porous mixture was soxhlet extracted for 24 hours with an 1:1 (v/v) mixture of acetone and hexane. After soxhlet extraction, the percent fat of the extract was determined by weighing 100 μ L of the extract, allowing it to evaporate for 24 hours, and reweighing the residual. Two to three ml of the extract were used for GPC separation. The solvent mixture used to elute the column was 3:2 (v/v) cyclohexane and methylene chloride. These methods have been described elsewhere (7). The solvent was removed by rotary evaporation, and a final cleanup was performed on 1% water deactivated silica. The final volume was reduced using a dry N_2 stream. Gas chromatographic analyses were performed on an HP 5890 instrument equipped with a 30 m x 0.25 mm ID, J & W DB-5, fused silica, capillary column and an electron capture detector. Electron capture, negative ionization, gas chromatographic mass spectrometry was performed on an HP 5985B mass spectrometer using the following conditions: The ion source temperature was set at 100°C; the ion source methane pressure was monitored using an MKS Baratron capacitance manometer and adjusted to 0.43 torr. The chromatographic conditions were the same as given above. The GC/MS interface was maintained at 285°C. For trace analyses, selected ion monitoring of the most abundant ions in the mass spectra of the component of interest was used. Full scan mass spectra were obtained by scanning from 34 to 650 daltons in 1.5 seconds.

Results and Discussion. During a study of human dosimetry, human tissues were being analyzed for chlordane components with selected ion monitoring GC/MS in the electron capture, negative ion mode. One ion we were using was m/z 235 due to $C_5Cl_5^-$, a common fragment ion in the mass spectra of chlordane-like compounds (8). Responses were observed for the common Cl_7 - Cl_9 chlordane-like components; in addition, responses were observed for components with much higher GC retention time. We also noted the same responses during the analysis of technical chlordane. We were curious about the possible identity of these high retention time compounds. Thus, we obtained complete mass spectra of these compounds by injecting 190 ng of technical chlordane and scanning the mass spectrometer only during the retention time window of interest. The electron capture, negative ion mass spectrum of the most abundant component is shown in Figure 1.

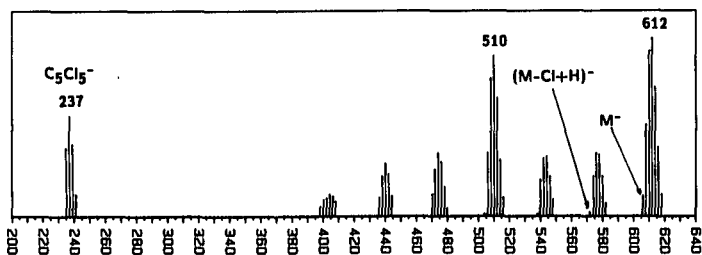


Figure 1. Mass spectrum of the $C_{15}H_6Cl_{12}$, chlordane-like molecule.

This spectrum clearly indicates that this compound has a molecular weight of 606 and 12 chlorines. The molecular ion isotope cluster agrees with that predicted from 12 chlorines. This compound could have at most 15 carbon atoms; this suggests the elemental composition of $C_{15}H_6Cl_{12}$. From this elemental composition we calculate that this molecule has 7 rings and/or double bonds. (An alternative composition of $C_{14}H_8Cl_{12}$ would be fully saturated; this seemed unlikely and was not considered

further.) This compound shows fragment ions corresponding to the sequential loss of 34 daltons. This corresponds to the loss of a chlorine atom and the addition of a hydrogen; this is a typical fragmentation process for highly chlorinated compounds under negative ion conditions (8). The prominent ion cluster at m/z 235, $C_5Cl_5^-$ due to the retro Diels-Alder fragmentation of the molecular ion (8), is also apparent in the spectra.

At this point in the interpretation, we thought it wise to consider the industrial synthesis of chlordane and chlordane. Technical chlordane is produced by the chlorination of chlordene, a $C_{10}H_6Cl_6$ compound formed by the Diels-Alder condensation of hexachlorocyclopentadiene (hex) and cyclopentadiene (see Figure 2, top). The further chlorination of chlordene produces technical chlordane (see Figure 2, lower right). We believe that the compound we observed is synthesized during the formation of chlordene. If an additional molecule of hex were to add to the double bond of chlordene at C_1 - C_2 , it would give a C_{12} compound.

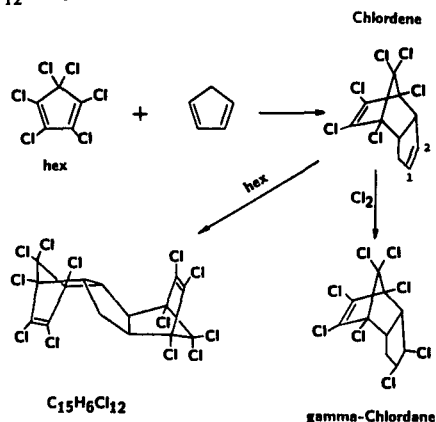


Figure 2. The synthesis steps for chlordene, gamma-chlordane, and the $C_{15}H_6Cl_{12}$ molecule.

Of course, the structure given in Figure 2 was only a hypothesis. To confirm this structure, we synthesized this compound. Hex and chlordene were mixed in equimolar amounts in a small sealed vial and then heated to $180^{\circ}C$ for 30 min. The mixture turned from a pale yellow to a deeper orange-brown. The mixture solidified upon cooling. The product was isolated from the precursors by repeated washings with hexane, in which the product is only slightly soluble. The product was recrystallized from trichloroethylene. The synthetic compound had the same mass spectrum and GC retention index (2958) as that found in the technical chlordane mixture. The structure of the synthetic compound was confirmed by x-ray crystallography. In fact, the conformation given in Figure 2 was the result of this x-ray study.

As mentioned above, these C_{15} compounds were also present in human adipose tissue. To quantify these compounds, the major ions in the mass spectra of these compounds were monitored during GC/MS analysis of a composite human adipose tissue sample. The same ions were monitored during the analysis of a technical chlordane standard. The average concentration in technical chlordane was 300 ppm, and in human fat it was 0.7 ng/g of fat. It seems as though this compound is not easily metabolized and is well retained in fatty tissues. The initial metabolic step may hinge on the availability of adjacent unchlorinated carbons for epoxidation.

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Evaluation of a Hollow Fiber Membrane for the
Determination of Volatile Organics in Water

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Semipermeable membranes are an effective and simple mechanism for isolating liquid matrices from the high vacuum requirements of the mass spectrometer. Many early applications, which used planar membranes, exhibited detrimental response times and memory effects. Recent work involving hollow fiber membranes has demonstrated improved sensitivity and response time. Small diameter hollow fibers provide thin membranes and excellent dimensional stability. We have evaluated a hollow fiber interface which uses a flow of helium to purge the membrane interior and transport analytes into the mass spectrometer.

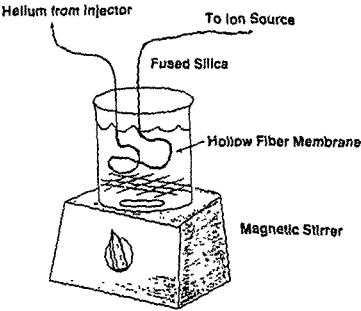
The sampling system under development consists of a length of Silastic™ tubing (0.17 mm thick) connected between two lengths of untreated fused silica capillary. One end of the fused silica capillary is inserted through the GC/MS interface and into the ion source. The other end is attached to the injection port of a GC which is used to maintain a flow of helium through the membrane system. The membrane portion of the system is immersed in the liquid matrix for analysis. The apparatus used for these experiments is shown in Figure 1.

Experiments were conducted to evaluate sensitivity and response time as a function of He flow rate, fiber configuration, and fiber length. It was determined that, while analytes were detectable if no helium was used to purge the interior of the fibers, the intensity of the ion signal was much stronger if helium was used. The response time (time for the ion current to reach 90% of maximum) is independent of the helium flow rate, within the accuracy of the timing equipment used. The response time is likely only proportional to membrane thickness. Sensitivity is, however, inversely proportional to helium flow rate. As shown in Figure 2, the intensity of the observed signal decreases with increasing helium flow. Maximum intensity is obtained when the flow is reduced to near zero, as long as there is some positive flow. It is suggested that the decrease is attributable to both physical dilution of the analyte delivered to the ion source, and helium suppression during the ionization process. Similar experiments determined that sensitivity is proportional to fiber length (with an upper practical limit of between 20 and 30 cm) and that sensitivity does not improve with parallel fibers. A comparison of one 30 cm fiber vs. six parallel 5 cm fibers indicated that the only difference is a slower response time attributable to the increased dead volume introduced by the added plumbing needed.

Using this system, we have determined several volatile organic compounds, including chloroform, trichloroethylene, and benzene, in water at the part-per-billion level. Figure 3 shows the total ion current response collected from the sequential injection of the analytes into reagent water. The individual extracted ion profiles for the base peaks of the four analytes are also shown. After the final injection had plateaued, the membrane was quickly removed from the spiked water and quickly transferred to a separate beaker of clean reagent water. The peak at the beginning of the benzene response curve suggests that the benzene did not disperse evenly and that a some of the concentrated solution may have reached the membrane before the solution equilibrated. The m/z 97 ion profile shows two plateaus, the first is due to a fragment ion of trichloroethylene, while the second is due to the trichloroethane. These compounds are distinguishable however by examining their background subtracted spectra (not shown), and suggest a reason why this interface would be useful when attached to a tandem mass spectrometer.

Figure 4 shows the total ion and a selected ion profile from the analysis of laboratory tap water. Repetitive scan data acquisition was initiated while the silicon membrane was immersed in reagent water. After several minutes the membrane was quickly transferred to a beaker of tap water. There is a noticeable rise in the signal intensity that occurs when the membrane is placed in the tap water. An averaged background subtracted scan is also shown. The ions at m/z 83 and 85 have an abundance ratio suggesting the presence of chloroform.

The hollow fiber membrane sampling system under development has significant potential for continuous monitoring of aqueous systems for trace levels of certain organic compounds. Potential applications include continuous monitoring of industrial process and waste streams, drinking water treatment streams, natural surface water streams, and subsurface groundwater.



Apparatus used for membrane response experiments

FIGURE 1

Sensitivity vs. He Flow

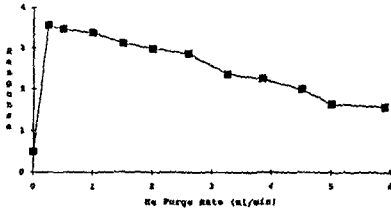


FIGURE 2

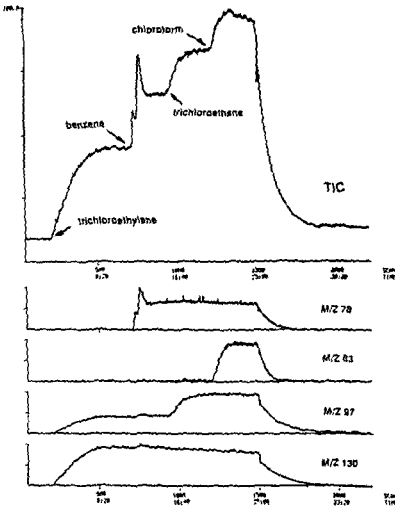


FIGURE 3

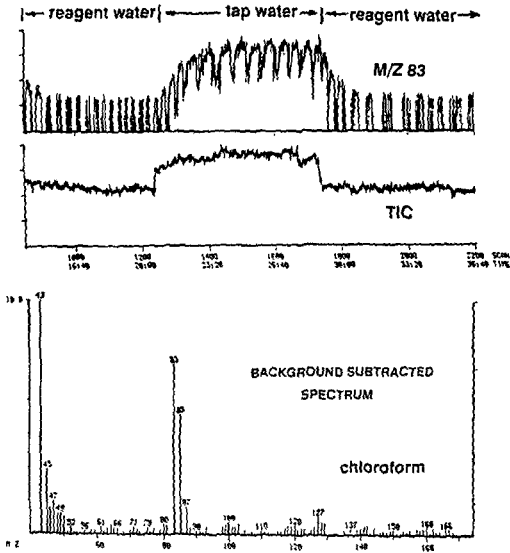


FIGURE 4

A COMPARISON OF PART PER TRILLION LEVEL ANALYSIS OF
POLYNUCLEAR AROMATIC COMPOUNDS IN DRINKING WATER USING
PREVIOUS GC/MS FULL-SCAN METHODS AND NEW SELECTED ION
MONITORING METHODS

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With the increasing concern of suspected health effects from chemicals, several states now require monitoring of ground water supplies for certain polynuclear aromatic compounds (PNAs). PNAs represent a class of compounds which include benzo(a)pyrene, benzo(g,h,i)perylene, benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene and others which are suspected carcinogens. PNA contamination of ground water supplies may occur with the release from creosote coated materials (eg., telephone poles and railroad ties) and other sources. In areas where creosote has been spilled or dumped, carcinogenic PNAs may seep through soil or fractures and contaminate ground water supplies. Recommended Allowable Limits (RALs) for PNAs are generally expressed as total nanograms of PNAs present, with individual compound detection limits of 1 or 2 parts per trillion. These low concentrations are typically difficult to routinely achieve.

The detection of PNAs have been achieved (as reported by Cole, Price and Hall; 1985) using full-scan mass spectrometry with a ppinici source. To obtain the necessary sensitivity, instrument conditions must be fully optimized and an extremely experienced operator must perform this analysis. The high level of interfering compounds precludes automated quantitation and requires analyst intervention for spectral interpretation and quantitation. The nature of selected ion monitoring (SIM) reduces interferences and provides greater sensitivity. Consequently, instrument conditions need not be so rigorously optimized and maintained to perform this analysis.

This presentation will report results from the analysis of field samples that were analyzed by both methods. Samples were chosen over a wide concentration range. The results provided by the two analytical methods were surprisingly similar.

Given these results, the SIM method for part per trillion analysis of PNAs in drinking water provides an excellent alternative to full-scan monitoring. The SIM method provides similar quality data but does not push the detection limits, as does the full scan methodology. SIM also reduces the number of interfering compounds. Consequently, if Selected Ion Monitoring of PNAs provides similar quality data as compared to full-scan methodology, than SIM analysis is a superior method.

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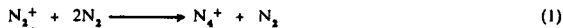
Very Low Energy Chemistry of Gas Phase Ions

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Ion-molecule reactions have been widely studied in bulk media over the past 30 years, however, few studies have been made at temperatures below 80 K. Ion-molecule chemistry is now accepted as being an important part of molecular synthesis in interstellar gas clouds. It is important to develop methods for the direct study of ion reactions in the 5 to 50 K temperature regime within which many of these clouds exist. In addition, temperature dependent rate studies have done much to elucidate mechanisms of complex chemical reactions. Extension of rate studies for gas phase ion chemistry to temperatures below 5 K promises to improve upon our understanding of ion-molecule reactive collisions.

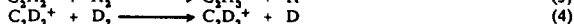
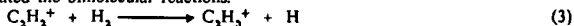
We have recently developed a free jet flow reactor method which allows the measurement of absolute ion-molecule rate coefficients in the temperature regime below 30 K.¹⁻⁴ A general schematic of the reaction zone of this instrument is shown in Figure 1. A mixture containing a small fraction (< 1%) of a suitable ion precursor, 1 to 5% of a neutral reactant gas and a buffer gas is expanded through a pulsed molecular beam valve into a vacuum chamber. The resulting isentropic expansion cools the molecules and leads to a supersonic jet with relative intrajet translational energies characterized by temperatures below 30 K. These temperatures may be controlled by variation of the beam nozzle diameter, stagnation pressure of the gas behind the nozzle, or the nature of the carrier gas species. Selective laser multiphoton ionization of the precursor molecules downstream of the nozzle (at position LF in Figure 1) produces a packet of selected cations and free electrons without perturbing the local energy distributions. The subsequent collisions of these ions with neighboring reactant neutrals in the jet can lead to chemical reactions at the low thermal energies of the expansion. This chemistry is monitored, as a function of flow distance, by periodic extraction and mass analysis of the travelling ion packet using time-of-flight mass spectrometry. The resulting temporal history of the ion density and mass distribution can be converted to absolute reaction rate coefficients for the observed reactions. To accomplish this, it is necessary to account for the thermal anisotropies inherent to free jet expansions.⁵

Figures 2 and 3 show recent results for the reaction rate coefficients obtained in this manner for, respectively, the reactions:



Both of these reactions follow strict T^{-n} rate coefficient laws with $n=1.92$ and 1.86 , respectively. These rate laws are found to be very well supported by statistical phase space theory (the calculations in the zero pressure limit are shown as the solid lines). The results strongly support the statistical nature of four atom cationic collision complexes formed at total collision energies down to 4 K.

We have also recently investigated the bimolecular reactions:



Reaction 3 proceeds with a collision efficiency less than 1% at 300 K and is not observed to proceed at 80 K. When C_3H_2^+ in its ground vibrational state is prepared in an H_2 expansion with a characteristic translational temperature of 4 K, we observe reaction to proceed at 6% of the collision rate. Reaction 4 is not observed to proceed under similar conditions in D_2 jets. When one quanta of the C-C stretch is placed in C_3D_2^+ , reaction 4 is found to proceed at near 10% of the collision rate. Coupled with the earlier bulk studies of Reaction 3, a mechanism is proposed involving production of a long lived ethylene radical cation collision complex. A barrier in the reaction exit channel, of approximate height $2.5 \text{ kcal mole}^{-1}$, inhibits direct reaction below 300 K. At very low temperatures, products from H containing complexes are formed by a slow tunnelling channel through this barrier in competition with complex dissociation back to reactants. Bulk rate coefficients cannot yet be provided, owing to the strong disequilibrium present between rotation and translation in H_2 jets. Current studies using HD and para- H_2 should to resolve this. It is very clear, however, that extremely long lived complexes play an important role in the low temperature chemistry of C_3H_2^+ . These complexes allow new mechanisms to emerge and efficient reaction channels to activate, mechanisms which may not be effectively open to higher energy reactants.

Acknowledgements: This work has been supported by financial assistance from the donors of the Petroleum Research Foundation, administered by the American Chemical Society, the Finnigan-MAT Corporation through the American Society for Mass Spectrometry and the National Science Foundation through Grant No.: CHE-8820740.

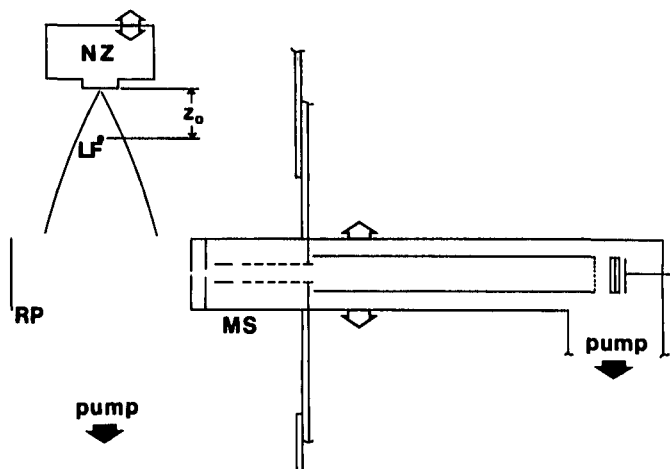


FIGURE 1

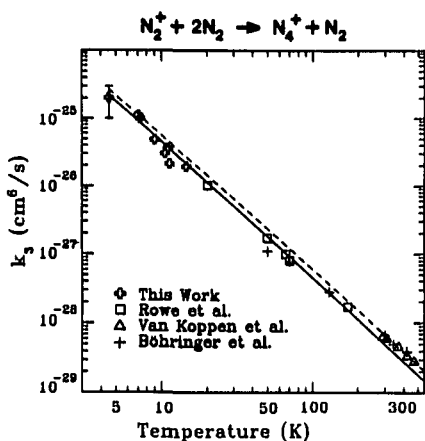


FIGURE 2

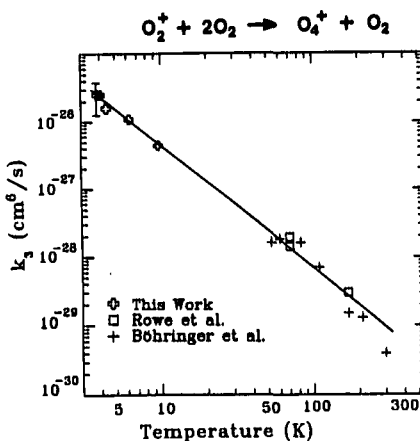


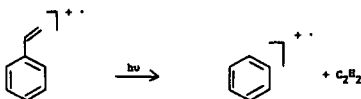
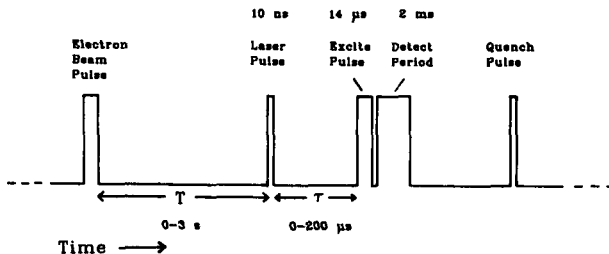
FIGURE 3

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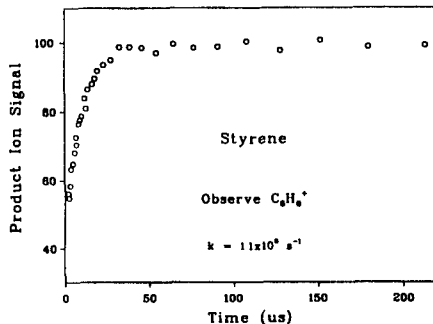
Time-Resolved Photodissociation and Energy Relaxation Processes in Styrene Ions

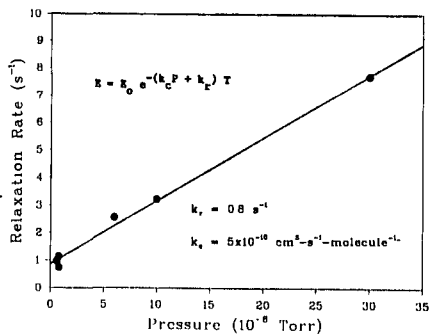
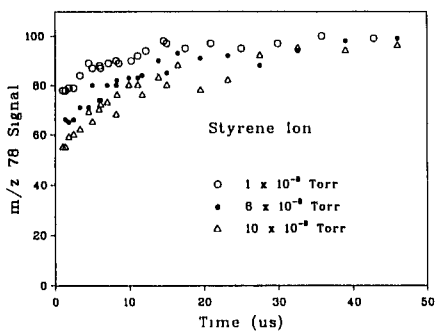
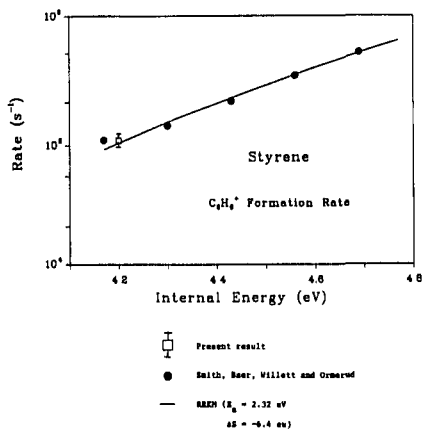
Robert C. Dunbar
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Using time-resolved photodissociation in the ICR ion trap, the fragmentation rate of styrene ions excited by an excimer-laser pulse at 308 nm was measured. Ions which were thoroughly thermalized by numerous collisions with parent neutral showed a dissociation rate constant of $1.1 \times 10^8 \text{ s}^{-1}$. This was in accord with the RRKM prediction assuming a somewhat tight transition state and an activation energy (0 K) of 2.32 eV. At low pressure and short time after ion formation the dissociation rate was greatly increased, due to unrelaxed excess energy from electron impact ionization. By following the dissociation rate as a function of pressure from 3×10^{-9} to 3×10^{-8} Torr, and as a function of delay after electron impact from 0 to 3 s, both collisional and IR-radiative relaxation of this excess energy could be followed. The transition from collision-dominated relaxation to IR-radiation-dominated relaxation occurs around 1×10^{-8} Torr of parent neutral.



Excimer laser 308 nm (4.02 eV)
Time-resolved ICR detection





Relaxation results

Initial energy E_0 (11.5 eV electron impact)	0.50 eV
Collisional Relaxation	5×10^{-10}
Radiative Relaxation	$0.65 s^{-1}$

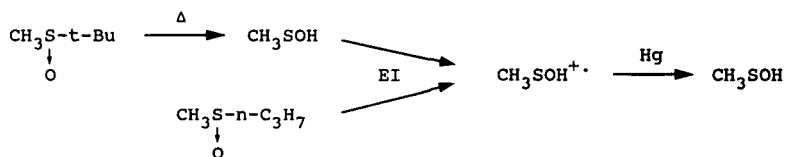
Conclusions

- Time-resolved PDS of styrene ion measures internal energies to $\pm .03$ eV in the 0.5 to 0.1 eV range
- Dissociation in the 10^5 to $10^6 s^{-1}$ region follows RRKM with a tight transition state, $E_0 = 2.32$ eV, $\Delta S^0(1000K) = -6.4$ eu
- Relaxation requires 2 collisions or 1.3 s.

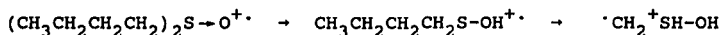
**Gas-Phase Chemistry of CH_3SOH , CH_2^+SHOH , $\text{CH}_3\text{SO}^\bullet$ and $\text{CH}_2\text{SOH}^\bullet$
by Neutralization-Reionization Mass Spectrometry**

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Methanesulfenic acid, CH_3SOH , has been suggested to occur as a reactive intermediate in photolytic oxidative degradation of methyl sulfide and dimethyldisulfide that belong to major natural and man-made atmospheric pollutants. We have generated CH_3SOH by flash-vacuum pyrolysis of methyl tert-butyl sulfoxide, or by neutralization of CH_3SOH^+ prepared from methyl n-propyl sulfoxide.



Ions CH_3SOH^+ decompose unimolecularly to give $\text{CH}_2=\text{S}^+\text{OH} + \text{H}^\bullet$, $\text{CH}_2=\text{SH}^+ + \text{OH}^\bullet$, and $\text{CH}_2=\text{S}^+ + \text{H}_2\text{O}$. The ion products were identified through their CAD spectra in MS/MS and MS/MS/MS experiments. The losses of $\text{OH}^\bullet$ and H_2O are preceded by isomerization of CH_3SOH^+ to a distonic isomer, $\text{CH}_2^+\text{SH-OH}$, which was generated independently from di-n-butyl sulfoxide and characterized by its CAD spectrum.



Neutralization of fast CH_3SOH^+ and CH_2^+SHOH with mercury vapor yielded the corresponding molecules, CH_3SOH and $\text{CH}_2^+\text{SH-OH}$, which upon reionization with O_2 gave distinct $^m\text{NR}^+$ mass spectra (Figure 1). Decompositions of neutral CH_3SOH and $\text{CH}_2^+\text{SH-OH}$ were further enhanced by collisions with helium in $^m\text{N/CAD/R}^+$ experiments (Figure 2). Following CAD, CH_3SOH undergoes C-S bond cleavage to give $\text{CH}_3^\bullet + \text{SOH}^\bullet$ ($\Delta H_f^\circ = 357 \text{ kJ/mol}$). CAD of $\text{CH}_2^+\text{SH-OH}$ yields mainly two types of products: $\text{CH}_2\text{SH}^\bullet$ and $\text{OH}^\bullet$ are formed by simple cleavage of the S-O bond ($\Delta H_f^\circ \approx 86 \text{ kJ/mol}$). $\text{CH}_2\text{S}^\bullet$ and H_2O arise by hydrogen 1,2-migration in $\text{CH}_2^+\text{SH-OH}$ to transiently form unstable $\text{CH}_2\text{S}^+-\text{OH}_2$ which decomposes spontaneously by a highly exothermic reaction. Semiempirical MNDO calculations indicate that $\text{CH}_2^+\text{SH-OH}$ is less stable than CH_3SOH , while the isomers are separated by a significant energy barrier.

CAD and $^{+}NR^{+}$ mass spectra were also used to distinguish isomeric ions $CH_3S^{+}=O$ and $CH_2=S^{+}OH$, and radicals $CH_3SO^{\cdot}$ and $\cdot CH_2SOH$. According to spectral evidence and MNDO calculations, the order of stability is $CH_2=S^{+}OH > CH_3S^{+}=O$, while $CH_3SO^{\cdot} > \cdot CH_2SOH$.

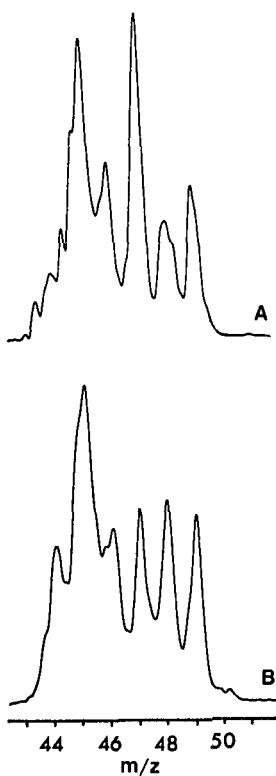


Figure 1. $^{+}NR^{+}$ (Hg(90%T)/O₂(50%T)) spectra of (A) CH₃SOH and (B) $\cdot CH_2S^{+}HOH$.

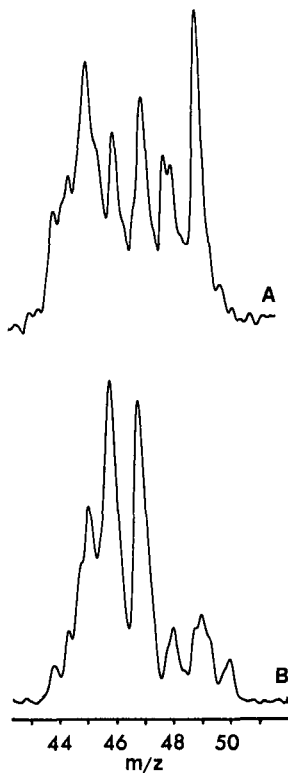
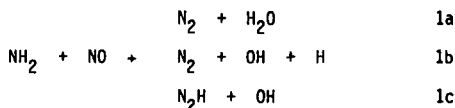


Figure 2. $^{+}NCR^{+}$ (Hg(90%T)/He(50%T)/O₂(50%T)) spectra of (A) CH₃SOH and (B) $\cdot CH_2S^{+}HOH$.

Observation of Dissociative and Radiative States of N_2H by
Neutralization Ion Beam Techniques

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An important industrial process for reducing nitric oxide emission from smoke stacks involves the addition of ammonia to the flue gas.¹ One of the critical reactions in this so-called thermal $DeNO_x$ process is believed to be that of the amidogen radical with nitric oxide:²



Previous kinetic experiments have determined that H_2O and OH are products of reaction 1³, but despite careful searching, product hydrogen atoms have not been identified.⁴ This negative evidence suggests indirectly that the previously unknown N_2H radical may be produced and live long enough to undergo bimolecular chemistry. In the present study, the first direct experimental information on this radical is obtained.

The $N_2H(D)$ radical was studied experimentally by measurement of the kinetic energy release in its unimolecular dissociation following formation by electron transfer from metal atoms to high velocity, mass-resolved ion beams and theoretically by *ab initio* techniques. Calculations of the minimum energy pathway for the ground state of the radical at the MP4/6-311G**//MP3/6-311G** level of theory indicate that the radical is unstable with respect to N_2 and H by 0.6 eV but separated from the dissociation products by a 0.4 eV barrier. One dimensional tunneling lifetimes are determined to be 7.0×10^{-12} s for N_2H and 3.6×10^{-10} s for N_2D . Neutralization of the ion with Zn targets produces predominantly radicals in the $^2A'$ ground state with dissociative lifetimes $\tau < 0.5 \mu s$, in agreement with the calculations. However due to the large ion/neutral geometry change (linear to 116° bent) it can be questioned whether the local minimum of the neutral surface was accessed in these experiments.

Mg targets were found to produce the radical in a mixture of the $^2A'$ ground and $^2A''(\pi)$ excited states with a branching ratio dependent on the internal energy of the precursor ion. A higher excited state of the radical, suggested to be an $n=3$ Rydberg level, is produced with K targets and is inferred to undergo radiative transitions.

Very recently, a new theoretical investigation of the N_2H radical has determined a significantly greater dissociation barrier (0.53 eV) using the CASSCF/CCI methodology.⁵ This new potential energy surface coupled with our tunneling analysis leads to predicted lifetimes of 3×10^{-8} s and 9×10^{-5} s for N_2H and N_2D respectively. We intend to test these predictions by producing the N_2H^+ ion from dissociative ionization of diimine thereby hoping to selectively excite the bending mode of the ion.

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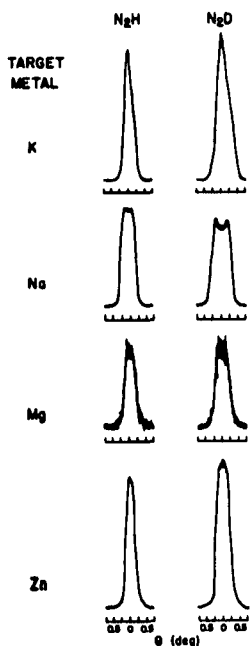


FIG. 1. Neutralized ion beam profiles obtained for N_2H^+ and N_2D^+ with K, Na, Mg, and Zn target atoms and an ion source temperature of 110 K.

TABLE I. Calculated fragmentation energies (eV).

Species	Source Temp	Target metal			
		K	Na	Mg	Zn
N_2H	300 K	2.3 ± 0.2	2.7 ± 0.1	1.8 ± 0.2	1.3 ± 0.1
	100 K	1.8 ± 0.1	2.8 ± 0.2	2.0 ± 0.2	1.4 ± 0.1
	300 K	2.6 ± 0.2	2.9 ± 0.1	1.3 ± 0.1	0.84 ± 0.06
N_2D	110 K	2.4 ± 0.1	3.0 ± 0.1	1.7 ± 0.2	1.14 ± 0.06

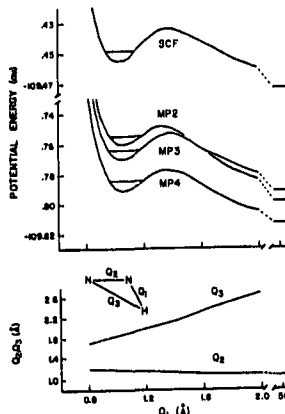


FIG. 2. (upper) *Ab initio* dissociation coordinates for $N_2H^+ \rightarrow N_2 + H$ calculated at the indicated level of theory using the 6-311G** basis set. Quasi bound levels indicate the one dimensional zero point energy of N_2H^+ in this coordinate. (lower) Variation of the internal coordinates Q_2 and Q_3 with Q_1 along the geometry optimized MP3 dissociation coordinate Q_1 . Q_2 and Q_3 are defined diagrammatically by the insert figure.

TABLE II. Theoretical analysis of $N_2H \rightarrow N_2 + H$ dissociation coordinate.*

	SCF ^b	MP2 ^b	MP3 ^b	MP4 ^c	Ref. ^{7d}
NNH					
Energy(au)	- 109.455 18	- 109.759 80	- 109.769 77	- 109.790 20	- 109.749 02
$r(NH)$ Å	1.031	1.058	1.049	1.060	1.045
$r(NN)$ Å	1.171	1.172	1.181	1.181	1.157
$\theta(NNH)$ deg	113.2	118.5	115.9	115.8	118.0
D_e (kcal/mol)	- 10.5	- 23.4	- 9.3	- 14.2	- 14.4
NN-H(TS)					
$E_s^\ddagger$ (kcal/mol)	13.4	7.4	10.7	8.5	10.5
$r(NH)$ Å	1.385	1.340	1.386	1.378	1.413
$r(NN)$ Å	1.111	1.134	1.134	1.135	1.112
$\theta(NNH)$ deg	113.9	117.4	115.3	115.3	119.9
ID-ZPE(cm ⁻¹)					
NNH	1519	1281	1381	1310	1538
NND	1136	959	1034	978	..
τ (s)					
NNH	2.9×10^{-9}	1.2×10^{-12}	5.1×10^{-11}	7.0×10^{-12}	5.0×10^{-11}
NND	2.2×10^{-9}	2.3×10^{-11}	5.3×10^{-9}	3.6×10^{-10}	

* Present calculations done with the 6-311G** basis set.

^b Geometry optimized at that level of theory.

^c Calculated at the MP3 optimized geometries.

^d MP4 calculations with the 6-31G** basis at the optimized geometry of MP3 calculations with the 6-31G**.

THE LASER MICROPROBE FTMS

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Surface characterization is important in material science and solid-state chemistry. The laser microprobe provides an alternative to the ion microprobe for the qualitative analysis of metals, polymers, organic or inorganic contaminants, and for localized elemental distributions in various matrices. Isobaric mass spectral interferences and matrix effects are often observed with these techniques. To overcome these limitations, we have combined the high mass resolving power of Fourier Transform Mass Spectrometry (FTMS) with the selectivity of laser desorption, ionization, or ablation processes, as well as the resonant two-photon process.

Instrumentation: The laser microprobe FTMS [1] is comprised of a dual FTMS source/analyzer FTMS-2000 mass spectrometer (Nicolet Instruments Corporation), a specially designed sample introduction system and state-of-the-art laser and viewing optics. The instrument consists of two parts (Figure 1):

i) The source side at medium pressure (10^{-8} Torr) contains an external manipulator 5 that is attached to the transfer rod, a sample introduction chamber pumped by a turbomolecular pump, a sample stage that is attached to an x-y-z internal Peltier effect manipulator 3 [1], and a blocking mechanism 4 that is attached to the sample manipulator to eliminate vibration the manipulator.

ii) The analyzer side at low pressure (10^{-9} Torr) comprises of laser focusing optics 6 that can be adjusted in the z-direction as a function of the laser wavelength, inverted "Cassegrain" mirror optics for the visualization and illumination of the sample, an endoscope 2 with illumination (improved contrast) for use in positioning the manipulator, and an external optics bench that contains a telescope, beam expanders and movable mirrors.

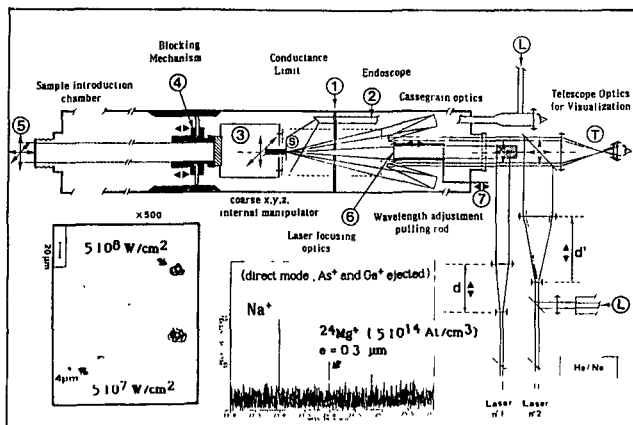


Fig 1: Laser microprobe FTMS a) minimum size of laser impact; b) detection of ^{24}Mg (5×10^{14} atoms/cm³) in GaAs matrix.

The ionizing laser (wavelength 190 nm to 360 nm) is focused normal to the sample surface. The placement of the sample at the focal point of the laser occurs from the data station by adjusting the current to the Peltier cells placed between two performed memory alloy blades [2]. The current induces a differential heating of the two blades and consequently a movement of the sample stage. A magnified view (X250) of the sample is available by using a telescope equipped with a zoom lens or by a CCD camera connected to the high-resolution video monitor near the data station.

The laser spot size is controlled by adjusting the distance between the two lenses in the laser beam expander. This design also permits resonant or nonresonant postionization of the expanded microplasma (or desorbed neutrals).

Experiments: Ionization was performed by using an excimer laser charged with KrF (249 nm) or ArF (193 nm). To enhance the detection of minor abundance ions, the major ions were ejected prior to the excite/detect event.

Results: The minimum laser spot size at low energy is 4 microns (Figure 1a). High resolution permits the easy separation of isobaric ions. For example, by operation the instrument at a resolving power of 160,000, $^{44}\text{Ca}^+$ was easily be separated from $^{88}\text{Sr}^{2+}$ at m/z 44; and $^{58}\text{Ni}^+$ and $^{60}\text{Ni}^+$ were distinguished from $^{116}\text{Sn}^{2+}$ and $^{120}\text{Sn}^{2+}$. For trace elements, the direct mode detection with a large number of data points (128 K) is preferable.

The sensitivity of LDMS is dependent on the ionization potential and work function of the element and matrix effects. For example, ^{24}Mg implanted by ion bombardment in Ga/As or In/P semiconductors (0.02 ppm or $5 \times 10^{14} \text{ atoms/cm}^3$) was only detected in the Ga/As matrix (Figure 1b). The detection threshold of Mg is 1-to-10 ppm per laser shot.

The ease of defocusing the laser and the precise control of the laser spot size (consequently the laser intensity) enables laser ablation and depth profile studies. For example, we have obtained a good separation between a Al/Cu layer and chromium layers on a silicon wafer.

Conclusion: The laser microprobe FTMS with Cassegrain optics has the following features: sample visualization (X250) for accurate positioning of the sample at the focal point of the laser; a homogeneous energy distribution for all experiments is guaranteed by the laser beam normal to the sample surface; and the automated micromanipulator that is controlled remotely from the data station. The detection sensitivity is comparable to other laser microprobe mass spectrometers such as LAMMA or LIMS.

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PROPERTIES OF CARBON CLUSTER IONS, C_n^+ FORMED BY DISSOCIATIVE IONIZATION

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Carbon cluster ions C_n^+ have previously been formed by laser vaporization of graphite and their properties studied through MIKE¹ and CAD² spectra, ion molecule reactions³, *ab initio* calculations³ and photodissociation⁴. C_n^+ ions can also be formed by dissociative electron impact ionization of molecules. In fact, a large fraction of mass spectrometry has been devoted to carbon containing ions. It is interesting to enquire whether the structure, stability and fragmentation properties of carbon cluster ions formed by dissociative electron impact ionization of carbon containing molecules are the same as those made by laser vaporization of graphite. Overcrowded perchloro hydrocarbons are a good source of C_n^+ ions, which are generated by exhaustive chlorine elimination⁵. In the present study we have made C_n^+ ions with $n = 3, 5, 6, 7, 10, 11, 12, 14$ and 16 from six different precursor molecules. Some of the ions were made from more than one precursor molecule. These C_n^+ ions were studied by means of MS/MS experiments including unimolecular MIKES, CAD MIKES, kinetic energy releases and charge stripping experiments, employing a VG ZAB-2F double-focusing mass spectrometer of reversed geometry.

The average kinetic energy releases as well as the instability towards fragmentation were observed to increase with increasing cluster size (Figs. 1 & 2), and to be independent of the precursor molecule. A characteristic metastable peak shape is represented for $C_5^+ \rightarrow C_3^+ + C_2$ in Fig. 3. MIKE and CAD spectra demonstrated characteristic 'magic' neutral losses. For example, C_5^+ eliminates unimolecularly C_2 ; C_6^+ , C_7^+ , C_{10}^+ and C_{14}^+ eliminate C_3 each while C_{11}^+ and C_{12}^+ eliminate C_1 . The fragmentation pattern of C_{16}^+ involves C_1 , C_2 , C_3 and C_5 losses.

Charge stripping (CS) was observed for C_{10}^+ and C_{16}^+ (Fig. 4) yielding minimum values for the energy required to remove the second electron, $Q_{\min} = 15.5$ eV, for both ions.

All of the data are in remarkable agreement with available data on C_n^+ ions formed by laser vaporization of graphite, suggesting that the same ion structures are formed by both methods. Furthermore, MIKES, CAD and CS data indicate a change in the C_n^+ structure between $n = 9$ and $n = 10$ in agreement with earlier findings based on photodissociation spectra and ion/molecule reactions which suggest that C_n^+ ions with $n = 3-9$ are linear while those with $n \geq 10$ are cyclic.

The unimolecular reaction $C_{11}^+ - C_{10}^+ + C_1$ is suggested to be a carbon atom extrusion from a cyclic ion to form another cyclic ion - a reaction possessing a relatively low critical energy of activation and tight transition state. Under collisional activation C_3 elimination is preferred; while being a reaction possessing a higher energy requirement it transforms a cyclic structure (C_{11}^+) to a linear ion (C_8^+) via two consecutive C-C cleavages with loose transition states. The possibility of two coexisting structures of $n = 6, 7, 8$ has not been pursued by MS/MS techniques.

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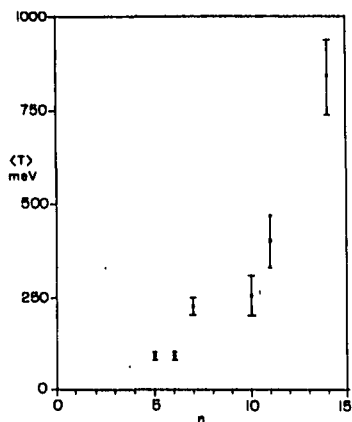


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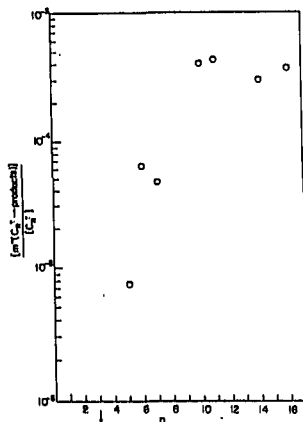


Figure 2:

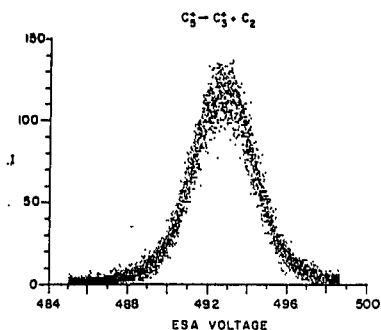


Figure 3:

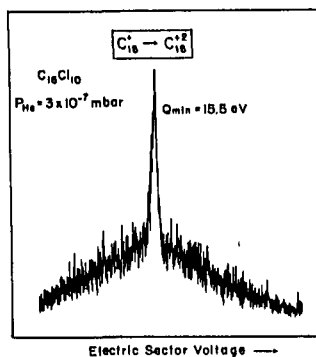


Figure 4:

STRUCTURAL EXAMINATION OF ALUMINUM CLUSTER IONS, Al_n^- for $n = 3-50$ *

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Analytical Chemistry Division
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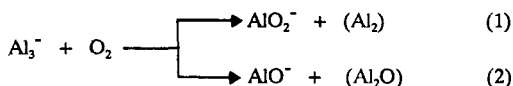
Aluminum cluster negative ions can be generated by laser ablation (without utilization of a bath gas or supersonic expansion) and studied with Fourier transform mass spectrometry (FTMS). The ease of aluminum cluster ion formation combined with the capabilities of FTMS for accurate mass measurements and ion trapping provide a powerful method for examining the structures and reactions of these ions. Specifically, charge exchange reactions, collision-induced dissociation, and ion-molecule reactions provide structural information for these clusters.

Laser ablation of a flat aluminum disk generated an abundance of negatively charged aluminum cluster ions ranging continuously from Al_3^- (m/z 81) to Al_{50}^- (m/z 1349), with Al_{13}^- observed as the most abundant cluster, Figure 1. Accurate mass measurements indicated that these clusters do not contain hydrogen or other ligands. Trapping these cluster ions for times up to 4 seconds with or without argon present indicated no change in the relative intensities of the cluster ion distribution, suggesting that any "hot" clusters have dissociated prior to ion detection. Figure 1 indicates that the odd-numbered clusters are more intense than the even-numbered clusters in the range of $n = 3$ to 15.

The aluminum cluster ions were reacted with either nitrogen dioxide (electron affinity, E.A. = 2.30 ± 0.10 eV) or para-benzoquinone (E.A. = 1.89 ± 0.10 eV) in order to investigate charge exchange. All clusters for Al_n^- where $n = 3-23$ indicated charge exchange with NO_2 . Al_3^- also indicated charge exchange with p-benzoquinone, implying E.A. (Al_3) < 1.89 eV. All of the other cluster ions were unreactive with benzoquinone, suggesting $1.89 \text{ eV} < \text{E.A.} (Al_n \text{ for } n = 4-23) < 2.30 \text{ eV}$, which are in good agreement with other experimental and theoretical values.

The collisional dissociation spectrum for most of these cluster ions is characterized by loss of one or more aluminum atoms. For example, Al_{13}^- fragmented to give products corresponding to Al_n^- where $n = 7$ to 12, shown in Figure 2. No fragments smaller than Al_7^- were observed. The fragmentation of these cluster ions is a competition between aluminum atom elimination (which is determined by the bond energy) and electron detachment (which is determined by the electron affinity of the neutral clusters). For clusters Al_n^- where $n = 4, 5, 8-13$, aluminum atom elimination was observed, implying that the bond energies for these ions are lower than the electron binding energies. For Al_3^- , Al_6^- , and Al_7^- , electron detachment was the primary fragmentation observed, indicating that the electron binding energies for these three ions are lower than their respective bond energies.

These aluminum cluster ions are unreactive with CH_4 and N_2O , but react quite specifically with O_2 . Clusters of Al_n^- for $n = 3-8$ react with O_2 to generate AlO^- and AlO_2^- , shown in equations 1 and 2 for Al_3^- . Large aluminum clusters ($n = 8-32$) also exhibit interesting oxygen reactions, shown in Figure



3. The even-numbered clusters react with O_2 to generate both AlO^- and AlO_2^- , whereas the odd-numbered clusters are unreactive. This size dependent reactivity may be a reflection of the stability of the neutral products which are generated (i.e. the neutral Al_n and Al_nO molecules).

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Figure 1

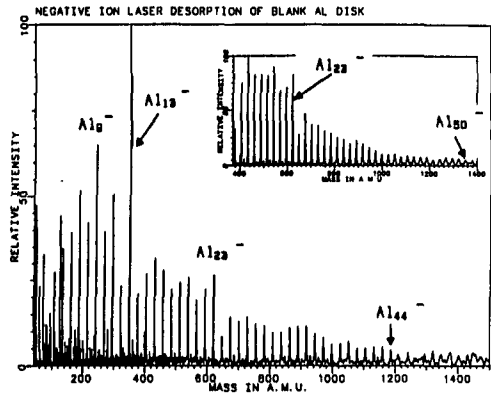


Figure 2

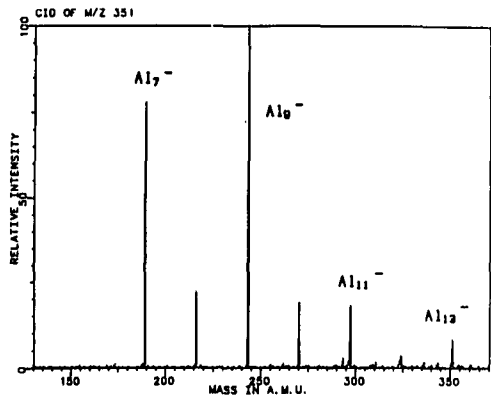
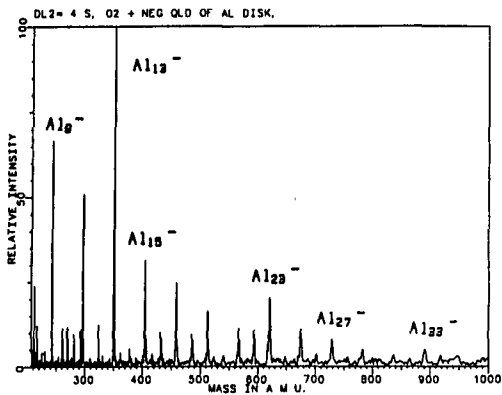


Figure 3



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THE FORMATION, DISSOCIATION, AND REACTION
OF INDIUM CLUSTER IONS, In_n^+ ($n = 2-9$)
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INTRODUCTION

Investigations of indium, gallium, and aluminum cluster ions were conducted in order to identify behavior characteristic of cluster ions composed of Group IIIB elements. These species and their oxides are important in a variety of technological applications ranging from catalysis to optoelectronics where performance has been linked closely to particle size. From the wide variety of techniques available for the production and characterization of clusters we chose fast-atom bombardment for production and mass spectrometry for characterization.

EXPERIMENTAL

Fast-atom bombardment is a relatively simple technique permitting the rapid production of an abundant cluster ion population. Al_n^+ , Ga_n^+ , and In_n^+ were produced by bombardment of the respective pure metals with an 8-keV Xe beam produced by a saddle field discharge gun. Likewise, In_xO_y^+ clusters were generated by the direct bombardment of the pelletized oxide powder, In_2O_3 , target with the 8-keV Xe beam.

Investigations of cluster ion abundance were conducted utilizing a reverse geometry (BE) double focussing mass spectrometer. The unimolecular dissociation of selected cluster ions was studied by mass-analyzed ion kinetic energy spectrometry (MIKES) in the absence of a collision gas. Low energy collision-induced dissociation (CID) and collision-induced reaction studies of the cluster ions were conducted on a triple quadrupole geometry (QQQ) mass spectrometer. The QQQ instrument was tuned to reproduce the abundance observed with the BE instrument in order to minimize potential tuning effects.

RESULTS AND DISCUSSION

Herzog reported the production of Al_n^+ cluster ions ($n = 2-18$) from the bombardment of an Al target with a rare gas ion beam.¹ A decrease in cluster ion abundance was observed as cluster ion size increased, except for cluster ions having $n = 7, 9$, and 14 which exhibited anomalous abundances. The nascent distribution of cluster ions we observed to result from the sputtering of Al, Ga, and In targets is shown in Figure 1. The Al_n^+ abundance distribution is similar to that reported by Herzog, as are the Ga_n^+ and In_n^+ abundance distributions. The data indicate that such a distribution is characteristic of Group III B cluster ions produced by sputtering.

Begemann, Meiwes-Broer, and Lutz investigated the unimolecular dissociation of sputtered aluminum cluster ions, $n = 2 - 23$, and found that the $n = 9$ and $n = 14$ cluster ions decayed to a lesser extent than adjacent sized cluster ions.² In their study they observed no unimolecular dissociation for species having $n < 8$. In MIKES investigations of Al_n^+ , Ga_n^+ , and In_n^+ we observed the unimolecular dissociation behavior for $n = 2$ to 9 . The $\log \%$ unimolecular dissociation is plotted as a function of parent cluster ion size, n , in Figure 2. Similar behavior was exhibited by Al_n^+ , Ga_n^+ , and In_n^+ . Clusters containing 2, 7, 9, and 14 atoms showed enhanced stability

relative to adjacent cluster sizes. The principal dissociation process observed was the loss of a metal atom. In general, the charge remained on the polyatomic fragment leaving a neutral atom as the other fragment. For all cluster ions $n = 5$ to 7 and for Al_4^+ , unimolecular dissociation yielded an ionized atom and a neutral polyatomic fragment. However, in low energy CID studies the $n = 9$ cluster ion dissociates to yield the ionized atom. In low energy CID studies of Al reported in the literature, similar behavior has been reported.³ The difference in ionization potentials between the $n = 8$ fragment and the atom may be so small that the additional energy from CID is sufficient to favor the higher energy fragmentation channel. In general, these results indicate that for group III B clusters the ionization potentials peak above $n = 2$ and below $n = 7$ rather than gradually decreasing from the atomic ionization potential toward the bulk work function.

As has been reported for Al cluster ions⁴, collisions of In_n^+ , $n = 2-5$, with O_2 are dissociative at most collision energies studied. The principal species observed in the daughter spectrum are the same as those observed for unimolecular dissociation or CID as described above. At low collision energies products indicative of reaction, such as In_2O^+ and In_3O^+ , were observed. In_2^+ and In_3^+ yielded the product ion, In_nO^+ , in which the parent cluster remained intact adding a single O atom. In_3^+ was the only species for which a product containing more than one O atom was observed, In_3O_2^+ .

Dissociation of In_xO_y^+ clusters, produced by the sputtering of an indium oxide target, generally yields neutral fragments of InO , In_2O , and In_2O_2 . For such species the loss of O or O_2 is a minor process. In contrast, dissociation of In_xO_x^+ , $x = 2 - 5$, yields only neutral loss of O and/or O_2 .

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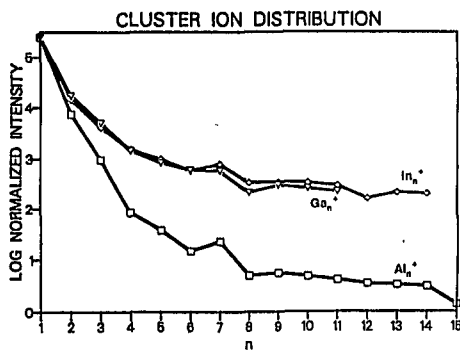


Figure 1.

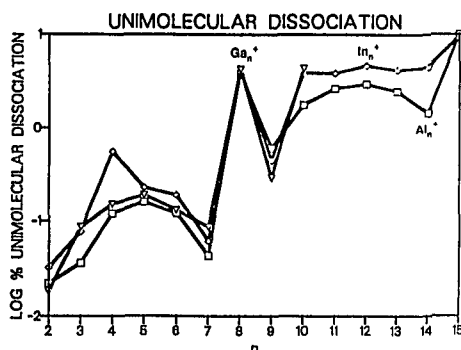


Figure 2.

DETECTION OF REACTION INTERMEDIATES IN THE
CONVERSION OF CYCLOHEXANE TO BENZENE ON Pt(111)

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Robert T. McIver, Jr., and John C. Hemminger
Institute for Surface and Interface Science and Department of Chemistry
University of California, Irvine, California 92715

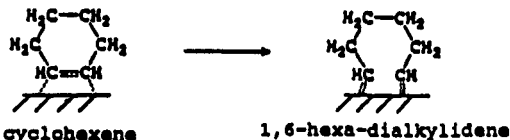
Dehydrogenation reactions catalyzed by transition metal surfaces have long been of great interest because of their importance in petroleum processing. In the conversion of cyclohexane to benzene on Pt, cyclohexene has been suggested as an intermediate, but it has never been directly detected. Presented here is the first direct observation of cyclohexene as an intermediate in this reaction. In addition, a second intermediate which is believed to be a bis(alkylidene) species is also detected. The reaction is studied as a function of temperature and time by laser-induced thermal desorption and Fourier transform mass spectrometry (LITD/FTMS). In the experiments described below, an excimer laser operated at low power (20 MW/cm², 248 nm, approx. 20 ns pulsewidth) is used to desorb molecules adsorbed on a Pt(111) single crystal. The Pt surface is not ablated and the adsorbates are removed as neutrals. The desorbed neutrals are ionized by a 70eV electron beam and detected by FTMS. The use of FTMS allows one to obtain a complete mass spectrum (m/z 10 to 600) for each single laser shot. Analysis of the mass spectra can provide molecular information about the composition of adsorbates on the surface.

A clean Pt(111) surface, supported in an ultra-high vacuum system, was cooled to 190K and exposed to gas-phase cyclohexane. Figure 1 contains four mass spectra obtained from laser-induced thermal desorption from the Pt(111) surface following a cyclohexane exposure which results in submonolayer coverage. Figure 1a is the LITD/FTMS spectrum obtained with a single laser shot after the cyclohexane has been on the surface for 3 minutes at 190K. This spectrum shows all the peaks expected for cyclohexane (mass 84 is the parent mass) and confirms that cyclohexane is stable on the surface at this temperature. In addition, there is no indication in Figure 1a of any unexpected peaks due to laser-induced cracking or pyrolysis of the cyclohexane adsorbate. A survey was then carried out by warming the sample in steps of about 3K. Several LITD/FTMS spectra were taken at each temperature (each at a different spot on the crystal) until there appeared to be no change between successive spectra taken several minutes apart. Three representative mass spectra are shown in Figures 1b-d. Figure 1b was obtained a few minutes after warming to 201K. There is no noticeable change in the cyclohexane intensity in Figure 1b relative to Figure 1a, as expected from TDS experiments by Rodriguez and Campbell which indicate no significant desorption between 190 and 201K.¹ In addition to the peaks due to cyclohexane, however, there are new peaks at m/z 50, 77, and 78 which indicate that benzene has formed on the surface. Also apparent in Figure 1b are new peaks at m/z 54, 67, and the expanded view shows a peak at m/z 82. These peaks are indicative that there is cyclohexene on the surface. To our knowledge, this constitutes the first direct observation of cyclohexene as a surface intermediate in this reaction. Additional experiments have shown larger cyclohexene concentrations, and that the relative concentrations of benzene and cyclohexene in the reaction mixture at this point are sensitive to the details of the reaction conditions. Previous direct desorption of benzene in this reaction was only recently achieved by Campbell, et al., using Bi-postdosing techniques.² Conventional thermal desorption conditions (heating rates 5-100 K/sec) result in dehydrogenation of the benzene product and also of cyclohexene at low coverages, as shown by Rodriguez and Campbell,³ so that neither product would be observed as desorption products in traditional thermal desorption spectroscopy of this reaction.

The spectrum in Figure 1c was taken after 20 minutes at 201K. The absence of a peak at m/z 84 indicates that cyclohexane is no longer present on the surface, and the absence of a peak at m/z 82 indicates that cyclohexene has also disappeared. There are several peaks that may be assigned to benzene (m/z 77 and 78), but the most prominent peaks in the spectrum at m/z 39, 41, 42, 54, and 67 are not due to benzene. This is clearly shown by

comparing Figure 1c and 1d. Figure 1d, which was obtained after warming the crystal to 314K for an additional 3 minutes, has all the peaks normally expected for benzene and is quite different from Figure 1c. The additional peaks in Figure 1c indicate that a distinctly different surface intermediate has formed. Although many C_6 hydrocarbons produce electron ionization fragment ions with masses similar to those in Figure 1c, the relative abundances are quite distinctive for each parent compound. Thus, if one compares Figure 1c with reference mass spectra for the C_6 hydrocarbons, only 1,5-hexadiene shows a close correspondence for both mass numbers and relative abundances. The distinctive features of the 1,5-hexadiene mass spectrum are a lack of parent ion and abundant fragment ions for cyclopropenyl ($C_3H_3^+$, m/z 39) and allyl ($C_3H_3^+$, m/z 41), as well as significant intensities at m/z 54 and 67.

Di-sigma bonded cyclohexene may undergo conversion analogous to olefin metathesis to form the bis(alkylidene), 1,6-hexa-dialkylidene, shown below. This species is similar to several u-bis(alkylidene) compounds identified in the organometallic literature. Laser desorption of this species would be expected to give a mass spectrum similar to that of 1,5-hexadiene and Figure 1c. Further support for the suggested mechanism is afforded by Macomber, et al., who have observed the reverse of this type of reaction.⁴



The high sensitivity of the LITD/FTMS technique has enabled us to identify the reaction product benzene, and two intermediates which, when laser desorbed, give cyclohexene and 1,5-hexadiene. Further LITD/FTMS experiments, including kinetics measurements, are being carried out to give a more complete understanding of the dehydrogenation processes involved.

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- (4) Macomber, D. W.; Hung, M.-H.; Verma, A. G.; Rogers, R.D. *Organomet.* 1988, **7**, 2072.

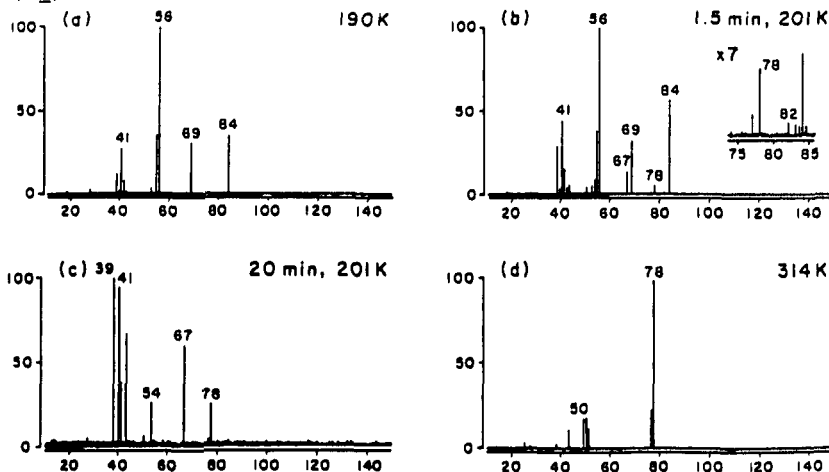


Figure 1: LITD/FT mass spectra, each from a single laser shot at a Pt(111) crystal surface, showing effect of warming after dosing with cyclohexane.

IONIZATION TECHNIQUES BASED ON PROPERTIES OF SUPERCRITICAL FLUIDS:
I. MECHANISM OF IONIZATION.

by
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The Ohio State University
Columbus, Ohio 43210

One of the largest barriers in the analysis of large molecules by mass spectrometry is the difficulty of volatilizing molecules without decomposition. This decomposition is often caused by thermal and/or electronic or vibrational addition of energy to the molecule.

We are developing new types of soft-ionization techniques. These techniques are based on the premise that the ionization properties can be controlled by varying the properties of the solvent, which is a supercritical fluid. The supercritical fluid expands into the vacuum by traversing through a supersonic jet expansion. Molecular clusters are present in the expansion due to cooling in the expansion but also due to their presence in the original solvent - the supercritical fluid. The size of the cluster that is present in the vacuum can be varied considerably by varying the properties of the supercritical fluid.

In this report, the ability to ionize the cluster systems which appear at the point of expansion was demonstrated. For the cases of carbon dioxide mixed with polar modifiers, such as methanol, both positive and negative cluster ions were produced. In the case of pure carbon dioxide only negatively charged clusters were produced. Ion beams with nanoampere currents were produced in all cases. Initial data on the possible mechanisms of cluster ionization were also shown.

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

ABRAMSON, F.P.	714	BABCOCK, L.M.	646
ABRAMSON, F.P.	1314	BABCOCK, L.M.	1385
ABRAMSON, F.P.	712	BACH, S.B.H.	1375
ACKER, M.	919	BAETEN, W.	923
ACKERMANN, B.L.	1008	BAILLIE, T.A.	1322
ACKERMANN, B.L.	383	BAILLIE, T.A.	955
ADAMS, J.	654	BAILLIE, T.A.	850
ADAMS, J.	907	BAIRD, W.M.	1324
ADURU, S.	784	BAKER, J.F.	959
AGGARWAL, S.K.	597	BAKER, T.R.	153
AGRANAT, I.	1508	BALASUBRAMANIAN, V.	1397
AHMED, M.S.	338	BALLARD, K.D.	696
AI, J.	289	BAMBERG, M.	456
AL-RAHI, M.	616	BARBER, R.C.	1143
ALBER, G.M.	1236	BARINAGA, C.J.	844
ALCARAZ, A.	298	BARINAGA, C.J.	586
ALDOUS, K.M.	1356	BARINAGA, C.J.	100
ALDRIDGE, J.C.	286	BARINAGA, C.J.	430
ALDRIDGE, J.C.	977	BARINAGA, C.J.	391
ALEXANDER, A.J.	104	BAROFSKY, D.F.	1151
ALEXANDER, A.J.	224	BAROFSKY, D.F.	1071
ALEXANDER, A.J.	226	BAROFSKY, D.F.	909
ALEXANDER, L.R.	1489	BAROFSKY, E.	1151
ALFRED, R.L.	460	BARONAVSKI, A.P.	157
ALLEMANN, M.	1254	BARRY, T.L.	1423
ALLGOOD, C.	1112	BARSHICK, C.M.	192
ALLISON, J.	1089	BARTELS, M.J.	1100
ALLISON, J.	1031	BARTMESS, J.E.	337
ALLISON, J.	1063	BARTMESS, J.E.	325
ALLISON, J.	256	BARTMESS, J.E.	326
ALLISON, J.	527	BARTNER, P.	893
ALLISON, J.	666	BARTON, R.	680
ALLISON, J.	32	BATEMAN, R.H.	790
AMER, M.A.	180	BATEMAN, R.H.	1017
ANACLETO, J.	1439	BATEMAN, R.H.	272
ANDEREGG, R.J.	917	BATEMAN, R.H.	1019
ANDEREGG, R.J.	881	BAUER, M.R.	1495
ANDERSON, K.	1363	BAYKUT, G.	1220
ANDERSON, S.	216	BEAN, M.F.	895
ANDERSON, S.G.	218	BEAN, M.F.	1131
ANDERSON, S.G.	214	BEATO, B.D.	636
ANDREWS, P.C.	854	BEATY, T.C.	1108
ANDRZEJEWSKI, D.	1445	BEAUCHAMP, J.L.	830
ANJANEYULU, P.S.:R.	1332	BEAUGRAND, C.	313
ANNAN, M.	614	BEAUGRAND, C.	1149
ANNAN, R.S.	996	BEAUGRAND, C.	1104
ANNAN, R.S.	736	BEAUGRAND, C.	466
ANTKOWIAK, J.G.	84	BEAVIS, R.C.	568
ANURADHA, D.	602	BECKER, C.H.	294
APLIN, R.T.	1342	BECKER, G.W.	875
APPEL, T.	1290	BECKER, H.	750
ARBOGAST, B.	90	BECKER, S.T.	1240
ARBOGAST, B.	1151	BEECHER, G.R.	772
ARNOLD, N.S.	304	BEHYMER, T.D.	141
ARNOLD, N.S.	1425	BEHYMER, T.D.	381
ARNOLD, N.S.	444	BEIER, R.C.	744
ASANO, K.G.	1260	BELL, C.	682
ASANO, K.G.	464	BELLAR, T.A.	141
AUDI, G.	1240	BELLAR, T.A.	381
AVERY, M.	1359	BELLARD, S.	361

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BEN-HUR, D.	1461	BOLGAR, M.S.	852
BENNETT, B.	676	BOLLEN, G.	1240
BENNETT, B.D.	535	BONI, R.	1039
BENNINGHOVEN, A.	1051	BONNER, R.F.	1181
BENNINGHOVEN, A.	204	BOSMAN, J.	984
BENNINGHOVEN, A.	1153	BOSS, W.F.	94
BENNINGHOVEN, A.	1081	BOTT, P.A.	1019
BENNINGHOVEN, A.	901	BOTTRELL, D.	1459
BENTZ, B.L.	1057	BOUCHARD, F.	321
BENZIGER, D.P.	1298	BOURELL, J.H.	1015
BERBERICH, D.W.	1250	BOWEN, R.D.	644
BERBERICH, D.W.	1147	BOWERS, M.T.	830
BERMAN, S.S.	143	BOYD, R.K.	226
BERTHIER, G.	313	BOYD, R.K.	224
BERTRAND, M.J.	395	BOYD, R.K.	104
BERTRAND, M.J.	346	BRADSHAW, N.	1102
BERTRAND, M.J.	860	BRAGG, N.	114
BERTRAND, M.J.	887	BRAJTER-TOTH, A.	371
BERTRAND, M.J.	668	BRAND, W.A.	33
BERTRAND, M.J.	792	BRAND, W.A.	1155
BERTRAND, M.J.	1019	BRASEN, D.	175
BERTRAND, M.J.	652	BRASSARD, B.	1478
BETH, A.H.	1332	BREAU, A.P.	680
BETOWSKI, L.D.	965	BREAU, A.P.	1304
BETOWSKI, L.D.	963	BREAU, A.P.	933
BETTAZZOLI, L.	754	BRENNA, J.T.	258
BEU, S.C.	1276	BRENNA, J.T.	167
BEYNON, J.H.	88	BRENTON, A.G.	88
BEYNON, J.H.	16	BRENTON, A.G.	401
BHARGAVA, A.K.	84	BRICKER, D.L.	222
BIBLE, K.C.	1135	BRILIS, G.	1408
BIEMANN, K.	537	BRILIS, G.M.	1418
BIEMANN, K.	230	BRODBELT-LUSTIG, J.	830
BIEMANN, K.	523	BROSZ, C.	1336
BIEMANN, K.	1077	BROWN, K.A.	1310
BIEMANN, K.	1122	BROWN, N.K.	1284
BIEMANN, K.	27	BROWN, N.K.	1286
BIEMANN, K.	990	BROWN, S.M.	1053
BIER, D.M.	517	BROWNER, R.F.	128
BIER, M.E.	151	BROWNER, R.F.	130
BIERL, T.W.	556	BROWNER, R.F.	131
BIGGERSTAFF, J.	216	BROWNER, R.F.	46
BIGGERSTAFF, J.	1369	BROWNER, R.F.	171
BILLER, J.E.	1077	BROWNER, R.F.	126
BILLS, N.D.	244	BRUINS, A.P.	984
BIRKINSHAW, K.	1369	BRUMLEY, W.C.	1408
BITSCH, F.	925	BRUMLEY, W.C.	1418
BITSCH, F.	919	BRUNER, F.	990
BJARNASON, A.	1194	BRYANT, D.	913
BJARNASON, A.	842	BRZAK, K.A.	1100
BJARNASON, A.	562	BUCHANAN, M.V.	1484
BJORKHEM, I.	927	BUCHANAN, M.V.	86
BLACK, D.B.	1344	BUCHANAN, M.V.	387
BLACKBURN, T.R.	1399	BUCHANAN, M.V.	1435
BLAIR, I.A.	92	BUCHANAN, M.V.	1402
BLAIR, I.A.	700	BUCHKO, G.	730
BLOM, K.	176	BUCHNER, J.D.	108
BLOOM, J.	1302	BUDE, W.L.	381
BLUNDSCHLING, M.	1240	BUDE, W.L.	798
BODOR, N.	1354	BUDE, W.L.	141

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

BUDE, W.L.	1495	CHAI, W.	790
BUJANOWSKI, V.J.	1451	CHAI, W.	788
BUNK, D.	432	CHAIT, B.T.	784
BURLINGAME, A.L.	873	CHAIT, B.T.	864
BURLINGAME, A.L.	1190	CHAIT, B.T.	1186
BURLINGAME, A.L.	870	CHAIT, B.T.	862
BURSEY, J.	969	CHAKEL, J.	1177
BURSEY, M.M.	1399	CHAMBERS, D.	54
BUS, C.A.	988	CHAMBERS, D.M.	56
BUSCH, K.L.	268	CHAN, K.	367
BUSCH, K.L.	694	CHAN, S.Y.	1476
BUSCH, K.L.	502	CHANG, J.S-C.	911
BUSCH, K.L.	35	CHANG, J.S-C.	638
BUSCH, K.L.	1053	CHANG, S.	1002
BUSMAN, M.	1118	CHANG, S.Y.	1350
BUTLER, I.	1023	CHANG, T.T.	539
BUTLER, L.C.	1455	CHARLES, J.C.	682
BUTTERWORTH, B.C.	1420	CHARLES, M.J.	1491
BUTTERWORTH, B.C.	1463	CHARLES, M.J.	441
CAESAR, J.P.	971	CHEN, D.C.	943
CAIRNS, T.	1423	CHEN, L.	1207
CALDWELL, G.W.	624	CHEN, L.	778
CALDWELL, K.A.	529	CHEN, L.	545
CALLAHAN, J.H.	157	CHEN, S-P.	323
CALLERY, P.S.	975	CHEN, T-M.	1008
CALLERY, P.S.	642	CHEN, T-M.	383
CAMPANA, J.	1506	CHESS, E.K.	688
CAMPANA, J.E.	1244	CHHEDA, G.B.	84
CAMPANA, J.E.	562	CHHEDA, G.B.	738
CANNON, T.M.	1429	CHIARELLI, M.P.	409
CANTLE, J.E.	594	CHOTT, R.C.	1348
CANTLE, J.E.	1102	CHOU, M.W.	967
CAPDEVILA, J.	92	CHOU, P.	834
CAPPIELLO, A.	990	CHOWDHURY, A.K.	260
CAPRIOLI, R.M.	722	CHOWDHURY, S.	862
CAPRIOLI, R.M.	1002	CHRISTENSEN, R.G.	1308
CAPRIOLI, R.M.	106	CHRISTIE, W.H.	1139
CAPRIOLI, R.M.	1474	CHUNG, H.L.	286
CARDIN, D.B.	1427	CHUNG, H.L.	977
CARR, S.A.	782	CISPER, M.E.	182
CARR, S.A.	895	CLAEREBOUDT, J.	923
CARR, S.A.	1131	CLAEYS, M.	923
CARTER, D.	809	CLARK, L.B.	1469
CARUSO, J.A.	48	CLARK, M.S.	1497
CARUSO, J.L.	921	CLAY, K.L.	756
CARY, A.S.	266	CLEMENT, R.E.	438
CASHMORE, G.	788	CLENCH, M.	1004
CASSADY, C.J.	161	CLENCH, M.	1414
CASSADY, C.J.	169	CLIFFORD, A.J.	244
CASTRO, M.E.	1274	COBURN, T.T.	298
CASTRO, M.E.	1272	CODY, J.T.	363
CASTRO, M.E.	458	CODY, R.B.	68
CASTRO, M.E.	1270	CODY, R.B.	1262
CASTRO, M.E.	1268	COFFEY, D.P.M.	1342
CERNIGLIA, C.E.	1306	COGGIOLA, M.J.	1242
CERNY, R.L.	102	COGGIOLA, M.J.	560
CHACE, D.H.	714	COHEN, M.J.	957
CHACE, D.H.	712	COLBY, S.M.	819
CHACE, D.H.	1314	COLE, M.J.	776
CHACE, M.	264	COLE, R.B.	246

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

COLEMAN, W.M.	977	CURRY, P.	30
COLTHUP, N.B.	1400	CURTIS, J.M.	104
COMISAROW, M.B.	455	CURTIS, J.M.	1135
COMISAROW, M.B.	323	CURTIS, J.M.	1128
COMISAROW, M.B.	1194	CUTTITTA, F.	889
COMPTON, R.N.	434	CZUK, C.I.	764
CONBOY, J.	436	D'AGOSTINO, P.A.	1439
CONBOY, J.J.	905	DAGUE, B.	106
CONTADO, M.J.	654	DAHM, K.H.	740
CONWAY, D.C.	718	DAS, P.	893
COOK, K.	1373	DASS, C.	1482
COOK, K.D.	1201	DASS, C.	885
COOK, K.D.	533	DASS, C.	600
COOK, K.D.	425	DASS, C.	602
COOKS, R.G.	369	DAVIDSON, T.	48
COOKS, R.G.	828	DAVIS, G.	915
COOKS, R.G.	151	DAVIS, G.	1290
COOKS, R.G.	98	DAVIS, P.P.	642
COOKS, R.G.	206	DAVIS, S.C.	1108
COOKS, R.G.	1027	DAVIS, S.C.	1371
COOKS, R.G.	25	DAVIS, S.C.	1246
COOKS, R.G.	228	DAVOLI, E.	754
COOKS, R.G.	60	DAWSON, P.H.	479
COOPER, B.	1350	DAY, R.	535
CORKILL, M.	361	DAY, R.A.	676
COSTELLO, C.E.	770	DE BIEVRE, P.	422
COSTELLO, C.E.	1204	DE KANEL, J.	1292
COSTELLO, C.E.	1192	DEAKYNE, C.A.	1367
COTTER, R.J.	545	DEARTH, M.A.	1493
COTTER, R.J.	1207	DEGA-SZAFRAN, Z.	248
COTTER, R.J.	547	DEIMEL, M.	1081
COTTER, R.J.	1258	DEINZER, M.L.	90
COTTER, R.J.	43	DEINZER, M.L.	1480
COTTER, R.J.	375	DELAETER, J.R.	1170
COTTER, R.J.	877	DELAPORTE, T.	1104
COTTER, R.J.	778	DELOACH, J.R.	744
COTTER, R.J.	1098	DELONG, S.E.	1238
COTTER, R.J.	576	DEMARK, B.R.	698
COTTR, R.J.	574	DERRICK, P.J.	1246
COTTRELL, J.	1000	DERRICK, P.J.	1371
COUTANT, J.E.	1008	DESIDERIO, D.M.	885
COUTANT, J.E.	383	DESIDERIO, D.M.	883
COVEY, T.	1014	DESPOT, K.	556
COVEY, T.	889	DETERDING, L.J.	385
COVEY, T.	118	DETERDING, L.J.	114
COVEY, T.	1177	DETERDING, L.J.	1006
COVEY, T.R.	558	DEUTSCH, J.	708
COVEY, T.R.	905	DEVANT, G.	466
COVEY, T.R.	1181	DEVANT, G.	1104
CRAWFORD, R.W.	298	DEVANT, G.	1149
CREASY, W.R.	167	DEVASTRO, J.	539
CREASY, W.R.	258	DHAHIR, P.H.	680
CREECH, J.	879	DHEANDHANOO, S.	1441
CREED, J.	48	DI BROZOLO, F.R.	287
CRESCENTINI, G.	990	DIDONATO, G.C.	852
CROCKETT, J.S.	240	DILTS, M.J.	139
CROW, F.W.	866	DILTS, M.J.	1045
CROW, F.W.	640	DIXON, M.	1083
CUMMINGS, R.	1408	DOBB, D.E.	1455
CUMMISKEY, J.	176	DOBSON, R.L.M.	698

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

DODIA, V.	782	ENKE, C.G.	1465
DOELLING, V.	893	ENKE, C.G.	1067
DOHERTY, S.J.	1053	ENKE, C.G.	348
DOHERTY, S.J.	694	ENKE, C.G.	1065
DOI, J.D.	1012	ENKE, C.G.	32
DOLNIKOWSKI, G.G.	1145	ENS, W.	232
DOLZINE, T.W.	1316	ENS, W.	399
DOMON, B.	770	ENS, W.	1059
DONNELLY, J.	1418	ENS, W.	566
DONNELLY, J.R.	1412	ERICKSON, E.D.	1067
DONNELLY, J.R.	800	ESCHER, E.	895
DONNELLY, J.R.	1455	ESMANS, E.L.	923
DONOHUE, D.L.	584	ETHIER, J.-C.	1344
DONOHUE, D.L.	22	EVANS, C.A.	812
DOUGLAS, D.J.	50	EVANS, C.J.	909
DOURDEVILLE, T.A.	897	EVANS, J.O.	1184
DOURDEVILLE, T.A.	992	EVANS, M.A.	1310
DOWN, S.	361	EVANS, S.	1000
DOWN, J.J.	1459	EWING, A.G.	30
DOWN, J.J.	356	EYLER, J.R.	1216
DREIFUSS, P.A.	929	EYLER, J.R.	1137
DRENTH, B.F.H.	984	EYLER, J.R.	1375
DRINKWATER, D.E.	1502	EYLER, J.R.	248
DRINKWATER, D.E.	1387	EYLER, J.R.	250
DRISCOLL, S.M.	961	EYMAN, D.P.	186
DUCKWORTH, D.	1141	FACKLER, J.P.	1133
DUCKWORTH, D.C.	188	FALCINI, M.R.A.	1145
DUCKWORTH, H.E.	1143	FALCINI, M.R.A.	1381
DULAK, J.G.	580	FALES, H.M.	674
DULAK, J.G.	1441	FALES, H.M.	632
DUNBAR, R.C.	1500	FALICK, A.M.	870
DUNBAR, R.C.	254	FALICK, A.M.	872
DUNBAR, R.C.	252	FAN, T.P.	300
DUNCAN, M.W.	1339	FARNHAM, I.	1408
DUTTA, S.P.	84	FARROW, P.E.	949
DUTTA, S.P.	738	FASSETT, J.D.	417
DUTTON, G.G.S.	1194	FAULK, J.D.	254
DYCK, G.R.	1143	FAULL, K.F.	909
DYCKES, D.F.	543	FEISTNER, G.J.	909
EASTON, M.P.	293	FEIZI, T.	788
ECKENRODE, B.A.	462	FEIZI, T.	790
ECKENRODE, B.A.	1094	FELLOWS, L.E.	1342
ECKENRODE, B.E.	1065	FENN, J.B.	389
EDMAN, K.R.	130	FENN, J.B.	428
EDMONDS, C.G.	391	FENNESSEY, P.V.	359
EDMONDS, C.G.	430	FENNESSEY, P.V.	411
EDMONDS, C.G.	844	FENNESSEY, P.V.	523
EDMONDS, C.G.	586	FENSELAU, C.	846
EDOM, R.W.	684	FENSELAU, C.	576
EDSTROM, R.	1488	FENSELAU, C.	786
EGELHOF, P.	1240	FENSELAU, C.C.	877
ELABBASSI, B.	466	FENSELAU, C.C.	375
ELLEFSON, R.E.	595	FERGUSON, A.	742
ELLERBE, P.	1308	FERRARIS, J.	178
ELLIOTT, W.H.	1358	FINK, S.W.	543
ELLIS, G.	1083	FINK, S.W.	541
ELSON, C.M.	1406	FIRTH, J.	1000
EMARY, W.B.	574	FISCHER, R.T.	1336
EMARY, W.B.	778	FISHER, J.J.	1391
ENKE, C.G.	776	FISK, J.	1459

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

FISK, J.	356	GETEK, T.A.	1302
FITE, W.L.	1441	GETEK, T.A.	1306
FJELDSTEAD, J.	1092	GETEK, T.A.	551
FLEET, G.W.J.	1342	GETEK, T.A.	1318
FLORY, D.A.	523	GHOSH, A.	917
FLURER, R.A.	335	GHOSH, D.	88
FODOR, L.	606	GIAM, C.S.	338
FOLTZ, R.L.	1352	GIBLIN, D.	238
FOLTZ, R.L.	1296	GIBSON, B.W.	1188
FRAENKEL, G.	1397	GIESE, R.	736
FRANZEN, J.	564	GIESE, R.W.	996
FRASURE, W.	1098	GILBERT, J.	1031
FREAS, R.B.	660	GILES, H.N.	310
FREAS, R.B.	543	GILL, G.	350
FREAS, R.B.	541	GILLECE-CASTRO, B.	1190
FREAS, R.B.	173	GILLIAM, J.M.	875
FREAS, R.B.	1280	GILMARTIN, D.	293
FREEMAN, H.S.	1453	GLAZER, A.N.	913
FREEMAN, J.A.	1449	GLISH, G.L.	1260
FREEMAN, J.P.	551	GLISH, G.L.	1094
FREEMAN, J.P.	1302	GLISH, G.L.	77
FREEMAN, K.H.	33	GLISH, G.L.	796
FREEMAN, K.L.	1155	GLISH, G.L.	464
FREISER, B.S.	824	GLISH, G.L.	584
FRENKING, G.	1367	GLISH, G.L.	462
FREY, R.	564	GLISH, G.L.	806
FRIESEN, M.	82	GLISH, G.L.	234
FRY, C.	282	GLISH, G.L.	335
FU, P.P.	1410	GLISH, G.L.	22
FUJIWARA, H.	1348	GLISH, G.L.	1029
FUKUDA, E.K.	642	GOERINGER, D.E.	1260
FULGINITI, M.	291	GO NYEA, G.J.	959
FULLERTON, M.	1461	GOODLETT, D.R.	879
FURA, A.	612	GOODLEY, P.C.	1092
FUTRELL, J.	1369	GOODMAN, S.D.	1218
FUTRELL, J.	216	GOODWIN, R.M.	933
FUTRELL, J.H.	218	GOODWIN, R.M.	1304
FUTRELL, J.H.	214	GORD, J.R.	824
FUTRELL, J.H.	496	GORDON, B.M.	977
GAGE, D.A.	752	GOSWAMI, T.	180
GAGE, D.A.	750	GOULD, V.	1110
GALBRAITH, W.	915	GRABOWSKI, J.J.	838
GALE, P.J.	1057	GRANGE, A.	1418
GALLEGOS, E.J.	296	GRANGE, A.H.	1412
GANDE, M.	608	GRANT, B.C.	1029
GANGULI, B.	1120	GRAY, J.W.	1085
GARDNER, G.J.	143	GRAYSON, M.A.	485
GARDNER, J.M.	359	GRAYSON, M.A.	282
GARREN, L.	82	GREATHEAD, R.J.	1246
GASKELL, S.J.	758	GREATHEAD, R.J.	1371
GASKELL, S.J.	760	GREAVES, J.	803
GASKELL, S.J.	897	GREAVES, J.	1488
GASKELL, S.J.	848	GREEN, B.N.	1491
GAUDRY, D.	1047	GREEN, B.N.	1017
GAUMANN, T.	65	GREEN, L.W.	588
GAUTHIER, J.	860	GREEN, M.L.	175
GELINAS, Y.	178	GREIG, N.H.	708
GELLENE, G.I.	1504	GREIG, N.H.	704
GENO, P.W.	720	GRESE, R.P.	768
GETEK, T.A.	967	GRIFFIN, D.A.	1480

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

GRIFFIN, P.R.	1125	HANSEN, G.	1035
GRIFFIN, P.R.	899	HANSEN, S.	1092
GRIFFIN, P.R.	821	HANSON, C.D.	458
GRIFFITHS, W.J.	1393	HANSON, C.D.	1268
GRIGSBY, R.D.	310	HANSON, C.D.	1272
GRIMSRUD, E.P.	554	HANSON, C.D.	1274
GRIMSRUD, E.P.	1379	HANSON, C.D.	1270
GRINDSTAFF, Q.G.	1457	HARADA, K-I.	1346
GROSS, M.L.	1224	HARBOL, K.L.	656
GROSS, M.L.	1222	HARDEN, C.S.	1365
GROSS, M.L.	754	HARGISS, L.O.	982
GROSS, M.L.	102	HAROLDSSEN, P.E.	758
GROSS, M.L.	240	HARRIS, F.M.	1393
GROSS, M.L.	654	HARRIS, F.M.	88
GROSS, M.L.	315	HARRIS, W.E.	128
GROSS, M.L.	768	HARRIS, W.E.	126
GROSS, M.L.	529	HARRISON, A.G.	553
GROSS, M.L.	238	HARRISON, W.W.	70
GROSS, M.L.	1416	HARRISON, W.W.	194
GROSS, M.L.	840	HARRISON, W.W.	36
GROSS, M.L.	628	HARRISON, W.W.	196
GROSSHANS, P.B.	1230	HARRISON, W.W.	198
GROSSHANS, P.B.	1232	HARRISON, W.W.	192
GROSSMANN, P.	1254	HARRISON, W.W.	52
GROSSMANN, P.	1252	HART, K.J.	348
GROTEMEYER, J.	973	HARTMAN, T.G.	1469
GROTEMEYER, J.	570	HARTMAN, T.G.	1486
GROTEMEYER, J.	568	HARTMAN, T.G.	1338
GRUTZMACHER, H-F.	1157	HARTMAN, T.G.	1341
GUAN, S.	1210	HARVEY, D.J.	1282
GUAN, S.	1212	HARVEY, D.J.	1284
GUAN, S.	451	HARVEY, D.J.	1286
GUERIN, M.R.	1402	HARVEY, D.J.	1288
GUERIN, M.R.	1484	HASS, J.R.	1491
GUIDO, D.M.	764	HATCHER, H.H.	302
GUIDO, D.M.	891	HAW, J.F.	280
HAAS, R.	873	HAWES, E.M.	1300
HABFAST, K.	1155	HAWES, E.M.	684
HABFAST, K.	33	HAWLEY, M.	1498
HABFAST, K.	1033	HAWTHORNE, S.B.	1049
HABIBI-GOUDARZI, S.	25	HAYES, J.	1155
HABIBI-GOUDARZI, S.	228	HAYES, J.M.	33
HAGENHOFF, B.	204	HAYES, M.J.	1047
HAGENHOFF, B.	901	HAYES, R.N.	628
HAGNAUER, G.L.	270	HAYES, R.N.	315
HAIL, M.E.	1449	HAYES, R.N.	840
HAIL, M.E.	1147	HEADLEY, J.V.	1478
HAKANSSON, P.	399	HEARN, B.A.	1216
HALL, B.J.	1143	HEATH, T.G.	752
HALL, J.E.	682	HEATH, T.G.	706
HALL, T.	1292	HEEREMANS, C.E.M.	935
HALUALANI, R.	856	HEINE, C.E.	1182
HAMDAN, M.	401	HELD, S.D.	1361
HAMMARGREN, W.M.	690	HELLER, D.N.	1258
HAMMOND, M.J.	1025	HELLER, T.	1153
HAN, D-H.	850	HEMBERGER, P.H.	60
HAND, O.W.	206	HEMBERGER, P.H.	1429
HANEY, C.A.	1453	HEMBERGER, P.H.	182
HANSEN, E.B.	967	HEMLING, M.E.	782
HANSEN, E.B.	1306	HEMLING, M.E.	1131

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

HEMMINGER, J.C.	202	HOUNSELL, E.F.	788
HEMMINGER, J.C.	1514	HOUNSELL, E.F.	790
HEMMINGER, J.C.	200	HOWALD, W.N.	1320
HENDERSON, L.E.	1098	HOWALD, W.N.	1322
HENION, J.	118	HOWARD, S.	216
HENION, J.	1014	HOWARD, S.	1369
HENION, J.	436	HOWARD, S.L.	214
HENION, J.	1181	HRUSKA, F.E.	730
HENION, J.	1472	HSU, F-F.	1115
HENION, J.D.	905	HUANG, E.	1014
HENION, J.D.	558	HUANG, E.	118
HENION, J.D.	365	HUANG, E.	1472
HENRY, K.D.	449	HUANG, L.Q.	1447
HENRY, K.D.	236	HUANG, L.Q.	1443
HENZEL, W.J.	1015	HUANG, S.	630
HEPBURN, J.W.	321	HUANG, S.	556
HEPPNER, R.A.	1431	HUANG, Z.H.	748
HER, G.	893	HUBBARD, J.W.	1300
HERALD, C.H.	102	HUDSON, C.E.	610
HERCULES, D.M.	409	HUGHES, B.M.	1012
HERCULES, D.M.	670	HUGHES, R.J.	220
HERCULES, D.M.	578	HULETT, L.D.	22
HEROLD, D.A.	597	HULETT, L.D.	584
HERROLD, D.	1377	HUNT, D.F.	899
HESS, K.R.	190	HUNT, D.F.	1125
HETTICH, R.L.	1510	HUNT, D.F.	821
HETTICH, R.L.	86	HUNTER, J.L.	868
HEUMANN, K.G.	414	HUNTER, R.L.	1220
HEYES, M.P.	774	HURD, M.L.	1455
HIEFTJE, G.M.	54	HYKAWY, J.G.	1143
HIEFTJE, G.M.	56	IJAMES, C.F.	1248
HIERL, P.M.	836	ILGNER, R.H.	1435
HJETTER, H.	925	INFANTE, R.	1467
HIGUCHI, T.	1087	INOUE, Y.	1330
HIGUCHI, T.	766	IPPOLITO, M.L.	1057
HILKER, D.R.	1356	ISAACSON, P.	1459
HILL, J.A.	230	ISAACSON, P.	356
HILL, J.A.	1077	ISHIHARA, M.	1087
HILLENKAMP, F.	1168	ISHIHARA, M.	1083
HITES, R.A.	809	ISHIHARA, M.	1077
HITES, R.A.	1493	ISONO, K.	724
HO, J.S.	1495	ISONO, K.	726
HO, T.	367	ITAGAKI, Y.	1087
HOGAN, J.D.	453	IYENGAR, B.S.	690
HOGAN, J.D.	1276	JACKSON, C.J.	1300
HOGG, A.	1106	JACOBS, D.B.	1427
HOGG, A.	506	JAKUBOWSKI, E.M.	1316
HOJRUP, P.	909	JAMESON, C.	969
HOLDER, C.L.	1302	JAOUEN, D.	466
HOLLAND, J.F.	1065	JAOUEN, D.	1149
HOLLAND, J.F.	1179	JARVIS, S.	1017
HOLLAND, J.F.	1182	JAYASIMHULU, K.	616
HOLLAND, J.F.	1063	JAYASIMHULU, K.	921
HOLLAND, J.F.	32	JENKINS, A.	88
HOLLAND, L.G.	1420	JENNINGS, K.R.	1145
HOLLANDER, D.	873	JENNINGS, K.R.	1381
HOLLE, A.	564	JENNINGS, K.R.	644
HONOVICH, J.P.	1256	JENNINGS, K.R.	165
HOOPER, J.B.	1085	JENSEN, N.J.	654
HORLICK, G.	58	JERTZ, R.	1240

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

JHO, C.	212	KENION, G.	1400
JIANG, L.F.	1151	KENNY, D.V.	147
JIANG, L.F.	1071	KENTTAMAA, H.I.	828
JIN, Q.	54	KENTTAMAA, H.I.	403
JOHN, C.M.	1188	KEOUGH, T.	658
JOHNSON, J.A.	1298	KERELY, E.L.	317
JOHNSON, J.A.	959	KERLEY, E.L.	458
JOHNSON, J.H.	852	KERLEY, E.L.	1272
JOHNSON, J.V.	1449	KERLEY, E.L.	1270
JOHNSON, J.V.	1294	KERN, F.	1240
JOHNSON, R.S.	1476	KETKAR, S.N.	1441
JOHNSON, R.S.	971	KETKAR, S.N.	580
JOHNSON, R.S.	1192	KEUHL, D.W.	1463
JOHNSON, W.	895	KHACHIK, F.	772
JOHNSTON, M.V.	1069	KHEMANI, L.	1047
JONES, A.D.	244	KILLINGER, W.E.	929
JONES, P.R.	451	KIM, H-Y.	762
JONES, P.R.	1212	KING, D.B.	1057
JONES, P.R.	1210	KING, F.L.	1512
JONES, T.L.	963	KING, F.L.	70
JONES, T.L.	965	KINGSTON, E.E.	88
JORGENSEN, J.W.	1006	KINOSHITA, T.	780
JORGENSEN, J.W.	114	KINSEL, G.R.	1069
JORGENSEN, J.W.	385	KINTER, M.	597
JOSEPH, R.S.I.	124	KIRK, J.D.	130
JUNG, G.	1079	KIRK, J.D.	131
JUNK, G.	1359	KISER, R.W.	512
JURGENS, U.	1153	KISER, R.W.	656
KAISER, R.E.	60	KITAGAWA, R.	1290
KAISER, R.E.	369	KITTELL, C.	230
KAISER, R.E.	1429	KLEIN, D.H.	556
KALINOSKI, H.T.	982	KLEMCHUK, P.	608
KAMBARA, H.	149	KLINGLER, J.A.	52
KAMINSKI, H.	564	KLINGLER, J.A.	196
KAMINSKY, L.	1356	KLUGE, H-J.	1240
KAMMEI, Y.	1087	KLYM, D.	1478
KAMONO, Y.	102	KNAPP, D.R.	696
KANAI, M.	953	KNOX, P.C.	135
KARANIAN, J.W.	762	KNOX, P.C.	379
KARAS, M.	1168	KO, S.S.	1336
KARI, F.W.	961	KOCH, D.	564
KARLINER, J.	212	KOCH, H.	599
KARLINER, J.	986	KOCH, W.	1367
KASPRZYK, P.	889	KODO, N.	1328
KASS, S.	834	KODO, N.	96
KASS, S.	1363	KOKKONEN, P.	998
KASTL, P.E.	1100	KONDRAT, R.W.	648
KATRITZKY, A.R.	248	KOPPE, M.	414
KATTA, V.	864	KORFMACHER, W.A.	1306
KAUR, S.	873	KORFMACHER, W.A.	1410
KAYGANICH, K.A.	706	KORFMACHER, W.A.	1318
KEBARLE, P.	832	KORFMACHER, W.A.	967
KEDDERIS, G.L.	1361	KORFMACHER, W.A.	551
KEEFE, C.D.	455	KORFMACHER, W.A.	1302
KEINONEN, M.	1470	KOSLIN, M.E.	80
KELLERHALS, H.P.	1254	KOSLIN, M.E.	1431
KELLERHALS, H.P.	1252	KOSTER, C.	973
KELLY, D.W.	1318	KOSTER, C.	568
KELLY, W.R.	417	KOTIAHO, T.	151
KEMP, T.J.	165	KOWALAK, J.A.	728

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

KRAUSE, R.T.	1445	LEE, T.D.	1075
KRIEGER, M.S.	1049	LEE, Y.C.	375
KRIER, G.	1506	LEEDS, J.	903
KUBOTA, E.	1087	LEGESSE, K.	582
KUEHL, D.W.	1420	LEGESSE, K.	1075
KUEHL, D.W.	1449	LEGESSE, K.	549
KUHARA, T.	1330	LEHMAN, T.H.	218
KUNIHIRO, F.	1087	LEIBMAN, C.P.	1429
KUNZ, K.	1240	LEIKER, T.J.	1433
KUO, W.S.	967	LEIKER, T.J.	1421
KURZ, E.A.	1073	LENAERS, G.	422
KUSMIERZ, J.	883	LEORI, G.E.	666
KUSMIERZ, J.	885	LEPAGE, P.	919
KYRANOS, J.N.	153	LEQUESNE, P.W.	614
KYRANOS, J.N.	270	LEV-ON, M.	1427
LAALI, K.	650	LEVERONE, L.A.	921
LABELLA, F.S.	686	LEWIS, S.	340
LACEY, M.P.	1266	LEYH, B.	612
LACEY, M.P.	658	LI, C.	329
LADISCH, S.	750	LI, L.	572
LAFORTUNE, F.	730	LI, L.	1041
LAI, K.	1037	LI, X.	342
LAING, G.A.	1455	LIANG, X.	660
LAKRITZ, L.	1338	LIANG, X.	173
LAM, Z.	1194	LIANG, Z.	1234
LAMMERT, S.A.	369	LIEBMAN, J.F.	1367
LAMPE, F.W.	931	LIECHTY, E.A.	1310
LAND, D.P.	200	LIFSHITZ, C.	1508
LAND, D.P.	1514	LIGHT, K.	256
LANDER, C.A.	1143	LIM, H.K.	1352
LANGVARDT, P.W.	1100	LIN, C.C.	1427
LANGVARDT, P.W.	1334	LIN, H.Y.	1377
LANT, M.S.	124	LIN, S-N.	1002
LANTING, A.	984	LIN, S-R.	911
LAPACK, M.A.	1465	LIND, A.C.	282
LAPPIN, G.	716	LINDNER, J.	568
LARAMEE, J.A.	90	LINDNER, J.	570
LARKINS, W.	1091	LINEMAN, D.N.	670
LARSEN, B.S.	915	LING, Y.-C.	1055
LARSEN, B.S.	1336	LINHARDT, R.J.	794
LARSEN, M.L.	1420	LINSCHIED, M.	947
LATTIMER, R.P.	650	LINTON, R.W.	868
LAUDE, D.A.	1276	LISCHWE, M.	915
LAUDE, D.A.	155	LO, H.H.	133
LAUDE, D.A.	453	LOEPFE, M.	1254
LAUE, H.J.	1033	LOGANATHAN, D.	794
LAWSON, A.M.	788	LOGUSCH, E.W.	1348
LAWSON, A.M.	790	LOH, S.	354
LAY, J.O.	1318	LONDROY, F.A.	460
LEARY, J.A.	913	LONG, E.J.	1043
LEBLANC, Y.	122	LOO, J.A.	120
LEBRILLA, C.B.	407	LOO, J.A.	586
LECH, J.	1341	LOO, J.A.	116
LECH, J.	1486	LOO, J.A.	844
LECH, J.	1469	LOO, J.A.	391
LEE, E.D.	365	LOPEZ, H.	718
LEE, J.J.	868	LOPEZ-MIRANDA, A.T.	1410
LEE, T.D.	582	LOPSHIRE, R.F.	1065
LEE, T.D.	549	LORETZ, T.J.	1073
LEE, T.D.	352	LOW, N.	358

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

LUBMAN, D.M.	1041	MARSHALL, A.G.	1397
LUBMAN, D.M.	572	MARSHALL, A.G.	1228
LUBMAN, D.M.	978	MARSHALL, A.G.	1226
LUM, R.C.-J.	838	MARSHALL, A.G.	1395
LUNDMARK, E.	1463	MARSHALL, A.G.	1236
LUSBY, W.R.	772	MARTIN, L.B.	702
MA, Y.C.	620	MARTIN, M.	106
MABUD, Md.A.	929	MARTIN, S.A.	1077
MACFARLANE, R.D.	210	MARTIN, S.A.	230
MACFARLANE, R.D.	432	MARTIN, S.A.	858
MACFARLANE, R.D.	1133	MARTIN, W.B.	1098
MACFARLANE, R.D.	289	MARTIN, W.B.	576
MACHIN, J.	1478	MARTINEZ, C.	180
MACLEAN, D.B.	606	MARTINEZ, R.I.	1120
MACMILLAN, D.K.	315	MARTINO, P.	1125
MAERKER, G.	1338	MASON, R.T.	674
MAGOLDA, R.L.	1336	MASON, R.T.	632
MAHAFFY, P.	1037	MASUCCI, J.A.	624
MAHONEY, J.F.	1075	MASUDA, K.	1346
MAHONEY, J.F.	549	MASWADEH, W.	304
MAHONEY, J.F.	582	MASWADEH, W.	444
MAJOR, H.J.	272	MATHEWS, W.R.	891
MAJOR, H.J.	790	MATHEWS, W.R.	764
MAJUMDAR, T.K.	206	MATSUMOTO, I.	1330
MALEKNIA, S.	453	MATSUMOTO, M.	1330
MALEKNIA, S.D.	155	MATTINA, M.J.I.	1443
MALLIS, L.M.	184	MATTINA, M.J.I.	1447
MALLIS, L.M.	186	MATTSOON, W.A.	1399
MALLIS, L.M.	794	MAXWELL, D.	1478
MALTBY, D.A.	872	MAYER, F.	566
MAMER, O.A.	1312	MAYER, F.	232
MAMER, O.A.	599	MAYNARD, S.J.	280
MAMER, O.A.	1196	MAYO, S.	417
MANDEL, L.	746	MCADOO, D.J.	338
MANN, M.	428	MCADOO, D.J.	610
MANN, M.	389	MCBAY, E.H.	1029
MANTUS, D.S.	1055	MCCARTY, H.	356
MAO, Y.	566	MCCARTY, H.	1459
MAO, Y.	232	MCCLENNEN, W.H.	1425
MARBURY, D.	626	MCCLENNEN, W.H.	444
MARBURY, G.D.	682	MCCLENNEN, W.H.	304
MARCH, R.E.	220	MCCLOSKEY, J.A.	726
MARCH, R.E.	460	MCCLOSKEY, J.A.	728
MARCUS, R.K.	1141	MCCLOSKEY, J.A.	724
MARCUS, R.K.	188	MCCORMACK, A.L.	899
MARECIC, T.	1045	MCCURVIN, D.M.	438
MARECIC, T.	139	MCDOWALL, M.A.	1025
MARINI, A.M.	1339	MCDOWALL, M.A.	1023
MARKEY, S.P.	1339	MCELVANY, S.W.	161
MARKEY, S.P.	994	MCELVANY, S.W.	169
MARKEY, S.P.	1256	MCELVANY, S.W.	163
MARKEY, S.P.	1039	MCELVANY, S.W.	159
MARQUIS, P.	1463	MCFADDEN, W.H.	470
MARQUIS, P.J.	1420	MCINTOSH, S.A.	1453
MARSH, K.N.	742	MCIVER, R.T.	407
MARSHALL, A.G.	1232	MCIVER, R.T.	202
MARSHALL, A.G.	1	MCIVER, R.T.	200
MARSHALL, A.G.	344	MCIVER, R.T.	1514
MARSHALL, A.G.	1230	MCIVER, R.T.	1220
MARSHALL, A.G.	1234	MCKAY, G.	1300

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

MCKAY, G.	684	MILBERG, R.M.	1135
MCKAY, R.G.	1336	MILBERG, R.M.	1128
MCKELLOP, K.	1035	MILLEN, W.G.	1214
MCKENZIE, D.E.	1012	MILLER, D.J.	1049
MCKOWN, H.S.	22	MILLER, L.V.	411
MCKOWN, H.S.	584	MILLER, M.L.	284
MCLAFFERTY, F.W.	449	MILLINGTON, D.S.	988
MCLAFFERTY, F.W.	1502	MILLINGTON, D.S.	746
MCLAFFERTY, F.W.	8	MILLINGTON, D.S.	1328
MCLAFFERTY, F.W.	612	MILLINGTON, D.S.	96
MCLAFFERTY, F.W.	354	MILLS, G.	1004
MCLAFFERTY, F.W.	1387	MILLS, T.M.	112
MCLAFFERTY, F.W.	236	MINARD, R.D.	30
MCLEAN, M.A.	937	MINARD, R.D.	1043
MCLOUGHLIN, P.W.	1504	MISSLER, S.R.	1383
MCLUCKEY, S.A.	796	MISSLER, S.R.	112
MCLUCKEY, S.A.	462	MISTRY, H.	1110
MCLUCKEY, S.A.	77	MITCHELL, D.W.	1238
MCLUCKEY, S.A.	584	MITROVICH, S.	110
MCLUCKEY, S.A.	1094	MOCK, K.	1000
MCLUCKEY, S.A.	22	MOINI, M.	712
MCLUCKEY, S.A.	464	MOINI, M.	666
MCLUCKEY, S.A.	335	MOLLEKEN, M.E.	553
MCLUCKEY, S.A.	234	MONSON, S.J.	1416
MCLUCKEY, S.A.	1260	MONTES, J.G.	592
MCLUCKEY, S.A.	1029	MOORE D.S.	286
MCLUCKEY, S.A.	806	MOORE, D.S.	977
MCMAHON, A.W.	460	MOORE, J.A.	1451
MCMAHON, T.B.	333	MOORE, L.J.	38
MCMAHON, T.B.	331	MOORE, M.	895
MCMAHON, T.B.	1160	MOORE, W.T.	106
MCMAHON, T.B.	329	MORAND, K.L.	25
MCMAHON, T.B.	1391	MORELLI, J.J.	282
MCMAHON, T.B.	321	MORIN, N.	1149
MCNEAL, C.J.	1133	MORING, S.E.	106
MCQUADE, P.S.	686	MORRISON, G.H.	1055
MEEK, J.T.	1214	MORTON, T.H.	1159
MEI, X.	122	MORTON, T.H.	618
MEI, Y.	198	MORTON, T.H.	648
MEIER, J.E.	344	MOSELEY, M.A.	1006
MEISELS, G.C.	668	MOSELEY, M.A.	114
MEL, S.F.	870	MOSELEY, M.A.	385
MENG, C.K.	428	MOSEMAN, R.	969
MENG, C.K.	389	MOZOLL, A.E.	1420
MERREN, T.O.	1023	MUCK, W.	365
MERREN, T.O.	1025	MUCK, W.	1014
MERRITT, D.A.	33	MULLER, J.F.	1506
MESTDAGH, H.	313	MULSHINE, J.	889
MESTDAGH, H.	1149	MUNSON, B.	493
MESTDAGH, H.	466	MUNSON, B.	620
MESTER, M.	692	MUNSON, B.	1112
MEUZELAAR, H.L.C.	302	MUNSON, B.	474
MEUZELAAR, H.L.C.	304	MUNSON, B.	491
MEUZELAAR, H.L.C.	1425	MURPHY, C.	786
MEUZELAAR, H.L.C.	444	MURPHY, C.M.	877
MEYER, S.	836	MURPHY, R.C.	754
MEYERHOFFER, W.J.	1399	MURPHY, T.J.	422
MIDHA, K.K.	684	MUSCHE, H.	901
MIDHA, K.K.	1300	MUSSELMAN, B.C.	1087
MILBERG, R.M.	1175	MUSSELMAN, B.D.	1083

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

MUSSER, S.M.	975	ORLANDO, R.	493
MYKYTYN, V.V.	734	ORLANDO, R.	786
NAGAKI, H.	780	ORLANDO, R.C.	877
NAKAMURA, T.	780	ORLEY, C.O.	692
NAKANO, K.	732	OSEI-TWUM, E.Y.	1312
NEAL, D.M.	698	OSEI-TWUM, E.Y.	1196
NEEDHAM, L.L.	1489	OSTERMAN, T.T.	1461
NELSON, C.C.	724	OTA, K.	1290
NELSON, P.R.	1114	OTSUKA, K.	1087
NELSON, R.J.	996	OYOFO, B.A.	744
NELSON, S.D.	1326	PALACZ, Z.A.	594
NELSON, S.D.	1322	PALMA, P.	990
NELSON, S.D.	1320	PAN, C.	46
NESTOR, J.	531	PAN, C.	171
NEVILLE, G.A.	1344	PAN, Y.H.	1198
NEWTON, R.P.	88	PANG, H.M.	978
NG, K.	212	PANNELL, L.K.	674
NG, K.	608	PANNELL, L.K.	632
NG, K.	986	PAPAYANNOPOULOS, I.A.	537
NIBBERING, N.M.M.	1163	PAPAYANNOPOULOS, I.A.	990
NIEHUIS, E.	204	PARKER, C.	969
NIEHUIS, E.	1081	PARKER, C.E.	961
NIEHUIS, E.	901	PATRIANAKOS, C.	986
NIEHUIS, E.	1153	PATRZYC, H.B.	84
NIER, A.D.C.	523	PATTERSON, D.G.	1489
NIESSEN, W.M.A.	377	PAUL, G.J.C.	832
NIESSEN, W.M.A.	373	PAULINO, J.A.	327
NIESSEN, W.M.A.	935	PEARSON, P.G.	850
NIESSEN, W.M.A.	998	PEARSON, P.G.	1322
NIEVES, E.	716	PEARSON, P.G.	1326
NIEVES, E.	1179	PEARSON, P.G.	1320
NISHIMURA, T.	668	PEDDER, R.E.	468
NISHIMURA, T.	640	PEDDER, R.E.	62
NOLAN, R.J.	1334	PEKAY, L.	312
NORD, R.	432	PELLETIER, M.	1506
NORD, R.F.	210	PELLIZZARI, E.D.	74
NORWOOD, D.L.	988	PENG, G.W.	678
NORWOOD, D.L.	96	PENTECOST, T.C.	1385
NORWOOD, D.L.	1328	PEREIRA, D.	688
NOURSE, B.D.	828	PEREIRA, W.E.	1433
NUNN, N.	1418	PEREIRA, W.E.	1421
NUNN, N.J.	1412	PEREL, J.	1075
NUWAYSIR, L.M.	1278	PEREL, J.	582
NUWAYSIR, L.M.	816	PEREL, J.	549
O'CONNOR, P.	1089	PERES, T.	1508
O'LEARY, I.A.	678	PERREAULT, H.	395
O'MALLEY, R.M.	672	PERREAULT, H.	1406
O'NEAL, J.M.	1461	PESCH, R.	1079
OCOLOWITZ, J.L.	875	PETTIETTE-HALL, C.L.	1514
ODOM, R.W.	287	PETTIETTE-HALL, C.L.	200
ODOM, R.W.	208	PETTIT, G.R.	102
ODOM, R.W.	812	PETZINGER, G.	1423
OHKURA, T.	1330	PEYTON, A.L.	1304
OKA, H.	1346	PHILLIPS, D.R.	726
OLESIK, S.V.	1091	PHILLIPS, W.H.	1290
OLESIK, S.V.	312	PIATEK, B.	608
ONISKO, B.	340	PIATEK, B.	986
OPPENHEIMER, S.	539	PIRKLE, J.L.	1489
ORKISZEWSKI, R.S.	897	PLAT, M.	264
ORKISZEWSKI, R.S.	760	PLEASANCE, S.	1006

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

PLEASANCE, S.	122	REYNOLDS, J.D.	533
POPPE, N.	232	RICCI, M.	1155
PORTER, C.J.	1019	RICCI, M.P.	33
PORTILLA, D.	746	RICHARDSON, D.E.	1137
POTTER, B.B.	1408	RIDGE, D.P.	1377
PRAKASH, C.	700	RIDGE, D.P.	1198
PRAMANIK, B.C.	992	RILEY, J.	626
PRAMANIK, B.N.	893	RINEHART, K.L.	1135
PREVOST, C.	246	RINEHART, K.L.	104
PREVOST, V.	82	RINEHART, K.L.	1175
PROKAI, L.	1354	RINEHART, K.L.	1128
PROVOST, L.R.	1439	ROACH, J.A.G.	1445
PU, Q-L.	674	ROBERTS, G.D.	782
PU, Q-L.	632	ROBERTS, G.D.	1131
PYLE, S.	1408	ROBERTS, J.	1478
QIAN, K.	216	ROBERTS, K.A.	304
QIAN, K.	214	ROBERTS, K.A.	1425
QIN, W.	903	ROBERTSON, A.	274
QUA, A.	622	ROBINS, R.H.	866
QUARLES, J.M.	718	ROBINS, R.H.	640
QUICK A.N.	359	ROBOZ, J.	716
QUILLIAM, M.A.	122	ROBOZ, J.	1179
QUILLIAM, M.A.	145	ROEPSTORFF, P.	909
QUILLIAM, M.A.	1404	ROHRBAUGH, D.K.	1437
QUILLIAM, M.A.	1406	ROHWER, E.R.	568
RAFTERY, M.J.	760	ROHWER, E.R.	973
RAJU, C.N.	600	ROITSCH, C.	919
RANDENIYA, L.	1498	ROLANDO, C.	313
RAPOPORT, S.E.	708	ROLANDO, C.	466
RAPOPORT, S.I.	704	ROLANDO, C.	1149
RASHED, M.S.	955	ROLLINS, K.	272
RATLIFF, P.H.	194	ROLLINS, K.	274
RAUTENSCHLEIN, M.	1155	RONG, L-S.	1175
RAUWALD, U.	710	RONG, L-S.	1135
RAYMER, J.	945	RONG, L.	1128
READ, P.A.	165	ROSARIO, O.	1410
REDDY, C.D.	602	ROSEN, J.D.	1469
REDDY, D.B.	604	ROSEN, J.D.	1486
REDDY, G.S.	600	ROSEN, R.T.	1469
REDDY, G.S.	602	ROSEN, R.T.	1486
REDDY, G.S.	604	ROSEN, R.T.	1338
REDDY, M.V.R.	604	ROSEN, R.T.	1341
REED, D.A.	1061	ROSNACK, K.J.	957
REENTS, W.D.	175	ROSS, B.	54
REICHERT, P.	893	ROSS, M.M.	157
REID, R.	361	ROSS, M.M.	1512
REILLY, J.P.	819	ROSS, M.M.	163
REIMER, M.L.J.	732	ROSS, R.	927
REINER, E.J.	438	ROST, K.	1079
REINHARD, J.	1350	ROSTAD, C.E.	1421
REINHOLD, V.N.	1476	ROSTAD, C.E.	1433
REINHOLD, V.N.	971	ROTHERAM, M.	1431
REINHOLD, V.N.	980	ROY, R.L.	1073
REINHOLD, V.N.	1192	RUDEWICZ, P.	953
REISER, R.W.	1010	RUDO, K.M.	961
REMER, W.A.	690	RUSHNECK, D.A.	523
REMPEL, D.L.	1222	RUSSELL, D.H.	1270
REMPEL, D.L.	1224	RUSSELL, D.H.	317
REYNOLDS, J.D.	1201	RUSSELL, D.H.	242
REYNOLDS, J.D.	425	RUSSELL, D.H.	1268

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

RUSSELL, D.H.	1272	SELLERS-HANN, L.	242
RUSSELL, D.H.	458	SELLERS-HANN, L.	319
RUSSELL, D.H.	1274	SERXNER, D.	190
RUSSELL, D.H.	319	SETCHELL, K.	927
RUSSELL, D.H.	1021	SEVERANCE, R.A.	147
RUSSELL, D.H.	222	SHABANOWITZ, J.	821
RUSSELL, D.H.	405	SHABANOWITZ, J.	899
RYAN, M.F.	1137	SHABANOWITZ, J.	1125
SADANA, K.L.	730	SHABANOWITZ, J.	1244
SAKAIRI, M.	149	SHALER, T.A.	618
SALEM, N.	762	SHAPLEY, J.R.	1135
SALVAGE, B.J.	88	SHARKEY, A.G.	409
SAMARA, E.	1288	SHARKEY, A.G.	670
SAMPSON, E.J.	1489	SHARKEY, A.G.	578
SANDER, P.	208	SHARMA, K.S.	1143
SANDER, P.	1061	SHARP, T.R.	740
SANDERS, P.R.	110	SHARP, T.R.	280
SANDERSON, N.E.	1102	SHARP, T.R.	742
SATO, K.	1083	SHARPE, P.	1137
SAUTER, A.D.	356	SHAW, R.W.	590
SAUTER, A.D.	1459	SHEEHAN, E.W.	139
SAVORY, J.	597	SHEEHAN, E.W.	108
SAYKALLY, R.J.	6	SHEEHAN, E.W.	133
SCHAEFER, M.S.	1310	SHEELEY, D.M.	971
SCHAUER, S.J.	186	SHEELEY, D.M.	980
SCELLENBERG, D.H.	438	SHEEN, J.T.	1457
SCHEY, K.L.	1027	SHEIL, M.M.	728
SCHEY, K.L.	98	SHEPPARD, B.	48
SCHEY, K.L.	25	SHERMAN, W.R.	517
SCHILLING, A.B.	137	SHERMAN, W.R.	1115
SCHLAG, E.W.	568	SHETTY, H.U.	704
SCHLAG, E.W.	570	SHIEA, J-T.	393
SCHLAG, E.W.	973	SHIEA, J.	531
SCHMIT, J-P.	180	SHIELDS, P.	1232
SCHMIT, J-P.	178	SHIH, M-C.	1339
SCHRAM, K.H.	732	SHIH, M.	994
SCHRAM, K.H.	690	SHIMANSKAS, C.	212
SCHRAM, K.H.	734	SHINKA, T.	1330
SCHREINER, A.F.	702	SHIRAISHI, H.	809
SCHRODER, E.	710	SHOEMAKER, J.D.	1358
SCHRYNEMEECKERS, P.J.	1497	SHOHET, J.L.	1244
SCHUELER, B.	208	SHU, Y-Z.	1300
SCHUELER, B.W.	1061	SHUKER, D.E.G.	82
SCHULTZ, G.	256	SHUKLA, A.	216
SCHULTZ, G.	527	SHUKLA, A.	1369
SCHULTZ, G.A.	1063	SHUKLA, A.K.	218
SCHURZ, H.H.	268	SHUKLA, A.K.	214
SCHWARTZ, J.C.	1027	SICHTERMANN, W.	1051
SCHWARTZ, J.C.	98	SIDKY, M.S.	1143
SCHWARTZ, J.C.	25	SIEGEL, M.	893
SCHWEIKHARD, L.	1240	SIEGEL, M.M.	1400
SCHWEIKHARD, L.	419	SIEGEL, M.M.	539
SCOTT, W.J.	184	SIEGEL, M.M.	350
SCRIVENS, J.H.	274	SIEGMUND, E.	1423
SCRIVENS, J.H.	272	SIGLER, J.	836
SEARS, L.J.	554	SIM, P.G.	1406
SEDGWICK, R.D.	1096	SIM, P.G.	122
SEENATAH, B.	604	SIMMS, J.R.	1266
SEGER, N.A.	1298	SIMMS, J.R.	1264
SELGREN, S.F.	1504	SIMONS, D.S.	417

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

SIMONSICK, W.J.	291	STERN, G.A.	664
SIMPSON, J.T.	740	STERN, G.A.	662
SIMPSON, R.C.	375	STOCKTON, G.W.	1214
SIU, K.W.M.	143	STOLL, M.	788
SIZEMORE, E.	1057	STOLL, M.	790
SJODIN, R.A.	592	STONE, J.A.	342
SLAGLE, R.E.	1457	STRAUB, K.	1165
SLATTER, J.G.	850	STRAUB, K.	367
SLAUGHTER, C.A.	992	STRIPE, R.J.	1266
SLIKKER, W.	1318	STRIPE, R.J.	1264
SLIVON, L.E.	1495	STROBEL, F.	222
SLIVON, L.E.	147	STROH, J.G.	957
SMITH, C.S.	135	STROUD, R.M.	870
SMITH, C.S.	379	STUDLEY, S.A.	33
SMITH, D.	1057	STULTS, J.T.	856
SMITH, D.C.	1110	STULTS, J.T.	1015
SMITH, D.H.	590	STULTS, J.T.	1065
SMITH, D.L.	903	SUARD, M.	313
SMITH, D.L.	1324	SUMPTER, T.	337
SMITH, D.L.	854	SUN, F.	1135
SMITH, G.G.	1184	SUN, F.	1175
SMITH, M.A.	1498	SUN, F.F.	764
SMITH, M.W.	929	SUN, Y.	854
SMITH, R.D.	430	SUNDQVIST, B.U.R.	399
SMITH, R.D.	100	SUNNER, J.	393
SMITH, R.D.	120	SUNNER, J.	531
SMITH, R.D.	116	SUNNER, J.	1118
SMITH, R.D.	391	SUSHAN, B.I.	558
SMITH, R.D.	844	SUSHAN, B.I.	1181
SMITH, R.D.	586	SUSLICK, K.S.	1135
SMITH, R.L.	190	SUTTON, C.	276
SMITH, R.W.	606	SUTTON, C.	308
SMITH, S.J.	616	SUTTON, M.	742
SMITH, W.J.	688	SUWANRUMPHA, S.	1280
SMYTH, M.H.	588	SUYANI, H.	48
SNYDER, A.P.	1365	SUZUKI, M.	1346
SOLSTEN, R.T.	1348	SWAISGOOD, H.E.	943
SONCRANT, T.T.	704	SWANSON, R.	913
SONCRANT, T.T.	708	SWEELEY, C.C.	750
SONODA, A.	640	SWEETMAN, B.J.	1332
SOPCHYSHYN, F.C.	588	SWEETMAN, B.J.	700
SORENSEN, D.M.	325	SZULEJKO, J.E.	333
SOVOCOL, G.W.	1412	TABET, J.C.	826
SOVOCOL, G.W.	800	TABET, J.C.	246
SOVOCOL, W.	1418	TACKETT, J.R.	595
SPATES, G.E.	744	TAGHIZADEH, K.	302
SPINDT, C.A.	560	TAGUCHI, V.Y.	438
SPINK, D.	1356	TAKITA, H.	84
SQUIRES, R.R.	327	TALAAT, R.E.	955
STACK, R.F.	1298	TANG, X.	232
STAMS, D.A.	618	TANG, X.	566
STAMS, D.A.	648	TANNER, M.J.	740
STANDING, K.G.	1059	TAYLOR, A.A.	758
STANDING, K.G.	566	TAYLOR, B.M.	764
STANDING, K.G.	232	TAYLOR, L.C.E.	1096
STANDING, K.G.	730	TAYLOR, L.C.E.	1350
STANDING, K.G.	399	TAYLOR, M.	274
STANDING, K.G.	686	TAYLOR, M.	272
STAROS, J.V.	1332	TAYLOR, W.S.	580
STEMMLER, E.A.	387	TAYLOR, W.S.	646

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

TECKLEBURG, R.	1063	TUINMAN, A.A.	796
TEESCH, L.M.	907	TURECEK, F.	1502
TEFFERA, Y.	1324	TURECEK, F.	1387
TETTA, D.	1408	TURNER, L.K.	1055
TEWS, E.C.	1451	TURNER, M.J.	1361
THANAWALA, P.	1478	TURNER, W.E.	1489
THIBAUT, P.	887	TWOREK, H.A.	84
THIBAUT, P.	224	TYCZKOWSKA, K.	945
THIBAUT, P.	226	TYLER, A.N.	1115
THIBAUT, P.	104	TYMIK, A.A.	852
THIBAUT, P.	1006	UBUKATA, M.	724
THIBAUT, P.	122	UDSETH, H.R.	586
THOELMANN, D.	1157	UDSETH, H.R.	100
THOMAS, D.	326	UDSETH, H.R.	116
THOMASSEN, D.	1326	UDSETH, H.R.	430
THOMPSON, A.G.	1135	UDSETH, H.R.	844
THOMPSON, A.G.	1128	UDSETH, H.R.	391
THOMPSON, A.G.	1175	UDSETH, H.R.	120
THOMPSON, T.	541	UECHI, G.T.	252
THOMSON, B.	145	UNGER, S.E.	852
THOMSON, B.A.	558	URAMOTO, M.	726
THOMSON, J.S.	310	UY, O.M.	1258
THORNE, G.C.	897	VALCARCE, R.V.	1184
THORNE, G.C.	848	VALKERS, S.	422
TIDWELL, R.	682	VAN BERKEL, G.J.	234
TIETJE, K-H.	1079	VAN BERKEL, G.J.	796
TINGLE, T.N.	294	VAN BREEMEN, R.B.	702
TJADEN, U.R.	377	VAN BREEMEN, R.B.	94
TJADEN, U.R.	935	VAN BREEMEN, R.B.	879
TJADEN, U.R.	998	VAN DEN EECKHOUT, E.G.	923
TJADEN, U.R.	373	VAN DER GREEF, J.	998
TODD, D.E.	1110	VAN DER GREEF, J.	935
TODD, J.F.J.	460	VAN DER GREEF, J.	377
TODD, P.J.	397	VAN DER GREEF, J.	373
TOLLENS, F.R.	1395	VAN DER HOEVEN, R.A.M.	377
TOLLENS, F.R.	1397	VAN DER HOEVEN, R.A.M.	935
TOMER, K.	969	VAN DORSSELAER, A.	925
TOMER, K.B.	114	VAN DORSSELAER, A.	919
TOMER, K.B.	226	VAN KOPPEN, P.A.M.	830
TOMER, K.B.	961	VANLEYEN, D.	204
TOMER, K.B.	446	VASSILIADOU, M.	112
TOMER, K.B.	385	VASSILIADOU, M.	1383
TOMER, K.B.	1006	VATH, J.E.	770
TONDEUR, Y.	1414	VEDEL, F.	460
TONDEUR, Y.	441	VELEZ, G.	945
TONDEUR, Y.	1491	VERMURI, S.	352
TONG, H.Y.	1416	VERHEIJ, E.R.	377
TORRELL, J.M.	1184	VESTAL, M.L.	41
TORRES, A.	1467	VESTAL, M.L.	937
TOU, J.C.	1465	VESTAL, M.L.	939
TOWNSEND, R.R.	375	VESTAL, M.L.	951
TREAGER, J.C.	1389	VESTLING, M.M.	846
TRESTON, A.	889	VIEIRA, N.E.	592
TROTTA, P.P.	893	VISENTINI, J.	860
TRZASKOS, J.M.	1336	VISENTINI, J.	887
TSAO, R.	539	VISWANADHAM, S.K.	670
TSAO, R.	893	VOGH, J.W.	310
TSARBOPoulos, A.	893	VOLK, K.J.	371
TUINMAN, A.	630	VOUROS, P.	270
TUINMAN, A.	1373	VOUROS, P.	736

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

VOUROS, P.	766	WELCH, M.J.	1308
VOUROS, P.	614	WELDON, P.J.	740
VOUROS, P.	153	WELLER, R.R.	562
VOUROS, P.	996	WENCLAWIAK, B.	1051
VOYKSNER, R.D.	868	WENCLAWIAK, B.	634
VOYKSNER, R.D.	1453	WESDEMIOTIS, C.	1387
VOYKSNER, R.D.	379	WESDEMIOTIS, C.	612
VOYKSNER, R.D.	945	WESOLOWSKI, J.	738
VOYKSNER, R.D.	135	WESTMORE, J.B.	662
VOYKSNER, R.D.	943	WESTMORE, J.B.	730
VRBANAC, J.J.	678	WESTMORE, J.B.	664
VRBANAC, J.J.	696	WETZEL, R.	856
WACHSMANN, M.	414	WHALEY, B.S.	722
WADE, N.	1290	WHEELER, J.J.	94
WAIDYANATHA, S.	881	WIEDENHEFT, C.J.	595
WALKER, R.J.	417	WILBANKS, S.	913
WALKER, V.	1004	WILKERSON, C.W.	819
WALTON, T.J.	88	WILKES, J.G.	939
WANCZEK, K.P.	456	WILKINS, C.L.	816
WANG, B.H.	236	WILKINS, C.L.	260
WANG, B.H.	449	WILKINS, C.L.	1278
WANG, D.T-S.	202	WILKINS, C.L.	1173
WANG, D.T-S.	407	WILKINS, C.L.	1248
WANG, H.M.	794	WILKINSON, F.E.	331
WANG, J-S.	622	WILKINSON, F.E.	333
WANG, J.	1369	WILLIAMS, E.R.	449
WANG, J.	606	WILLIAMS, E.R.	236
WANG, M.	1228	WILLIAMS, J.	369
WANG, M.	1226	WILLIAMS, T.	913
WANG, R.	545	WILLOUGHBY, R.	110
WANG, R.	547	WILLOUGHBY, R.C.	108
WANG, R.	778	WILLOUGHBY, R.C.	133
WANG, T-C.L.	1039	WILLS, M.R.	597
WANG, T-C.L.	994	WILSON, D.C.	868
WANG, T-C.L.	1339	WILSON, K.J.	106
WANG, X.T.	967	WILSON, S.	1004
WARD, S.R.	698	WINN, D.	939
WARDEN, D.	1290	WINN, D.	951
WARNER, C.D.	628	WINPENNY, R.E.P.	1133
WARRACK, B.M.	852	WINTERGRASS, D.	367
WASILCHUK, B.	614	WISE, M.B.	1484
WATSON, C.H.	250	WISE, M.B.	1435
WATSON, C.H.	248	WISE, M.B.	1402
WATSON, D.	927	WOLEN, R.L.	680
WATSON, J.T.	706	WOLF, C.J.	282
WATSON, J.T.	1063	WOLF, M.A.	1429
WATSON, J.T.	32	WONG, S.F.	428
WATSON, J.T.	1182	WONG, S.F.	389
WAYNE, R.S.	1214	WOOD, D.	1414
WEBER, R.S.	428	WOOD, D.	306
WEBER, W.D.	262	WOOD, G.	72
WEBER-GRABAU, M.	468	WOODARD, C.L.	1316
WEIL, D.	1506	WOODWARD, P.W.	310
WEIL, D.A.	1194	WOOLGAR, P.	124
WEIL, D.A.	562	WORDEN, R.D.	1057
WEININGER, S.	278	WRIGHT, A.D.	644
WEIR, O.	1414	WUNSCHÉ, C.	901
WEIR, O.	306	WYATT, J.R.	80
WEIR, O.	1004	WYSOCKI, V.H.	403
WEISS, G.	564	WYSOCKI, V.H.	163

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

WYSOCKI, V.H.	1512
XIA, S.J.	967
YAMASHITA, K.	92
YANG, L.	941
YANG, L.	1461
YANG, M.	819
YANG, S.S.	1224
YANG, Y.M.	748
YASAKA, T.	732
YATES III, J.R.	1125
YEFCHAK, G.E.	1067
YELLE, L.M.	949
YEN, T.Y.	1071
YEOMANS, W.	278
YERGEY, A.L.	592
YERGEY, A.L.	931
YERGEY, J.A.	774
YINON, J.	965
YINON, J.	963
YOST, R.A.	371
YOST, R.A.	636
YOST, R.A.	1294
YOST, R.A.	1250
YOST, R.A.	1147
YOST, R.A.	638
YOST, R.A.	62
YOST, R.A.	1449
YOST, R.A.	468
YOUNG, D.	766
YOUNG, J.P.	590
YOUNG, S.	1242
YUAN, Z-M.	576
ZAKETT, D.	369
ZAMECNIK, J.	1344
ZEEVAART, J.A.D.	752
ZEHNDR, S.	1290
ZHA, Q.	668
ZHA, Q.	652
ZHA, Q.	395
ZHA, Q.	792
ZHANG, J.Y.	748
ZHANG, J.Y.	1296
ZHANG, M-Y.	1387
ZHAO, S.	578
ZHOU, J.	913
ZHU, G.	46
ZHU, G.	171
ZHU, N.Z.	821
ZHU, N.Z.	899
ZHU, N.Z.	1125
ZIDAROV, D.	346
ZIEGE, E.A.	1304
ZIMMER, H.	616
ZIMMERMAN, J.A.	672
ZINKL, M.A.	1012
ZIRROLI, J.A.	756
ZOOK, D.R.	1379
ZUMBERGE, J.E.	308
ZUMBERGE, J.E.	276

1,1,4,4-TETROXIDE 604
1,2-SHIFTS 610
1,2-DIBROMO-3-CHLOROPROPANE 1320
1,25-DIHYDROXY-VITAMIN D3 927
1,3-BENZOTHAZINES 606
13-C LEUCINE 1310
13C-, 15N-, AND 2H- ENRICHMENT 1314
1,4-DITHIANE-1,1-DIOXIDE 604
14C-LABELED DRUGS 714
2-ALKYL-4,6-DINITROPHENOLS 626
2-ARYLOXY-3-CYCLOHEXYL-6-(1,1-DIMETHYLETHYL)-3, 4-DIHYDRO-2H-1 600
2,10-DICHLORO-6-ARYLOXY-DIBENZO [d,g] [1,3,6,2] DIOXATHIAPHOS 602
2,3:4,5-BIS-O-(1-METHYLETHYLIDENE)-D-FRUCTOPYRANOSE DERIVATI 624
3-METHYLADENINE 82
3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) 1352
5,6-DIHYDROURIDINE 732
7-R-O-METHYLNORGAROL 640

-A-

AB INITIO 313
ABSCISIC ACID 752
ACCELERATION REGION PROCESSES 220
ACES 348
ACRANTOPHIS DUMERILI 740
ACRYLONITRILE 1361
ACYL-CoA COMPOUNDS 988
ACYLCARNITINES 96, 1004, 1330
ADDUCT IONS 1443
ADINAZOLAM 678
AEROSOL TRANSPORT 130
AFFINITY 342
AIR MONITORING 77
ALCOHOL-FLUORIDE ADDUCT IONS 331
ALDOSE REDUCTASE 1175
ALKALI METALS 907
ALKYL PHOSPHATE ESTERS 1365
ALKYL PHOSPHONATE 1365
ALKYL PHENYL ETHERS 648
ALKYLAMINES 342
ALKYLATED NUCLEOSIDES 86
ALKYLBENZENESULFONATES 112
ALKYLPHENOLS 809
ALKYLINS 803
ALUMINUM CLUSTERS IONS 1510
AMINO ACIDS 911
AMMONIA CHEMICAL IONIZATION 1342
AMPICILLIN 1280
ANDROSTANES 742
ANION EXCHANGE LIQUID CHROMATOGRAPHY 375
ANTIBIOTICS 945, 1400
ANTIBODY-ANTIGEN 868
ANTIFUNGAL 726

ANTIMETABOLITES 128
ANTIPSYCHOTIC DRUGS 1300
APODIZATION FUNCTIONS 1216
ARABINITOL 1179
ARECOLINE 704
AROMAS 139
AROMATIC POLYMERS 272
AROMATIC DIAMIDINO ANALOGS 682
AROMATIC AMINES 387
ARRAY DETECTORS 27, 1065, 1077, 1079, 1083, 1085, 1087, 1190
ARTIFACTS 875, 879
ARTIFICIAL INTELLIGENCE 512
ARYL-N-METHYLCARBAMATES 1443
ATMOSPHERE ANALYSIS 80
ATMOSPHERIC MONITORING 1431
ATMOSPHERIC PRESSURE IONIZATION 1114, 1177, 1365, 1379, 1441
ATMOSPHERIC PRESSURE SPRAY IONIZATION 149
Au67(PH3)14Cl 1133
AUTOMATED GC/MS 1427
AUTOMATED SOLIDS INSERTION PROBE 1110
AUTOREGRESSION 344
AZIDOTHYMININE (AZT) 716
AZO SULFONATED DYES 212
AZODYES 798

-B-

B-Q TANEDM MASS SPECTROMETER 1033
BACTERIA 718
BACTERIAL CELL WALL GLYCOLIPIDS 778
BASICITY 658
BAYESIAN SPECTRAL ANALYSIS 344
BEAM-INDUCED REDUCTION 533
BENZENE CHARGE EXCHANGE 1441
BENZO(a)PYRENE METABOLITES 1324
BENZOPHENONE OXIME 620
BENZYLOXYCARBONYL METHYL ESTERS 955
BEqQ 848
BETA-ENDORPHIN 885
BETA-ADRENERGIC BLOCKERS 1354
BIDENTATE LIGAND ANIONS 646
BIOLOGICAL REDOX REACTIONS 371
BIOMOLECULES 405, 1017, 1021
BISMUTH CLUSTER IONS 163
BLOOD 1290
BLOODROOT EXTRACT 694
BONDED COMPLEXES 832
BRADYKININ METABOLITES 696
BRAIN 1350
BROMOCHLORO 800

-C-

C3H8O+ 610
C4H7 1389
C7H8 315
CA 240, 315, 840
CAD 684, 750, 752, 856
CALBINDINS 899
CALCASIEU RIVER ESTUARY 1421
CALCIUM HOPANTENATE 1330
CALIFORNIUM-252 PLASMA DESORPTION 289,
784, 893, 1133, 1186
CANCER CELL LINES 889
CANDIDIASIS 1179
CANNABICHROMENE 1286
CANNABIDIOL 1288
CANNABINOID METABOLISM 1282
CAPILLARY ELECTROPHORESIS 112, 114, 116,
385
CARBOCATIONS 1385
CARBOHYDRATE 782, 1476
CARBOHYDRATE SEQUENCE IONS 786
CARBON 13/12 RATIOS 1155
CARBON CLUSTER IONS 159, 167, 1375, 1508
CARBON DISULFIDE 186
CARDIAC GLYCOSIDES 256
CAROTENOID 772
CATECHOLAMINE METABOLITES 1350
CCD DETECTOR 1055
CESIUM ION SOURCE 1039
CESIUM ION LIQUID (SIMS) 893
CF-FAB 722, 1002, 1474
CGS-18416A 1047
CH5+ 1377
CHANNELPLATE DETECTORS 1081
CHARGE-REMOTE FRAGMENTATIONS 238,
240, 654
CHARGE EXCHANGE CI 1463
CHARGE STRIPPING 529
CHARGE STATE 367
CHARGE TRANSFER 1369, 1377
CHARGE INVERSION 222
CHD 599
CHEMICAL IONIZATION 652, 1262, 1437
CHEMICAL WARFARE 1439
CHIRAL ANTIARRHYTHMIC DRUGS 700
CHIRAL DIAMIDE PHASE 911
CHLORDANE 1493
CHLORINATED HERBICIDES 965
CHLOROALKYL PHOSPHATE 1433
CHLOROPHYLL 224, 768
CHLORPROTHIXENE 642
CHOLESTEROL 614
CHOLESTEROL OXIDES 1338
CHROMIUM- -DIKETONATES 634
CI MASS CALIBRATION STANDARDS 1115
CLUSTER IONS 157, 161, 329, 395
CLUSTER 165, 319, 670, 933

CLUSTERING REACTIONS 317
CO2 553, 816
CO2 LASER 574
COAL 304, 308
COAXIAL CONTINUOUS FLOW FAB 114,
1006
COCAINE 1296
COCONUT AMINE SALTS 949
COLD-ON-COLUMN 1457
COLICIN Ia 870
COLLISION INDUCED DISSOCIATION 104,
155, 173, 214, 216, 218, 403, 654, 656,
766, 796, 905, 907, 1190
COLLISION 858
COLLISIONAL ACTIVATION 222, 224, 248,
346
COMPUTER APPLICATIONS 1173
CONCERTED ISOTOPE PATTERN 176
CONSTANT NEUTRAL LOSS 877
CONTINUOUS DYNODE 1073
CONTINUOUS FLOW 383, 385, 728, 758, 760,
988, 992, 994, 996, 998, 1000, 1008, 1039,
1324, 1339
COPPER VAPOUR LASER 588
CORONA-TYPE MS SOURCE 1118
CRESOTE CONTAMINATION 1408
CROSSED BEAM 1369
CRUDE OIL 306, 310
CSF 1350
Cu+ 315
CURIE POINT PYROLYSIS 1184
CYANOGEN BROMIDE 879
CYCLIC PEPTIDES 102
CYCLIC SUGARS 792
CYCLIC NUCLEOTIDES 88
CYCLOALKANES 1395
CYCLOPHILIN 915
CYCLOPROPANE RADICAL CATION 628
CYCLOPROPYL 834
CZE/MS/MS 436
CZE/CF-FABMS 106
CZE/MS 1472

-D-

DAMAGE CONTROL 1201
DATA ACQUISITION 1023
DATA SYSTEM 350, 1059
DATA TRANSCRIPTION 359
DATABASE 1120
DAUGHTER ION 232, 464, 1029, 1059
DC QUADRUPOLE 468
DCI 1045, 1304
DEHYDROGENATION 1514
DEI 1045
DESORPTION IONIZATION 533
DESORPTION TECHNIQUES 43
DETROIT RIVER 809

DEUTERIUM ISOTOPE EFFECT 622
DEXAMETHASONE 706
DIAMINE BASICITIES 1397
DIASTEREOMERIC MH⁺ 826
DIAZABICYCLOHEPTANE 957
DIAZONIUM ION-CROWN ETHER 650
DIBENZOFURAN (PCDF) 1489
DIBENZOFURANS 800, 1491
DICARBOXYLIC ACID 1330
DICHLOSILANE 175
DIGOXIN 1322
DIMERIC PEPTIDES 925
DIMETHYL SULFITE 838
DIODE LASER 590
DIOXINS 438, 800
DIOXOURANIUM (VI) DINITRATE 165
DIPHENYL-1-PHENYLAMINO-1-PHENYLMET
HANEPHOSPHONATES 616
DIPOLE-ANION INTERACTIONS 337
DISSOCIATION 668
DISSOCIATION REACTIONS 242
DISSOCIATIVE IONIZATION 1508
DISTONIC ION 403, 628
DISULFIDE BONDS 860, 925, 943
DISULFIDE-CONTAINING PEPTIDES 854
DNA BASES 736
DOMOIC ACID 122
DOUBLE-QUADRUPOLE 188
DOUBLE FOCUSING DIMIDIATE GEOMETRY
1019
DOUBLE-CHARGE - TRANSFER 1393
DOUBLY CHARGED PORPHYRIN 234
DRINKING WATER 1497
DRUG METABOLISM 1165
DUMERIL'S GROUND BOA 740
DYES 963

-E-

EC-NI 752
ECNCI 1338
EH ION SOURCE 549
EICOSANOIDS 1264
ELECTROCHEMICAL ELECTROLYSIS 551
ELECTROCHEMICAL 854
ELECTROHYDRODYNAMIC 582
ELECTRON TUNNELING 210, 432
ELECTRON ATTACHMENT NCI 1463
ELECTRON IONIZATION 1112
ELECTRON CAPTURE CHEMICAL IONIZATION
553
ELECTRON CAPTURE NEGATIVE ION 772
ELECTRONIC AND VIBRATIONAL ENERGY
LEVELS 401
ELECTROPHORESIS 30, 118
ELECTROSPRAY 41, 389, 391, 430, 586, 844
ELEMENTAL COMPOSITION 180
ENDOTHELIAL CELL 758

ENDOTHERMICALLY FORMED DAUGHTER
IONS 246
ENERGY RELAXATION PROCESSES 1500
ENERGY 858
ENERGY DISSIPATION RATES 252
ENVIRONMENTAL CONTAMINANTS 441,
444, 798, 800, 1449
ENZYMATIC HYDROLYSIS 943
ENZYMATICALLY DIGESTED 1015
EOSINOPHILS 764
EPA METHOD 625 1457
EPOXIDE 614
EPOXYEICOSATRIENOIC ACID 92
EQUATIONS OF ION MOTION 1230
EQUILIBRIUM ISOTOPE EFFECTS 333
ERGOSTEROL 780
ESTERIFICATION 638
ETFE COPOLYMER 282
ETHANOL 774
ETHYLYLIDYNE FORMATION 200
ETHYL CARBAMATE (URETHANE) 1341
ETHYLENETHIOUREA (ETU) 1445
EXACT MASS 539
EXCITED STATE PROTON TRANSFER 409
EXPLOSIVES 806
EXTERNAL SECONDARY ION SOURCE
1248
EXTERNAL ION SOURCE 456, 1252, 1254

-F-

FAB/MIKES 88
FAB/MS/MS 776, 923, 1175, 1328
FAB 30, 96, 110, 383, 407, 411, 527, 531, 535,
537, 539, 650, 658, 716, 730, 750, 758, 846,
854, 860, 883, 885, 901, 903, 929, 988, 992,
996, 1000, 1008, 1010, 1043, 1106, 1175,
1182, 1300, 1324, 1373
FAST ATOM 94, 131, 165, 184, 186, 268, 270,
300, 385, 393, 395, 541, 543, 652, 728, 760,
792, 794, 887, 891, 915, 998, 1196, 1318,
1356, 1482
FATTY ACYL HYDROXYLATION 750
FATTY ACID 744, 748
FIELD DESORPTION 272, 274, 650, 1108
FIELD IONIZATION 296, 560
FIELD CORRECTED ION CELL 1274
FIELD ASSISTED ION EVAPORATION 558
FISH 1420, 1461
FIVE- AND SIX-RING NITRO-PAHs 1410
FLAME RETARDANTS 1433
FLAVORS 139
FLOWING AFTERGLOW-TRIPLE
QUADRUPOLE 327
FLUFF 1412
FLUORETHANE 1393
FLUORINATED HERBICIDES 1348
FLUORINE 182

FLUORINE-CONTAINING CATIONS 618
FLUOROCARBONS 630
FLUPHENAZINE 684
FLYASH 1416
FOLIC ACID 244
FOOD DYES 1346
FOSSIL-DERIVED FUELS 1402
FOUR-SECTOR 230, 858, 1128, 1188
FOURIER TRANSFORM 1, 86, 258, 260, 331,
449, 458, 821, 1220, 1254, 1268, 1272, 1274,
1391
FREE-RADICAL 312
FROG ALKALOIDS 632
FT/ICR 250, 262, 344, 453, 455, 1210, 1212, 1214,
1226, 1228, 1234, 1236, 1238, 1256, 1270, 1375
FT/MS 65, 68, 236, 338, 816, 824, 1137, 1218,
1222, 1224, 1244, 1248, 1506

-G-

GANGLIOSIDES 750, 770
GAS-PHASE CHEMISTRY OF CH_3SOH ,
- CH_2SHOH , CH_3SO AND CH_2SOH 1502
GASEOUS ION ENERGETICS 1160
GC-RIMS 714, 1314
GC/MS/MS 438, 698, 1264
GC-NCIMS 1359
GC-FTMS 1252
GDMS 192
GDMS(SOLIDS) 36
GETTERS 198
GLOW DISCHARGE 52, 70, 188, 190, 194, 196,
198, 355, 580, 1139, 1141
GLUCOSE CONJUGATES 1288
GLUCURONIDE METABOLITES 1318
GLUTATHIONE 955, 1322
GLUTATHIONE CONJUGATES 1320
GLYCOPEPTIDE 1008
GOLD-LEAF ELECTROSCOPE 506

-H-

HAIR DYES 961
HALOGENATED BENZENES 1381
HALOGENATED COMPOUNDS 1412, 1421
HAZARDOUS CONSTITUENTS 1467
HEAVY METAL ALKYLATES 947
HEME PRODUCTS 1207
HEMOGLOBIN 873
HEPARIN-DERIVED OLIGOSACCHARIDES 794
HEXACHLOROCYCLOPENTADIENE TRIMERS
1493
HIGH-BOILING WAXES 310
HIGH ENERGY DYNODE (HED) 1092
HIGH RESOLUTION TRANSLATIONAL
ENERGY SPECTROSCOPY 401
HIGH PRESSURE ELECTRON CAPTURE 554
HIGH RESOLUTION

HINDRED AMINE 608
HISTORY OF QUADRUPOLE MASS
SPECTROMETRY 479
HISTORY OF ANALYTICAL MS/MS 502
HISTORY OF GC/MS INTERFACES 485
HOLLOW FIBER MEMBRANE 1495
HPLC/MS 137, 949
HPLC/TSP/MS 965
HPLC/FABMS 1015
HUMAN GROWTH HORMONE 875, 1177
HYBRID 306, 1029, 1383
HYBRID MS/MS 682
HYDROCARBON 744
HYDROGEN-DEUTERIUM EXCHANGE
1400
HYDROXY-ALOINS 710
HYDROXYHYDANTOIN ISOMERS 340

-I-

IBEI-B/E LINKED 748
ICP-MS 171
ICR 254, 344, 1240
ICR DETECTOR 1232
IMMUNOAFFINITY 82
IMPULSE EXCITATION 1220
IN-SITU POINT MONITORING 1429
IN-SITU PYROLYSIS 1242
INCINERATORS 1467
INDIUM CLUSTER IONS 1512
INDOLE-3-ACETIC ACID 921
INDUCTIVELY COUPLED PLASMA/MS 36,
46, 50, 58, 171, 1102, 1104, 1455
INJECTORS 1457
INLET SYSTEM 1427
INORGANIC FINGERPRINT 178
INORGANIC 1135, 1204
INTERNAL ENERGIES 222
INTERNAL ENERGY 252
IODONIUM SALTS 268
ION TRAP 444, 462, 468, 638, 674, 704, 828,
1228, 1242, 1250, 1260, 1264, 1266, 1294,
1352, 1402, 1429
ION PARTITIONING 1270
ION/SOURCE SURFACE COLLISIONS 25
ION TRAP 60, 62, 77, 369
ION ABUNDANCE TUNING 356
ION MOBILITY SPECTROMETER 1094
ION TRANSMISSION 1025
ION TRAP CAVITY 1258
ION-ION INTERACTION 1222, 1224
ION TRAJECTORIES 1096
ION CYCLOTRON 1230
ION DECELERATION LENSES 1089
ION CYCLOTRON RESONANCE 456, 1246,
1391
ION SPRAY 143, 145, 365, 984

ION-MOLECULE REACTIONS 1309, 1383, 1449
ION KINETIC ENERGY 1065
ION EVAPORATION IONIZATION 889
ION INJECTION 1244
ION DETECTOR 1075
ION EVAPORATION SPECTRA 1181
IONIZATION POTENTIALS 1375
IONIZATION/TANDEM 889
IRIDIUM COMPLEXES 664
ISOCYANATE PREPOLYMERS 266
ISOELECTRONIC 1367
ISOGYRIC REACTIONS 1367
ISOMERIZATION PROCESSES 668
ISOQUINOLINES 606
ISOTACHOPHORESIS 120
ISOTOPE ANALYSIS 576
ISOTOPE ABUNDANCE 422
ISOTOPE DILUTION (IDMS) 417
ISOTOPE DILUTION 1308, 1312
ISOTOPE RATIO 33, 594, 1238
ISOTOPE ABUNDANCE MEASUREMENTS 1170
ISOTOPIC LABELING 608
ITD 302
ITMS 1435, 1484

-K-

K+IDS 291, 527
KEPONE 1488
KEROGEN 308
KETO ACIDS 1312
KILOVOLT COLLISIONS 226
KINETIC ENERGIES 672, 1272
KINETIC ENERGY RELEASE 224, 830
KINETICS 242
KRATOS MS80RFAQ 1035

-L-

LACTAM SYNTHESIS 676
LADDER SCALES 325
LANOSTEROL DEMETHYLASE 1336
LARGE IONS 236
LASER 86, 287, 304, 321, 409, 568, 670, 812, 821, 1506
LASER-INDUCED 202
LASER VAPORIZATION 157
LASER IONIZATION 819
LASER ABLATION 167, 258
LASER EVAPORATION 1041
LASER DESORPTION 248, 262, 572, 574, 576, 578, 778, 816, 1256, 1258, 1260, 1278
LC/API/MS 133, 984
LC/MS 108, 110, 139, 147, 149, 369, 373, 383, 931, 933, 935, 941, 947, 955, 957, 963, 967, 969, 986, 988, 998, 1008, 1012, 1014, 1045, 1302, 1306, 1308, 1322, 1354, 1472

LC-MS 967, 1008, 1302, 1306
LC/MS/MS 147, 436, 953, 1308
LD/FTMS 562
LDI-FT-ICR 1194
LEAFY SPURGE (EUPHORBIA ESULA L.) 1184
LENS PROTEINS 903
LEUCINE TRANSAMINATION 1310
LEUKOTRIENES 760
LEWIS ACIDS 646
LIGAND EXCHANGE 319
LIMS 287, 812
LINKED SCAN 270, 340
LIPIDS 302
LIPIDS 740
LIPOSIDOMYCIN ANTIBIOTICS 724
LIPOXYGENATION 762
LIQUID NH3 INJECTION 978
LIQUID CARBON DIOXIDE 284
LIQUID METAL ION 1071, 1151
LIQUID SIMS 90, 397, 720
LITD/FTMS 200, 1514
LONG-LIVED EXCITED STATES 216
LSIMS 90, 788, 872, 873, 1188, 1304
LTB4 764

-M-

MACPROMASS 352
MAGNESIUM ISOTOPIC RATIOS 592
MAGIC LC/MS 128
MANNOSE 1192
MANTLE MINERALS 294
MARINE NEUROTOXIN 122, 145
MARINE MAMMALS 1463
MARS 523
MASS-FLOW 373
MASS-PROBABILITY BASED MATCHING 354
MASS SPECTROMETRY DATA CENTRE 361
MASS-SELECTED REAGENT ION 1145
MASSPEC 350
MATRIX-FREE FAB 153
MATRIX REACTIONS 887
MAXIMUM ENTROPY METHOD (MEM) 1212
MECHANISM 652
MEMBRANE/MASS SPECTROMETRY 1465
MEMORY EFFECT 597
MERCAPTURIC ACID METABOLITES 1361
METABOLIC DISORDERS 1328
METAL CARBONYLS 1198
METAL-LINKED DIMERS 1207
METAL IONS 244, 321
METALLOPORPHYRINS 636
METALS 597
METEORITES 294
METHANE CHEMICAL IONIZATION 491
METHAPYRILENE 1318
METHOD 6020 1455

METHYL, CYCLOPENTADIENYL CHROMIUM
DINITROSYL 323
METHYL ESTERS 674
METHYLNITRITE 326
MICROBIAL CELL 302
MICROBORE 992, 1008
MICROCOLUMN LC/CONTINUOUS FLOW 1010
MICRODIALYSIS 1002
MICROPROBE 1506
MILK 180
MITOMYCIN C 690
Mn₂(CO)₁₀⁺ 660
MOLECULAR BEAM 216
MOLECULAR ION SPECTROSCOPY 6
MONO (2-ETHYL-HEXYL) PHTHALATE 1344
MONOCYTE CHEMOTACTIC AGENT 899
MORFINANS 984
MORPHINE ALKALOIDS 1294
MORPHINE 1290
MOVING BELT LC/MS 153
MS/MS/MS 98, 246, 1027
MS/MS 143, 240, 315, 348, 383, 441, 446, 674,
694, 716, 750, 752, 840, 866, 913, 929, 1025,
1128, 1190, 1228, 1266, 1290, 1322, 1326,
1414
MSCOINT 346
Mⁿ EXPERIMENTS 464
Mⁿ+1, ZERO-ANGLE (SID)_n 1149
MUCIN GLYCOPROTEINS 790
MULTIFUNCTIONAL GROUP MOLECULES (III)
1015
MULTIPHOTON DISSOCIATION 250
MULTIPHOTON IONIZATION 564, 572, 672,
973, 1254
MULTIPLE REACTION MONITORING 1290
MULTIPLY CHARGED IONS 100, 425, 428, 430,
432, 656
MULTIPLY CHARGED NEGATIVE IONS 434
MULTIPLY CHARGED PEPTIDES 905

-N-

N-BUTYLBENZENE 252
N-LINKED GLYCANS 980
NABILONE 680
NAPAMEZOLE 1298
NAPHENIC ACIDS 300
NCI 387, 971
NE-11740 698
NEGATIVE ION CHEMICAL IONIZATION 92,
462, 642, 708, 746, 917, 986, 1418, 1447,
1486
NEGATIVE ION FABMS/MS 756
NEGATIVE IONIZATION 1250
NEGATIVE THERMAL IONIZATION 414
NEGATIVE ION FAST ATOM BOMBARDMENT
702
NEGATIVE ION 680, 1443

NEGATIVE ION CLUSTERING 1379
NEREISTOXIN 967
NEUROPEPTIDES 1128
NEUROPHYSINS 909
NEUTRAL MOLECULES 570
NEUTRALIZATION-REIONIZATION 1387,
1502
NEUTRALIZED ION BEAM 1504
NEUTRINO PHYSICS 1143
NITRILES 840
NITRO-PHENYLISOTHIOCYANATE 881
NITROCELLULOSE 399, 545
NITROMETHANE 326
NO₃ 355
NON-AEROSOL VAPORIZATION 147
NONLINEAR 1094
NPPD ("SPY DUST") 969
NUCLEOPHILIC SUBSTITUTION REACTIONS
836
NUCLEOSIDE-PAH DNA ADDUCTS 996
NUCLEOSIDES 84, 734, 1196
NUCLEOTIDE ANTIBIOTIC 726
NUCLEOTIDES 738

-O-

O-ALKYL DI-AND TRINUCLEOTIDES 730
O-(2-DIISOPROPYLAMINOETHYL) O'-ETHYL
METHYLPHOSPHONITE 1437
OCTADECADIENOIC ACIDS 240
OCTAMETHYLCYCLOTETrasiloxane
1451
ODOURIFEROUS EMISSIONS 1478
OIL-SANDS 1478
OLIGONUCLEOTIDE 728, 844
OLIGOPEPTIDE 572, 877
OLIGOSACCHARIDE 720, 788, 790, 971, 1188,
1190, 1192, 1194
ON-LINE VAPOR MONITORING 1425
ONSET POTENTIALS 1399
OPIATES 1292
OPTICAL CCD CAMERA 1053
ORBITAL CALCULATIONS 1367
ORGANIC COMPOUNDS 151
ORGANIC SECONDARY ION 1071
ORGANIC NITROXIDE RADICALS 1332
ORGANOMETALLICS 1135, 1204
ORIFICE POTENTIAL 580
Os⁺, Os₂⁺, Os₃⁺ 1395
OUABAIN 686
OXALIC ACID 278
OXANTRHONE GLUCOSYLES 710
OXAZOLINE DERIVATIVES 748
OXIRANE ISOMERS 612
OYSTERS 803

-P-

- PALLADIUM COMPLEXES 184
PARALLEL COLUMN CHROMATOGRAPHY 1404
PARTICLE BOMBARDMENT 157, 399
PARTICLE BEAM 108, 110, 126, 130, 133, 135, 137, 139, 141, 379, 381, 963, 1012, 1045
PARTICLE-BEAM LC/MS 131
PC SOFTWARE 358
PC-BASED LINKED SCAN CONTROL 1098
PCDD 1416
PCDF 1416
PD/FAB 909
PD 658
PEAK MATCHING 1106
PENICILLINIC ACID THIOLACTONES 688
PENNING IONIZATION 190
PENTACHLOROPHENOL 1480
PENTAFLUOROBENZYL 736
PENTAQUADRUPOLE 1027
PEPPERS 1423
PEPTIDE HYDROPHOBICITY 545
PEPTIDE TOXINS 1128
PEPTIDE GROWTH HORMONES 889
PEPTIDE MAPPING 872
PEPTIDE SEQUENCING 852, 917
PEPTIDES 98, 100, 104, 106, 116, 352, 537, 543, 582, 722, 782, 850, 856, 858, 860, 864, 866, 881, 887, 895, 897, 901, 907, 943, 992, 1006, 1014, 1122, 1131
PERACETYLATED OLIGOSACCHARIDES 784
PERFLUOROCARBONS 556
PERFORMED IONS 531
PESTICIDE 135
PETROLEUM 300
PHARMACEUTICAL 379
PHARMACOKINETICS 1002
PHASE ANGLE SYNCHRONIZATION 1268
PHENYLGLYCIDYL ETHER NUCLEOSIDE ADDUCTS 923
PHENYLISOTHIOCYANATES 917
PHOSPHINOISITOLS 94
PHOSPHOLIPASE A2 (PLA2) 746
PHOSPHOLIPID 776
PHOSPHORYLATED 891
PHOTO INDUCED DISSOCIATION 230, 824
PHOTOAFFINITY 870
PHOTOCHEMICALLY-INDUCED 642
PHOTODEGRADATION PRODUCTS 1453
PHOTODISSOCIATION 252, 407, 816, 1500
PHOTODISSOCIATION FOURIER TRANSFORM 1278
PHOTOFRAGMENTATION 254, 666
PHOTOIONIZATION 294, 666, 1389
PHTHALATE 680
PHYCOCYANIN 913
PITUITARY 686, 885
PLANAR CHROMATOGRAPHIES 35
PLANT TOXINS 1339
PLASMA 48, 50, 58, 96, 580, 680, 704, 708, 1350
PLASMA-SOURCE 54
PLASMA DESORPTION 545, 547, 778
PLASMASPRAY 716
PLATELET ACTIVATING FACTOR 756, 758
PLATELETS 762
PLATINATED OLIGONUCLEOTIDES 702
POLLUTANTS 1441
POLY-3-METHYLTHIOPHENE 289
POLYAMIC ACIDS 262
POLYAROMATIC HYDROCARBONS 1482
POLYARYLACETYLENE RESINS 293
POLYATOMIC PRIMARY IONS 206, 228
POLYBROMINATED FURANS 1418
POLYBUTADIENES 260
POLYCHLORINATED DIBENZO-P-DIOXIN (PCDD) 1489, 1491
POLYCYCLIC AROMATIC HYDROCARBONS 670
POLYHYDROXY AMINES 1342
POLYIMIDES 264
POLYISOPRENES 260
POLYMER 167, 204, 258, 270, 278, 287, 1108
POLYMER SURFACES 210
POLYNUCLEAR AROMATIC COMPOUNDS 1497
POLY[bis(PHENOXY)-PHOSPHAZINE] 280
PORPHYRIN PYRROLE 796
PORPHYRINS 636
POSITRON 22, 584
POST-TRANSLATIONAL 875
POST SOURCE PULSE FOCUSING 1069
POTENTIAL ENERGY SURFACES 646
POTWS 1469
POWDERED METAL OXIDES 196
PRIVADOLINE 959
PRECISE ATOMIC MASS MEASUREMENTS 1143
PREHISTORIC RESEARCH 692
PRESSURE 458
PROPANE 218
PROSTAGLANDIN 137, 754, 774
PROTEASE 846
PROTEIN SEQUENCING 821
PROTEIN STRUCTURE 1131
PROTEINS 100, 116, 352, 870, 879, 893, 1000, 1014, 1015, 1122, 1125, 1181
PROTEOLYTIC DIGESTS 1175
PROTON TRANSFER REACTIONS 1371, 1399
PT(111) 1514
PULEGONE 1326
PULSED ION 864, 1276
PUMILIOTOXIN 632
PYRIDYL GROUP MIGRATION 606
PYRILAMINE 1302

PYROLYSIS 286, 293, 304, 718

-Q-

Q-FTMS 407

QUADRUPOLE ELECTROSTATIC DEFLECTOR 1037

QUADRUPOLE DOUBLET(QPD) 1087

QUINONES 986

-R-

RADIATIVE ASSOCIATION REACTIONS 1385, 1391

RADIATIVE STATES OF N₂H 1504

RADICAL IONS 403, 1157

RADIOGAS CHROMATOGRAPHY 1100

RAPID SCREENING 1328

RATIO OF GLYCEROL TO ANALYTE 1182

RATTLESNAKE (CROTALUS ATROX) 740

REACTANT ION CI 1147

REACTION MONITORING 706

REACTION INTERFACE 712, 714

REAL-TIME REACTION MONITORING 365

REFLECTING TIME-OF-FLIGHT 232, 1059

REFLECTION 228

REGULATORY REQUIREMENTS 72

RESIDUAL GAS ANALYZERS 1031

RESONANCE IONIZATION 588, 590

RESPONSE FACTOR 1459

RHODIUM CHELATES 662

RIMS 38, 417, 419

RUSKA THERMA-CHROM 1057

RUTHENIUM 173

-S-

SACCHARIDES 1196

SALMONELLA TYPHIMURIUM 744

SAMPLING RATE 1236

SCOPOLAMINE 708

SECONDARY ION MICROSCOPE 208, 1061

SECONDARY ION 204, 206, 268, 868, 1051, 1053

SELECTIVE METHODS OF IONIZATION 474

SELVEDGE REGION 527

SERUM 1344

SFC/MS 266, 971, 975, 977, 1476

SHEET DIRECT INSERTION MEMBRANE 151

SILANE 175

SIMS 417, 730

SIMS IMAGING 1055

SITE-SPECIFIC REARRANGEMENTS 1365

SKIMMER GEOMETRY 58

SLUDGE 310

SOLID PROBE 622

SOLID FAB 529

SOLIDS ANALYSES 1141

SOLVOLYSIS 1159

SORBITOL 535

SOY SAUCES 1341

SPACE CHARGE EFFECTS 453

STABLE ISOTOPES 850

STABLE-ISOTOPE DILUTION 927

STATIC FAB 212

STERIODS 742

STRUCTURES OF IONS IN THE GAS PHASE 1163

STYRENE-7,8-OXIDE 873, 1334

STYRENE BASED POLYMERS 276

STYRENE 1500

SUBMARINES 80

SUBSTANCE 883

SUGAR SULFAMATES 624

SULFATED PEPTIDES 891

SULFUR-CONTAINING 712

SUPERCritical FLUID 312, 377, 945, 975, 980, 982, 1049, 1051, 1091

SURFACE-CATALYZED REACTIONS 387

SURFACE 228

SURFACE ACTIVITY 658

SURFACE ANALYSIS 208

SURFACE TEMPERATURE 537

SURFACTANT 539, 929, 982

SWIFT WAVEFORMS 1216

SYDNEY TAR PONDS 1406

SYNTHETIC LUBRICANT 274

SYNTHETIC PEPTIDES 862

-T-

TANDEM 96, 100, 102, 118, 340, 365, 385, 405, 496, 678, 710, 724, 754, 758, 760, 768, 780, 846, 848, 850, 895, 897, 899, 990, 1021, 1077, 1122, 1125, 1131, 1165, 1290, 1294, 1302, 1320, 1322, 1326, 1361, 1445, 1449

TANDEM QUADRUPOLE 1023, 1025

TANTALUM CARBIDE CLUSTER IONS 169

TCDD AND TCDF 1414

TEMPERATURE JUMP 202

TEMPERATURES 828

TETRAALKYL AMMONIUMSURFACTANTS 1373

TETRAHYDROCANNABINOL 1284

TETRAMETHYLSILANE 493

THENYLDIAMINE 1306

THERMAL DESORPTION 202

THERMAL SLICING 308

THERMAL DEGRADATION 268

THERMAL IONIZATION 595

THERMAL DESORPTION 1435

THERMAL CHROMATOGRAPHY 276

THERMOCHEMISTRY 327

THERMOSPRAY 41, 367, 369, 543, 551, 716, 927, 929, 931, 933, 935, 937, 939, 941, 947, 951, 953, 959, 963, 965, 967, 975, 988, 1304, 1306, 1318, 1354, 1400

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May 21-26, 1989, Miami Beach, FL

THIN FILMS 194
THIN LAYER CHROMATOGRAPHY 1041
THIODIGLYCOL 1316
THIOPHENOL IONS 254
THORPE REACTION 840
TIBENELAST 1304
TIME-RESOLVED ION MOMENTUM 1065
TIME-OF-FLIGHT 204, 208, 294, 588, 730, 864,
931, 1061, 1063, 1067, 1069, 1071, 1153
TIME-OF-FLIGHT FTMS 1276
TIME ARRAY DETECTION 32, 1063
TIMS 38
TOF-SIMS 204, 901
TOFMS 32
TRACE METAL 411
TRANSFER LINE 1425
TRANSITION METAL 313, 317, 155, 830, 843,
1137
TRAPPED IONS 460
TRIAZINES 1447
TRICHLOROPHENYL ETHANOL 1423
TRIFLUOROACETIC ACID 860
TRIMETHYLSILYL GROUP MIGRATIONS 614
TRIMETHYLSILYL IUM 342
TRIPLENNAMINE 1306
TRIPLE QUADRUPOLE 438, 866, 1147, 1294,
1399
TRYPTIC 118, 383, 1128
TSP LC/MS 961
TWO DIMENSIONAL FT-ICR 451
TWO-PHOTON IONIZATION 978
TWO DIMENSIONAL ION-TRAP 466

-U-

ULTRAVIOLET LASER DESORPTION 1168
UNIMOLECULAR 834
URETHANES 644
URIC ACID 1308
URINE 82, 84, 732, 1288, 1292, 1296, 1312, 1316,
1350, 1358
UV LASER 566
UV LASER IONIZATION 1041

-V-

VERY LOW ENERGY 1498
VIETNAM VETERANS 1489
VINYL ANION 1363
VITAMIN D 766
VOLATILE ORGANICS 1465, 1484

-W-

WARFARIN 1356
WATER 298
WAVEFORMS 1218

-X-

XENOBIOTIC CONJUGATES 446, 955
XENOBIOTIC 1420

-Z-

Z-EJECTION 1226
ZEIN 921
ZOOMING MASS 1087

AMERICAN SOCIETY FOR MASS SPECTROMETRY
BUSINESS MEETING

Fontainebleau Hilton
Miami Beach, Florida
May 24, 1989

President Ron Hites called the meeting to order at 11:20 a.m. Susan Weintraub presented the Secretary's report. She first announced the results of the 1989 ASMS elections. The new Board members will be: Robert Lattimer, Vice President for Arrangements; Veronica Bierbaum, Secretary; Richard Caprioli, Member-at-Large. Thanks were also expressed to the candidates who were not elected - David Millington, John Eyler and Charles Wilkins. Next, the list of winners of the 1989 Student Travel stipends was displayed. The recipients are: Amit Ghosh, University of Maine; James Ronald Gord, Purdue University; Richard P. Grese, University of Nebraska - Lincoln; Shenheng Guan, University of the Pacific; Lenaers Guido, Antwerpen University, Belgium; Richard J. Hughes, Trent University, Ontario, Canada; Martin Arthur Moseley, III, University of North Carolina - Chapel Hill; Johnny D. Reynolds, University of Tennessee - Knoxville; Kevin L. Schey, Purdue University; Lutz Schweikhard, University of Mainz, West Germany; Anthony G. Thompson, University of Illinois. The students were congratulated on their achievement.

Michelle Buchanan then gave the Treasurer's report. Charts depicting ASMS income and expenses were shown, and Buchanan explained that a net profit of approximately \$2,000 was realized last year. She added that the ASMS Board tries to maintain year-end assets at a level equal to one year's expenses.

The Vice President for Programs, Robert Murphy, spoke next. He first thanked the members of the Program Review Committee for their efforts in reviewing the abstracts. Those serving on the committee were: Michelle Buchanan, Richard Caprioli, Keith Clay, Charles DePuy, Gary Glish, Michael Gross, Ron Hites, Vernon Reinhold, Doug Ridge and Joe Zirrolli. Murphy continued by saying that initially, 50 abstracts were rejected; however, 26 were resubmitted and were included in the program. Murphy said that he feels that the quality of the abstracts has improved significantly since the review process was initiated.

Murphy next told about various aspects of the program. He explained that the structure of the program is based, to a large extent, on information gained from the interest groups and workshops. He added that he is currently soliciting suggestions about symposia and plenary speakers for next year. Murphy then displayed a chart which showed the number of oral and poster presentations from 1984 to 1989, noting that posters have increased dramatically over this period. He said that he feels that the society benefits greatly by the growth in the number of posters, as posters are such a good way to transmit information. Murphy then announced that there were 1623 registered attendees at the 1989 Conference. This number is slightly less than the total from the San Francisco meeting, but it follows the trend seen in the past.

Murphy concluded by thanking people who are assisting him with audiovisual and other arrangements at the conference - Kevin Hart of Michigan State, Casey Grimm of Oak Ridge, Michael Shirley of Colorado and George Yefchak of Michigan State. At this point, Mike Grayson commented favorably on the high quality of the poster boards being used at this conference, and he encouraged Murphy to obtain similar ones in the future. Murphy added that if this type of board is available, presenters would be able to use pieces of Velcro to mount their displays.

Gary Glish, the Vice President for Arrangements, gave his report next. He first explained about the beach party scheduled for that evening and said that members and their guests should wear their green name badges for admission to the party. He also said that various games would be held throughout the evening, and he listed the prizes which could be won.

Glish next showed slides of the hotels which will be used for the 1990 Conference in Tucson - the Ventana Canyon and the La Paloma. He then listed the dates and sites for future ASMS meetings. These are: 1991, Opryland Hotel in Nashville (May 19 - 24); 1992, the Hilton in Washington, D.C. (May 31 - June 5); 1993, the Riviera Hotel in Las Vegas (June 6 - 11). He noted that none of these dates includes the Memorial Day holiday.

Glish concluded by thanking the Board for their help. Sandy Markey then congratulated Glish on this year's arrangements, adding that the site was very pleasant. He was concerned, however, about the choice of Las Vegas for a future meeting. Glish said that the Board had carefully evaluated Las Vegas and felt that the outstanding

quality of the meeting space, the low room rates and the availability of meals at modest prices outweighed other aspects of the city. Glish continued by saying that there are not many facilities which can accommodate our meeting in a single hotel and that we are not yet large enough to use a convention center. Graham Cooks suggested that a straw vote be taken to measure the members feelings about Las Vegas. Ron Hites asked for those present at the meeting to applaud appropriately if they were "for" or "against" Las Vegas. The response was approximately equal, and Hites said that the Board will review the situation and make a final decision at their August meeting.

The Member-at-Large with responsibility for Education, Mike Story, was next to speak. He explained that he serves as chairman of the Education Committee. The members of this committee are Paul Goodley, Ann Goetz, Harry Hertz, Alan Marshall and Al Yerger. He reported that 5 short courses were offered this year and that there were 175 participants. Story reminded ASMS members to fill out and return the questionnaire about short courses which was distributed at registration.

Story next described a brochure about mass spectrometry which was developed by the Education Committee. This publication is a promotional booklet which will be given to undergraduate students in an attempt to interest them in mass spectrometry. Story said that special thanks were owed to Ann Goetz for her efforts on this project. Story then told about 2 "educational" symposia which were being presented this year - one on state-of-the-art research in mass spectrometry, organized by Harry Hertz, and the other, introductory lectures about various topics in mass spectrometry, organized by Al Yerger. Story said that there had been good attendance at these symposia and that he felt that they had been well received. In conclusion, Story expressed his thanks to Harry Hertz, Paul Goodley and Al Yerger, whose terms on the committee are ending.

Willard Harrison, the Member-at-Large responsible for the Proceedings, was next to report. He reminded the members to turn in their extended abstracts. Harrison said that in the past, the submission rate for extended abstracts has been about 95%, enhancing the quality of the Proceedings. He said, however, that he hopes to increase the number of abstracts from the plenary lecturers. Harrison then said that for 1988, the Proceedings cost the society \$11 per book and that approximately 2900 copies were printed. Harrison estimated that the cost to the society for the 1989 Proceedings will be \$12 and that 2900 copies will again be printed.

The Member-at-Large with responsibility for Measurements and Standards, Rodger Foltz, gave the next report. He described recent activities of the Measurements and Standards Committee, of which Foltz serves as chairman. In one area, they have worked with Phil Price to compile an updated list of definitions of terms in mass spectrometry. They have attempted to resolve conflicts of opinion in this regard and are in the process of revising their document. Work is also underway with a subcommittee directed by Jon Onstot to develop a standard computer format for transfer of mass spectral data. In addition, the committee is sponsoring a workshop, organized by Richard Martinez, concerned with CAD databases. Foltz then listed the members of the Measurements and Standards Committee: Bill Budde, Hank Fales, Ron Grigsby and Doug Cameron. Foltz explained that the terms of the latter three members are due to expire and that anyone interested in serving on this committee should contact Ron Hites.

ASMS President Ron Hites was next to speak. He began by expressing deep appreciation to Judith Watson for all of her work for the society. He said that the Board felt that she deserved added recognition and decided to change her title to be the "Executive Director of the American Society for Mass Spectrometry". Hites then asked Watson to come forward, and he presented her with a gift as a token of the Board's gratitude for her outstanding work. Hites next thanked the outgoing Board members, Gary Glish, Susan Weintraub and Willard Harrison, and presented each with a plaque from the society.

Hites next announced the winners of the 1989 ASMS Research Awards for young or beginning mass spectrometrists. Martin Janiak presented the VG Award, explaining that he was grateful to have the opportunity to provide support and say thanks to the mass spectrometry community. The recipient of the VG Award was Steven Kass of the University of Minnesota. The title of his project is "Unimolecular Rearrangements of Carbanions". Bob Finnigan then conferred the Finnigan Award to John Amster of the University of Georgia. His project is entitled "Gas Phase Reactions of Laser-Desorbed Neutral Molecules with Transition Metal Ions".

Hites then described several new activities for ASMS. First, he explained about the establishment of the ASMS Award for Distinguished Contribution to Mass Spectrometry. He said that ASMS expects to be able to present the first award at the Tucson meeting and that nominations are due by November 1, 1989. He added that the award is designed to recognize focused achievement or contribution to fundamental and applied areas of mass spectrometry. Hites said that a committee consisting of Jane Gale, Mike Grayson, Sandy Markey and Ron

Mitchum, chaired by Willard Harrison, had formulated the plans for the award. Hites also said that the ASMS President will appoint a special committee to select the recipient of the award. The composition of this committee will not be made known to the members.

Hites next presented information about a major innovation for ASMS - publication of an ASMS Journal. Hites displayed a representation of the cover of the new journal which will be called the Journal of the American Society for Mass Spectrometry and said that he expects that the first issue will be published in March of 1990. He said that the journal will be a great benefit to ASMS members and that publication of the journal will not prompt any dues increase in 1990. Hites further explained that the publisher of the journal will be Elsevier, and Hites was appreciative for the easy negotiations with that company. He added that the scope of the journal will include all areas of mass spectrometry; there will be a complete peer review process, and the editor will be Mike Gross. Four associate editors will be appointed along with a Board of Advisors. Hites then answered in advance two questions that he anticipates receiving. First, "Is another journal needed?" The answer is "yes". The ASMS Journal will be important for unifying the field by providing comprehensive coverage of a wide range of topics including applications and fundamentals. The journal will be available at a much lower cost than other mass spectrometry publications because of the large membership base (approximately 3000). In addition, the price for an institutional subscription is going to be \$230, much less than for other journals. The second question Hites expected to hear was, "Will costs go up significantly after the first year, necessitating a major dues increase?" Hites said that the answer to this is "no". He showed a graph depicting changes in ASMS dues over the past years. He explained that after correcting for inflation and indexing to 1989 dollars, there had, in fact, been little increase in the actual dues level. Hites did say that he anticipates that a \$10 increase in dues will probably be put into effect in 1991. He then said that from the results of the recent salary survey, the average member earns about \$48,000, making the cost of dues about 700 ppm!

Hites then invited questions from the audience. When asked about the number of issues, Hites responded that there will be 6 issues in 1990 and 12 in 1991 and that Mike Gross is already prepared to receive manuscripts. Hites also said that he expects it to be a great honor for an author to be included in the premier issue, and he urged the members to submit their work. Regarding the format, Hites answered that a 2-column, 8 1/2 x 11 inch format will be used with standard weight paper. Concerning the inclusion of short communications, Hites replied that he anticipates three types of presentations - brief reviews, research papers and short communications. One member wanted to know about advertising, and Hites said that a limited amount of advertising space will be available. Hites added that he has told the ASMS corporate members about the journal and advised them that they would be entitled to reduced rates for advertising in the journal. Hites continued by saying that it is important to keep the institutional costs down so that many libraries can subscribe to the journal. Since libraries are often dependent on their users for recommendations about acquisitions, Hites urged ASMS members to take the journal announcement that they will receive to their libraries and encourage institutional subscriptions. Hites said that there would not be any non-member rates since ASMS dues are so low. When asked about prices over the long term, Hites answered that costs and rate schedules have been built into the contract with Elsevier; arrangements have been made so that decisions about all major financial aspects of the journal are under control of the ASMS Board. Concerning the future of the Proceedings, Hites replied that at present, there would be no change in the way ASMS publishes the extended abstracts. Hites did say, however, that he anticipates that some changes may be instituted in five or ten years. A member wanted to know about financial exposure of the society because of the journal, and Hites answered that although there will be profit sharing, there is no risk sharing for the journal. The publisher assumes all of the risk and has the option to not renew at the end of the contract period. When asked about page charges, Hites said that there will not be any page charges and that authors will receive 50 free reprints of each paper they publish. A suggestion was then made that the Proceedings contain a regional directory of ASMS members. Hites said that such a directory could be organized by states and that this idea will be considered. In the final question from the floor, it was asked if the Spectaker would be incorporated into the journal. Hites responded that the journal will contain a society news page in addition to classified ads. The classified ads will be available at a nominal cost, by arrangements through the Elsevier redactory office. In conclusion, Hites thanked everyone for their attendance. The meeting was adjourned at 12:16 p.m.

Respectfully submitted,

Susan T. Weintraub

ASMS SURVEY OF 1989 SALARIES

At the behest of the members, the Board recently organized a salary survey of mass spectrometrists. A form was sent out with the most recent Spectaker; 757 responses were received. This is about 25% of all members. This is an excellent response and allows us to draw some valid conclusions about the compensation of mass spectrometrists. The data were entered by Judith Watson's office into a Lotus spreadsheet and analyzed by the Statistical Analysis System (SAS Institute, Cary, North Carolina) at Indiana University. Some of the categories on the original survey form were pooled with one another because of small numbers and because there were not significant differences between them.

The results are reported in the following table. Each entry is the 90th percentile (top), the median (50th percentile), and the 10th percentile. The data have been broken down by the four categories that are related to compensation: highest degree, gender, years of experience, and current employer. A blank cell in the table indicates that there were less than ten individuals in this category; for statistical validity, it seemed best not to report these data.

A couple of examples should make this table clear. The cell labeled "overall" tells us that the average member of the Society makes \$48,592; there are, however, 10% of the members who make in excess of \$75,000. The cell for university faculty with 15 to 19 years of experience tells us that these people make, on an average, \$60,000 but that there are 10% of these people who make less than \$34,000. Not surprisingly, those members with a Ph.D. make more money than members without this degree regardless of experience. Our typical male member makes \$9,000 more than our typical female member. The highest number on this table is \$170,000 for those in the 90th percentile with 15 to 19 years of industrial management experience; the lowest is \$12,761 for the 10th percentile of non-faculty, university employees. The compensation for university faculty is similar to those doing industrial research and development up until 20 years of experience, but after that university faculty are more well paid than their industrial counterparts. The lesson from these data should be clear to graduate students: Do not reject a faculty career because it is not as well paying. One merely has to be patient.

In the salary survey, two different questions were asked about salary: base salary and total compensation. With two exceptions, the total compensation was only 5 to 6% more than the base salary. The exceptions were university faculty, whose base salary was about 80% of their total compensation, and those working in the marketing and sales sector of industry, whose base salary was about 85% of their total compensation.

	Overall	Years of experience							Degree			Gender		Number (%)
		0-4	5-9	10-14	15-19	20-24	25-29	30+	Doctorate	Masters	Batchelor	Male	Female	
Overall	\$75,000 \$48,592 \$32,000	\$52,060 \$39,100 \$26,000	\$65,014 \$46,000 \$32,000	\$76,900 \$50,000 \$35,050	\$93,500 \$60,000 \$36,150	\$90,000 \$53,000 \$33,200	\$91,500 \$60,000 \$36,000	\$107,800 \$62,750 \$42,300	\$80,000 \$52,000 \$35,000	\$68,282 \$43,932 \$31,517	\$60,000 \$42,000 \$29,700	\$80,000 \$50,000 \$32,250	\$56,384 \$41,000 \$30,000	757 100
Government	\$67,400 \$46,700 \$31,148	\$44,100 \$32,250 \$26,800	\$61,934 \$45,200 \$29,814	\$60,050 \$45,650 \$36,582	\$71,987 \$53,000 \$30,700	\$75,070 \$51,300 \$37,000	\$101,000 \$53,148 \$35,200		\$70,826 \$52,106 \$34,750	\$58,192 \$40,673 \$29,907	\$58,000 \$42,600 \$29,963	\$69,300 \$49,400 \$31,700	\$56,630 \$40,000 \$29,926	141 19
University, faculty	\$102,500 \$54,526 \$35,600	\$63,700 \$40,550 \$31,623	\$98,400 \$45,000 \$36,950	\$82,500 \$50,000 \$36,500	\$128,000 \$60,000 \$34,000	\$114,000 \$75,000 \$35,700			\$102,500 \$54,526 \$35,600			\$103,000 \$56,000 \$36,300	\$45,900 \$41,000 \$32,200	104 14
University, others	\$52,500 \$35,000 \$25,700	\$37,850 \$29,500 \$12,761	\$51,000 \$33,000 \$28,880	\$53,600 \$37,200 \$25,220					\$51,830 \$37,350 \$26,000	\$54,750 \$34,250 \$13,132	\$61,500 \$30,100 \$17,785	\$53,600 \$36,000 \$23,660	\$51,000 \$34,500 \$26,040	76 10
Industry, res & dev	\$72,000 \$51,000 \$36,892	\$52,000 \$45,000 \$30,000	\$68,400 \$52,000 \$36,000	\$80,000 \$54,300 \$38,484	\$73,924 \$62,500 \$47,660	\$85,200 \$57,000 \$41,344	\$116,300 \$69,500 \$43,720		\$79,100 \$55,000 \$43,420	\$72,300 \$46,250 \$31,750	\$60,000 \$45,000 \$29,800	\$73,200 \$52,000 \$37,260	\$67,600 \$45,700 \$34,800	222 29
Industry, management & sales	\$86,000 \$60,000 \$40,000	\$67,500 \$43,800 \$37,000	\$71,400 \$52,000 \$37,750	\$87,774 \$68,062 \$47,200	\$170,000 \$75,000 \$44,877				\$95,000 \$69,400 \$43,560	\$84,500 \$60,000 \$41,968	\$83,600 \$50,000 \$37,100	\$88,000 \$64,000 \$41,700	\$80,422 \$43,500 \$36,650	99 13
Industry, laboratory & QC/QA	\$60,000 \$44,750 \$32,000	\$47,000 \$38,000 \$25,000	\$52,000 \$45,000 \$34,400	\$66,000 \$45,000 \$33,250					\$71,350 \$48,500 \$39,400	\$52,000 \$43,750 \$34,150	\$54,100 \$38,000 \$29,600	\$62,600 \$45,000 \$32,820	\$51,628 \$41,500 \$26,800	98 13
Doctorate	\$80,000 \$52,000 \$35,000	\$56,700 \$43,550 \$26,300	\$68,700 \$50,000 \$37,200	\$80,000 \$55,000 \$37,200	\$107,240 \$66,000 \$48,000	\$105,500 \$66,128 \$39,500	\$101,000 \$64,500 \$36,000	\$136,000 \$72,000 \$44,000				\$83,550 \$54,126 \$35,920	\$58,640 \$45,000 \$32,600	472 62
Masters	\$68,282 \$43,932 \$31,517	\$58,900 \$36,350 \$25,300	\$60,800 \$41,000 \$31,563	\$65,800 \$49,000 \$34,500	\$77,000 \$50,000 \$30,800							\$71,400 \$45,500 \$32,300	\$51,600 \$40,000 \$28,400	116 15
Batchelors	\$60,000 \$42,000 \$29,700	\$45,000 \$32,400 \$19,819	\$52,000 \$40,000 \$27,480	\$52,700 \$43,000 \$29,240	\$76,200 \$47,000 \$30,070	\$61,990 \$46,600 \$31,800	\$80,800 \$50,265 \$32,800	\$68,796 \$55,777 \$42,100				\$60,560 \$44,000 \$29,300	\$51,311 \$37,968 \$29,360	159 21
Male	\$80,000 \$50,000 \$32,250	\$56,800 \$40,000 \$26,680	\$65,627 \$48,000 \$34,840	\$78,200 \$50,000 \$36,000	\$95,000 \$62,000 \$38,600	\$96,000 \$57,000 \$33,600	\$96,500 \$62,000 \$37,500	\$109,000 \$66,960 \$42,000	\$83,550 \$54,126 \$35,920	\$71,400 \$45,500 \$32,300	\$60,560 \$44,000 \$29,300			644 85
Female	\$56,384 \$41,000 \$30,000	\$46,700 \$36,800 \$25,000	\$57,900 \$40,718 \$28,707	\$57,838 \$41,849 \$32,027					\$58,640 \$45,000 \$32,600	\$51,600 \$40,000 \$28,400	\$51,311 \$37,968 \$29,360			109 14
Number (%)	757 100	133 18	157 21	150 20	102 13	71 9	44 6	32 4	472 62	116 15	159 21	644 85	109 14	

ANALYTICAL UTILITY OF HIGH AND LOW ENERGY COLLISION REGIMES

A Workshop sponsored by the Biomedical Interest Group

Much recent work in tandem mass spectrometry has been performed in laboratories with principal interests in biomedical research. A variety of instrument types have been used, corresponding to substantially different conditions for the collisional activation (CA) of precursor ions. Accordingly, this Workshop was organized to provide a forum for discussion of the analytical implications of the distinctions between different CA regimes. The discussion was led by the following invited participants: Bob Boyd (National Research Council of Canada, Halifax), Simon Gaskell (Baylor College of Medicine, Houston), Don Hunt (University of Virginia, Charlottesville), Jim McCloskey (University of Utah, Salt Lake City) and Rick Yost (University of Florida, Gainesville). Approximately 70 people attended.

Simon Gaskell opened the proceedings with a discussion of the parameters which define CA conditions, not all of which are well understood. The collision energy (laboratory frame of reference, E_{lab}) and the masses of the precursor and target gas determine the maximum energy available (E_{center} , E_{cm}) for conversion to internal energy of the precursor ion, but other factors (such as time scale, multiplicity of collisions and efficiency of collection of scattered ions) are also important. Gaskell showed examples of analyses on a hybrid instrument where the high and low energy regimes are of complementary value. Some fragment types are observed only under high energy conditions.

Bob Boyd presented examples of peptide fragmentations (including 'charge site remote' fragmentations of amino acid side chains) which were observed under high E_{lab} (keV) conditions or (in modest yield) in a quadrupole collision cell with $E_{lab} > 200$ eV. On a reverse geometry hybrid instrument (BEQ), the products of high energy CAD (in the region between B and E) were best observed by simultaneous scanning of E and Q; a superimposed modulation of E facilitates efficient collection of all daughter ions.

Rick Yost described the comparison of daughter ion spectra from a triple quadrupole instrument using argon or nitrogen as collision gas under conditions of equal E_{cm} . The non-equivalence of the spectra reinforced the need to consider other experimental parameters. Yost suggested that the use of multiple collision conditions to increase in a stepwise fashion the internal energy of a selected precursor might be particularly effectively achieved in an ion trap MS. Drawing a contrast with common experience of CAD of positive ions, Yost reviewed data previously reported by Harrison (University of Toronto) which showed greater structural detail from low energy CA of small negative ions than was evident in the high energy CA spectra. The effect was attributed to the prevalence of electron detachment following electronic excitation under high E_{lab} conditions.

Jim McCloskey presented examples of tandem MS analyses of nucleosides at various values of E_{cm} and E_{lab} . The more energetic collisions clearly yielded increased structural information. The analytical utility of constant neutral loss scanning was particularly emphasized (executed in this case on a hybrid instrument).

Don Hunt discussed his analyses of peptides by CAD using a triple quadrupole instrument. The success achieved (albeit with very low resolution of both precursor and product ions) suggested that the limit of application was presently imposed by the mass range of the analyzer rather than by the ability to induce fragmentation. The decomposition mechanisms are certainly imperfectly understood and the apparent effectiveness of low E_{cm} CA of peptides to M_r 2000 requires further study.

In a brief contribution solicited at the time of the Workshop itself, Dick Smith (Batelle Northwest, Richland) described his recent findings on CAD of multiply charged ions (produced by electrospray) using a triple quadrupole instrument. He attributed the success of these experiments largely to the ability to generate "hot" precursor ions in the electrospray interface.

In summary, a number of issues were raised at this Workshop to emphasize the present limitations on our understanding of CA processes. Nevertheless, effective analytical applications are being made to a wide variety of biomedical research problems.

Submitted by Simon J. Gaskell

Report of the Biomedical Interest Group

This report is based on suggestions and comments made to me at the Miami Beach meeting, and, to a lesser extent, those made to me throughout the year.

Those expressing comments concerning the Miami Beach meeting were almost unanimously positive. The facilities were excellent, with sufficient space for posters to allow them to be examined at some leisure. The symposia and plenary lectures seemed to be well accepted and well attended. There were some difficulties with the slide equipment, but these were not of major consequence.

Only one clear suggestion was made for a symposium that would address the integration of mass spectrometry with other analytical techniques such as NMR, IR, etc. for deducing the structure of organic compounds of biological interest. This symposium would have a goal of elucidating the real-world approach to structural problems, for example, that a drug manufacturer might encounter in studies involving new drugs. A number of people greeted this idea for a symposium with enthusiasm.

Members are encouraged to send in additional suggestions for the 1990 meeting, particularly those involving biomedical interest, to the vice president for programs or to the biomedical interest group coordinator. Our group consists of a wide diversity of interests and applications, and our input is vital to the Board in preparing next year's meeting. If you feel there are areas not well represented and which you think are vital to the society, please write and give us your thoughts.

Submitted by
Richard M. Caprioli
Biomedical Interest Group
Coordinator

**A WORKSHOP
SPONSORED BY THE ACADEMIC LAB MANAGERS' INTEREST GROUP
BUDGET BLUES
OR
"WHERE DO I GET THE MONEY TO BUY A NEW MULTIPLIER?"**

Stemming from the results of our survey on the operation of mass spectrometry laboratories, and from several members' involvement in a study conducted by Robert Smith (West Virginia University) on operating cost variables for a mass spectrometry center, a workshop was held on the subject of budgets and the budgeting process. Approximately 50 people were in attendance throughout the 1.25 hour allotted time, with as high as 75 present at some time during the session. The discussion continued well beyond the designated stopping time.

To start the discussion, the question was asked: "How many have a 'formal' budgeting process?" Before any response could be measured, many members of the audience asked for clarification. In the ensuing discussion, it became clear that there were nearly as many different answers to the question of how one goes about this task as there were people in the audience. To provide some specific case studies, three individuals were introduced and asked to describe the structure of their organizations and the considerations they make in establishing a yearly operating budget.

William Howald (Dept. of Medicinal Chemistry, University of Washington) described the organization of his laboratory as a cost center. Having been established in 1979 as a research support lab for Medicinal Chemistry, they soon began providing services to other departments. Upon reaching a total of \$15,000 of extramural service work, the University required the laboratory to become a cost center. An operating budget was established whereby the real costs -- personnel salaries and fringe benefits, operating and maintenance costs, supplies, etc. -- were identified, predictions were made on the amount of work anticipated for a year, and users' fees were set in order to generate the necessary income to cover those costs (present fee \$50/hr, typical 1/2 hr for low res, 1 hr for acc mass, etc.). Further, the expectation was that the cost center account would achieve a zero balance at the end of the fiscal year, with $\pm 5\%$ tolerance. Deficits would be made up by deans and department heads involved, and profits would be given up to the general university fund, if not corrected by the end of the following fiscal year. The laboratory is forced to operate very much as a business, with all the penalties resulting from having lost money, but without the incentive of being able to retain profits to enhance the laboratory. Capital funds for new instrumentation or major upgrades must be generated from separate grants.

Robert Minard (Dept. of Chemistry, The Pennsylvania State University) contrasted his experience involving a remarkably open and organized budgeting process on his local public school board with the closed and disorganized nature of his departmental operating budget. Several years ago the old catch-as-catch-can budgeting process failed, and the Chemistry Department budget fell apart. Upon reorganizing, each of the shops and services supporting departmental activities collected information on past performances and established formal operating budgets for each facility. Since that time, Dr. Minard and others review the situation annually, and establish their best estimate of needs for the mass spectrometry laboratory, dealing mainly with operating expenses. An example, using this year's numbers, is given below. Part of the lab director's salary is covered by teaching duties. The present charge is \$16/hr actual cost to users from all departments. 40% is subsidized by the Department, making a per hour cost of *ca.* \$27/hr. The primary benefit derived by going through this formal process is that one knows ahead of time precisely what resources are available -- e.g. that there will be \$1,500 available for travel.

Dan Jones (Facility for Advanced Instrumentation (FAI), University of California - Davis) reported that the FAI includes significantly more than mass spectrometry services, and was originally started as an electron microscopy facility. It is a recharge center for campus-wide and departmental instruments. Administratively, the Center answers to a Vice Chancellor of the campus. The staff of the facility are all on "soft money" positions. The mass spectrometry staff consists of a lab director, a staff spectroscopist and a graduate student assistant. The director annually proposes a budget to the campus budget office. While the campus provides some subsidy, the facility is expected to generate the remainder of its budget through recharges paid by both on- and off-campus users (presently \$47/hr) or through extramural grants. A separate "insurance" fund provides for major instrument repair costs exceeding \$500 per event. Numbers for FY '89 are given below. The campus, with the FAI caught in the middle, is experiencing a stalemate: while the budget office endorses an increase in users' fees to decrease the deficit, the faculty are opposed to such a rate increase. While the existing facility continues to operate with a large deficit, numerous research groups are finding it easy to obtain funds to set up their own small gc-ms instruments, thus further diminishing the user base for a sophisticated central instrumentation facility. It is unclear how or when this vicious cycle can be broken.

After these presentations, the results of Robert Smith's study were summarized. The premise of Smith's study, using a Delphi analysis method, was to ask whether the structure of an operating budget proposed by Walling *et al.* in 1975 would still be valid for laboratories operating now, and whether new categories should be added. The cost variables list proposed by Walling *et al.* is given below. The values given are averages resulting from their survey

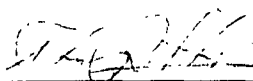
**Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL**

of operating costs for 192 laboratories in 1975, assuming one instrument per laboratory. They found an average \$402,000 start-up cost -- compared with \$392,000 reported by Florey *et al.* (1980) for their laboratory, and \$520,264 for the West Virginia University laboratory (Smith, 1987) -- and an average of \$184,300 per year in operating costs. NIH Biotechnology resources provided \$1.3 million in 1977 to establish eight mass spectrometry centers, with average funding of \$325,000 (1/2 provided by NIH, 1/2 provided by the institution) (Coulter, 1980). Smith found that the validity of most of these cost variables had not changed in the 12 years elapsing, but that several new categories and subcategories should be added to such a model budget. Smith's validated cost variables are given below. Smith quotes a number of observations from the Walling *et al.* report, which are still very appropriate for this discussion.

"Our research produced more questions than answers. There were few firm data on true costs of operating mass spectrometry centers available, and it seemed that there was reluctance on many of the centers to face up to the problem of keeping records showing the categories of such costs."

"The centers and labs investigated...substantially subsidize the cost of the instrumental analysis which they supply. We strongly recommend changes be developed in the funding patterns which will acknowledge these deficits and provide for the true costs of such services."

Interest Group Business Meeting: After the workshop, the following items were discussed. The group was polled for suggestions of Symposium and Plenary Lecture topics and speakers for submission to the ASMS board to plan for future meetings. The group was reminded that this route of communication with the ASMS board is very much alive and functioning. Group members should communicate their suggestions either through the group coordinator or directly to the ASMS board. Suggestions for future activities for this group were: (1) a salary survey of this group to compare with the ASMS general survey reported this year; (2) a discussion of the "job description" of an academic lab manager; (3) a workshop seeking to set up a dialogue with instrument companies to influence their development of new techniques and accessories; (4) a survey of capabilities of commercial analytical laboratories. Members having other ideas or strong feelings about any of these should communicate those feelings to the group coordinator.



Reported by
Thomas R. Sharp
Interest Group Coordinator

Coulter, C.L. (1980). "Biotechnology Resources in Mass Spectrometry," in G.R. Waller & O.C. Dermer, *Biomedical Applications of Mass Spectrometry*. New York: Wiley Intersciences

Florey, D.A., Scott, R.B. Lichtenstein, H.A. (1980). "Everything You Ever Wanted to Know About Setting Up a GC-MS-COM Laboratory -- and Were Afraid Someone Would Tell You." *Am. Chem. Soc. Abstracts*, #SCBP-5, San Francisco, CA, 24-29 August 1980.

Smith, R.R. (1987). "A Study to Validate Operating Cost Variables for a Mass Spectrometry Center within a State Land-Grant University." Master of Arts Thesis, West Virginia University, Morgantown, W. Va.

Walling, C., Grant, D.M., Futrell, J.H., Pugmire, R.J. (1975). "A Study to Improve the Management of Costly Instrumentation Centers." Washington, D.C.: National Science Foundation.

COST VARIABLES LIST Walling <i>et al.</i> (1975)	COST VARIABLES LIST Smith (1987)	PENN STATE MS LAB BUDGET
Mass Spectrometer Cost \$300,000		Salaries (partial) \$66,000
Life Expectancy (years) 7		Supplies & Services 4,200
1. Equipment Costs	1. Equipment Costs	Telephone 1,500
a. Amortization \$42,000	a. Amortization	Travel 1,500
b. Improvement/Update \$12,000	b. Improvement/Update	TOTAL 75,250
2. Personnel Costs \$83,000	c. Maintenance	Desired Income (60%) 45,000
a. Salaries	2. Personnel Costs	
b. Fringe Benefits	a. Salaries	
3. Operating Costs	b. Fringe Benefits	
a. Supplies \$12,000	c. Part-time Salaries	
b. Maintenance \$7,000	d. Administration	
4. Overhead	e. Education	
Hours Operation/week 38	3. Operating Costs	
Hours Operation/year 1850	a. Supplies	
Cost of operation/hour \$111	b. Maintenance	
5. Total Center Cost/year \$184,300	4. Travel Costs	
	5. Subsidization Costs	
	6. Total Center Operation/year	
		UC-Davis FY '89 MS OPERATIONS
		Academic Salaries \$41,100
		Staff Salaries 47,162
		Benefits 28,450
		Supplies 12,780
		Travel 1,000
		Total 130,692
		Subsidy 65,000
		Recharge goal 65,692
		Estimated recharges 38,000
		Net (deficit) 27,692

DESORPTION PROCESSES INTEREST GROUP REPORT

Two workshops were sponsored by this Interest Group. "Ion Damage in Liquid SIMS" was moderated by Frank Field and "High Mass Ions in Quadrupole Mass Spectrometry" was moderated by Peter Todd. Prior to each workshop, questionnaires were distributed to obtain input for next years' meeting. Volunteers were solicited for the chair of the interest group for the next two years.

The workshop on "Ion Damage in Liquid SIMS" was attended by ten people. Brian Bentz initiated the discussion with slides showing various aspects of ion damage. He pointed out that from static SIMS data, the onset of damage becomes apparent in spectra at a dose of 10^{13} to 10^{14} particles per cm^2 . Such a dose corresponds to a time of about one second or less in a typical FAB or liquid SIMS experiment. Uncertainty in the onset arises from the variation with different organic materials. The domain of damage includes not only the usual assorted reactions, but includes charging and primary particle implantation.

Thomas Keough addressed the apparent radical chemistry of glycerol under bombardment from primary ions and from gamma rays. Evidence from GC/MS indicates that the radical reactions initiated in liquid SIMS involved carbon-centered radicals. This is in contrast to reactions initiated by gamma rays in that they appear to involve oxygen-centered radicals.

Ken Busch pointed out that given the complexity of the reactions involved in liquid SIMS, it is possible to find evidence to support almost any kind of reaction. Furthermore, the intensity of ions characteristic of certain kinds of damage may be time dependent. Evidence of ion damage may be suppressed for one sample depending upon additives present in the sample. A good example was hypoxanthine. Reduction is apparent in LSIMS spectra of hypoxanthine/glycerol solutions when p-toluene sulfonic acid is present, but if HCl is substituted for the p-toluene sulfonic acid, no reduction is apparent in the LSIMS spectrum.

Peter Todd addressed the difference between "reversible" and "irreversible damage". The distinction is based upon how the mass spectrum of a solution changes with time and primary ion current density. A reduced primary ion intensity will often lead to an increase in relative abundance of analyte-characteristic ions, an effect that can be reversed by increasing the primary current density. This appears to be due to a combination of surface erosion, analyte damage at the surface, and perhaps sample charging, but in general reflects the competitive effects of the various reactions involved in LSIMS. Cumulative damage is apparent in the generation of new peaks in the mass spectrum, whose intensity continuously increases with time.

The workshop, "High Mass Ions and Quadrupole Mass Spectrometry" was attended by about one hundred fifty persons. Jack Henion outlined the experimental setup for Ion Spray Mass Spectrometry, which is a form of pneumatically assisted electrospray. The technique requires liquid effluent flow through a center capillary and simultaneous "sheath gas" N_2 flow from the annular region between the center capillary and a second coaxial capillary. Flow rates of the effluent is 40 - 50 $\mu\text{L}/\text{min}$ and 250 mL/s . for the sheath gas. By this method droplets of about 5 μm . are formed. A pressure of about 1 ATM is maintained in the source into which the effluent flows. This pressure prevents discharge when the coaxial capillary system is charged to a potential of ± 3000 V. with respect to a slightly off-axis counterelectrode and subsequent skimmer. This part of the system acts as an interface to the mass spectrometer which is maintained at a pressure of 10^{-3} torr. Dry N_2 is introduced as a collision gas between the counterelectrode and the skimmer to reduce clustering. This permits higher flow rates than might otherwise be used. Consequently direct coupling between liquid chromatograph and mass spectrometer is possible using this technique. The system is apparently very easy to construct and permits generation of ions having

molecular weights in excess of 10^4 . It is particularly suitable for biomedical applications. High charge states permit transmission through the quadrupole mass spectrometer. Limitations to the technique include a limited dynamic range in concentration (10^3) and orifice clogging at high concentrations. Furthermore, Jack pointed out that the system has been developed empirically, and a thorough understanding of the effects and causes of variations in operating procedures is lacking.

In the same workshop, Julius Perel outlined his work on improving efficiency in detecting high mass ions in quadrupole mass spectrometry. The thrust of his work involved using a post-acceleration dynode between the quadrupole mass analyzer and the continuous dynode electron multiplier (CDEM). He indicated that sensitivity could be improved by up to a factor of 100 for higher mass ($m/z > 500$) ions using this approach. Further research involving lowering the work function and modifying the ion optics of the dynode - CDEM region promise further improvements.

Submitted by:



Peter J. Todd

ENVIRONMENTAL INTEREST GROUP REPORT

Approximately 155 attendees met on Thursday, May 25, 1989 from 1:00 - 2:15 PM to participate in the annual business meeting and a workshop titled "Environmental Applications of Liquid Chromatography/Mass Spectrometry."

The business meeting consisted of a discussion of symposia topics, plenary speakers, and workshop topics for the 1990 meeting in Tucson. The consensus favored a symposium of invited and contributed papers on "Development of Liquid Chromatography (LC)/Mass Spectrometry (MS) Methods for the Determination of Non-Gas Chromatographable Compounds in Environmental Samples."

The workshop was moderated by W. L. Budde, and consisted of brief opening statements by the discussion leaders Thomas Behymer, Jack Henion, Robert Voyksner, and Larry Slivon. In keeping with the interactive and informal atmosphere of a workshop, a number of those present contributed to the lively discussion, especially Marvin Vestal who presented results from a new design particle beam interface.

Thomas Behymer (EPA, Cincinnati) led the discussion on the carrier effect of less volatile substances and coeluting compounds on more volatile compounds in particle beam interfaces. Most quantitative data available was obtained by the EPA-Cincinnati group using ammonium acetate as the carrier. Much more data is needed to fully document this effect.

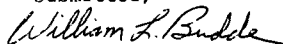
Jack Henion (Cornell University) led the discussion of the ion spray/atmospheric pressure ionization mass spectra of compounds of environmental interest, especially the intractable sulfonated azo dyes.

Robert Voyksner (Research Triangle Institute) led the discussion of techniques for the optimization of signal intensity in particle beam interfaces. This discussion included comparisons of spectra obtained using electron ionization, positive and negative chemical ionization, and thermospray. As always, some ionization techniques work better with some compounds than others.

Larry Slivon (Battelle Columbus Laboratories) described a new design LC/MS interface, and showed results of measurements of caffeine by direct LC injections of 5:1 aqueous dilutions of Diet Coke, Caffeine-Free Diet Coke, Dr. Pepper, and Battelle vending machine coffee.

One conclusion of the workshop was that there is a need for far more mass spectrometric data on compounds after separation with an LC column, and more quantitative information including precision, accuracy, detection limits, calibration stability, etc.

Submitted,


William L. Budde

FUNDAMENTALS OF PEPTIDE SEQUENCING BY MASS SPECTROMETRY

Pharmacology Interest Group Workshop Report

This workshop considered general aspects of peptide sequencing by mass spectrometry and was aimed at those scientists not necessarily highly experienced in this rapidly evolving area. The workshop was well attended and the audience posed numerous questions to the speakers. At this meeting it was also announced that at a meeting of the interest group chairmen and the Vice President for Programs, the decision was made that since the subject matter encompassed by the Pharmacology Interest Group, formerly Quantitative Organic Analysis, directly overlaps with the subject matter of the Biomedical Interest Group, formerly Qualitative Organic Analysis, these groups will be combined in the future and will be called the Biomedical Interest Group. Suggestions for the content of next year's ASMS Conference were solicited. Speakers were Steve Martin of Genetics Institute, Donald Hunt of The University of Virginia, Terry Lee of Beckman Research Institute and Frank Crow of The Upjohn Company.

Steve Martin presented an overview of the role of MS in protein sequencing and how this technology fits into the overall scheme of elucidating protein structure and complements other more traditional techniques. The most important advantage MS offers over other techniques is the ability to detect posttranslational modifications (e.g., N-acetylation, O-sulfation, methylation, etc.). Other advantages include the ability to analyze mixtures, rapid acquisition of data, sensitivity, and lack of ambiguity in data interpretation. Dr. Martin noted that when using the four-sector tandem instrument at M.I.T., usually 1 nmole was required to obtain useful sequence information but as little as 10 pmole has been observed to be sufficient.

Don Hunt described the procedure used in his laboratory for obtaining sequence information using a triple quadrupole tandem instrument. Usually 0.5 to 2 nmole of starting material was digested and fractionated using microbore HPLC. Half of the sample was propylated or methyl esters were formed (to facilitate spectral interpretation). It was stated that mass spectral analysis was usually completed in about 3 hours. Data identifying MH^+ was obtained with between 10-50 pmole and CAD spectra were obtained on 10-200 pmole. Manual interpretation of the data required between 2 to 3 days and typically 40-70% of the amino acid sequence for a 50k dalton protein was obtained in 4-5 days. Dr. Hunt stated that, in general, handling of the sample has been observed to be more problematic than the analysis. Other specific aspects of the methodology employed at the University of Virginia included a 6 kV cesium ion gun, a small amount of thioglycerol (enough to last approximately 40 seconds) on a gold plated probe, parent ion resolution of between 3-10 daltons, 2 mtorr of argon: xenon (2:1, approximately 10 collisions), collision energies between 15 and 40 eV (higher energies at higher mass), tuning of Q3 at less than unit resolution and scanning at 300 amu/sec, and the use of a 15 kV conversion dynode.

Terry Lee described methodology for the determination of peptide sequences using a double focusing sector instrument. If sample supply is not limited then complete sequence information has been obtained using this approach. Using B/E linked scans and background subtraction of the glycerol/thioglycerol matrix spectrum, sequence information was obtained on approximately 200 pmole peptide. This approach was observed to work well without the use of CAD up to 10 amino acid residues as a result of metastable decompositions. In general this methodology has been observed to be less sensitive than when using triple quadrupole or four-sector mass spectrometers.

**Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL**

Frank Crow presented an overview of investigations of the utility of TSP-MS and TSP-MS-MS in peptide sequence determination that he and Russel Robins have carried out over the past few years. Problems that were encountered initially included poor sensitivity, greatly attenuated response at high masses, and unstable source conditions. Several modifications to the standard manufactures TSP ion source aimed at increasing high mass response were made. These included using a needle repeller positioned approximately one mm directly behind the sampling cone, tightening of the source which allowed for higher flow rates, decreasing the size of the jet orifice, and use of a postcolumn cross gradient to stabilize ion source conditions. It was stated that this cross gradient (usually 75% aqueous, total flow of 3 ml/min) has been observed to be important for the successful analysis of peptide mixtures by TSP-MS. The mass spectrum obtained following TSP-MS-MS analysis of a doubly charged peptide ($MH_2^{++} = 1042$) was discussed and it was stated that this approach could allow for the MS-MS analysis of higher molecular weight peptides.

Respectfully submitted,

James Vrbanac
The Upjohn Company

HISTORICAL INTEREST GROUP REPORT — June 1989

The Historical Interest Group organized the *Symposium on the Historical Development of Instrumentation in Mass Spectrometry* for the 1989 Meeting in Miami Beach. A morning session covered four broad areas of development: 1) Combined Inlet Systems for Mass Spectrometry, 2) Alternative Ionization Techniques, 3) The Quadrupole Mass Filter, and 4) Computers in Mass Spectrometry with one oral presentation on each topic. The afternoon featured a poster session of nine authors, highlighting selected portions of the general topics outlined above. The poster "*Mass Spectrometers in Space, Rockets to Satellites to Mars: 1960-1976*" was presented, even though it had been inadvertently omitted from the final program. Poster authors were present with their work throughout Tuesday afternoon, but the posters remained in place through Thursday afternoon to provide interested parties an adequate opportunity to view them. Poster authors were asked to contribute their posters to the Historical Interest group for archival storage and use at a later date.

A meeting of the Interest Group was held Tuesday evening and ideas for the 1990 program were formulated. Suggestions for plenary speakers and workshop topics have been forwarded to the Vice-President for Programs. It was decided not to organize a symposium for the coming year.

A number of activities occurred during the year:

Center for the History of Chemistry :

ASMS is in the process of formally affiliating with the Beckman Center for the History of Chemistry in Philadelphia. Approval of affiliation was granted at the ASMS Board meeting in Miami Beach prior to the conference. The Historical Interest Group Chairman will be the focal point for interaction between the Society and the Center; and Member-at-Large, Mike Story, has been appointed liaison with the ASMS Board for the coming year.

Affiliation with the Center provides three resources to ASMS:

- A proper, permanent archival facility for papers and small physical items.
- A professional resource to consult with Interest Group members on the details of collecting papers and objects of historical value.
- Ready access to the Center's growing collection of materials for our members.

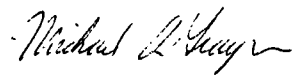
To all of our members who have papers, photos, manuals, data, literature or other items of historical value which they are considering donating to the Society, I encourage you contact me and do so now. You can dismiss any uncertainty which you may have had about the ultimate fate of such donations. If you are in the Philadelphia area, I encourage you to stop and visit the Center. It should be remembered that the Center represents a facility primarily for the collection of PAPERS and not EQUIPMENT.

Other ongoing projects during the past year are :

- Continuation of the 'Roots' survey of members.
- The collection of oral histories from:
 - John Beynon
 - O. P. Tanner
 - Al Nier
 - Bill Sherman
- The collection of photos of historical value.
- Consultation with the Smithsonian on the acquisition of a mass spectrometer for their collection.

A nucleus of interested individuals is at the heart of the Historical Interest Group. The activities enumerated above should continue throughout the coming year. Particularly, the collection of materials suitable for archival placement with the Center for the History of Chemistry should have a high priority among Interest Group members.

Respectfully submitted,



Michael A. Grayson
Chairman, Historical Interest Group
McDonnell Douglas Research Laboratories (314) 233-2541

WORKSHOP: "INSTRUMENT-INDEPENDENT CAD DATABASE"

(Attendance: ca. 80)

ORGANIZER: Richard I. Martinez, National Institute of Standards and Technology
(NIST; formerly National Bureau of Standards)

SPONSOR: Measurements and Standards Committee of ASMS.

SUMMARY:

The purpose of this Workshop was to assess whether or not the MS/MS community wants (and/or needs) a standardized, instrument-independent CAD database, and, if so, to discuss what standardized operating conditions and database format(s) one should use. The consensus of the participants who submitted written comments for this report can be summarized as follows: (a) it is important to generate instrument-independent spectra; (b) it is desirable to have a database of standard CAD spectra; and (c) the participants would contribute standard CAD spectra for inclusion in a NIST database.

Standardized, instrument-independent CAD spectra can be generated on XQQ (QQQ, BEQQ, etc.) tandem mass spectrometers by using the kinetics-based measurement protocol developed at NIST (1).¹ This protocol requires that measurements be made under single-collision conditions. Some workshop participants indicated they would be willing to use the single-collision conditions specified in the NIST protocol to generate standard spectra. However, most participants would prefer to explore the development of a protocol for generating standard CAD spectra under multiple-collision conditions. This latter approach would necessitate the participation of the MS/MS community in an additional round robin to explore the feasibility (or lack thereof) of measuring standardized, instrument-independent CAD spectra under multiple-collision conditions. The workshop participants indicated they were willing to participate in such a NIST-coordinated round robin.

PROCEEDINGS:

To provide a focus for the Workshop discussion, R.I. Martinez (NIST) gave an introductory presentation in which he:

- (a) reviewed the dynamical prerequisites (2) which must be observed to develop an undistorted, dynamically-correct (i.e., instrument-independent) MS/MS database (library) for XQQ instruments;²
- (b) reviewed the technical basis of the NIST kinetics-based measurement protocol (1) that has been proposed for the development of a standardized CAD database comprised of Characteristic Branching Ratios of Ionic Substructures (CBRIS Database Format (1));³
- (c) reported the results of the NIST-EPA International Round Robin for XQQ tandem mass spectrometers (>50% of QQQ instruments currently in the field can provide instrument-independent response (3), so a standardized CAD database is feasible);
- (d) presented data for the CAD of the acetone cation (m/z 58) under single-collision and multiple-collision conditions which indicated that single-collision conditions provide a unique MS/MS fingerprint characteristic of acetone, while multiple-collision conditions distort the structural information that is specific to a particular ion; and
- (e) proposed that the protocol for the second NIST round robin should consist of measuring four sets of branching ratios for the CAD of the acetone cation (m/z 58): viz., two fixed target thicknesses (corresponding to both single-collision and multiple-collision conditions) at two LAB energies (10 and 80 eV).

The participants then expressed their views (see SUMMARY). There was some confusion about the concept of a database of substructures (e.g., the CBRIS database) vs. a library of CAD spectra for source compounds (see Note 3(b)). One participant pointed out the imbalance between instrument time and analyst time when one is characterizing unknowns⁴ (viz., significantly less than 50% of instrument time is used for acquisition of the CAD spectra of unknowns, while significantly more than 50% of the analyst's time is devoted to interpretation of the CAD spectra of unknowns), and suggested that a CAD database may help to improve the correlation of analyst time to instrument time.

NOTES:

- ¹ The precepts of the NIST protocol (1) are also applicable to other types of tandem mass spectrometers which have strong focusing properties (e.g., quadrupole-hexapole-quadrupole MS/MS), so long as the energy range used for the pertinent ion chemistry is the same as for XQQ instruments.
- ² Unless the reaction dynamics of the CAD process are taken into account, results in XQQ instruments will be highly instrument dependent, even though reproducible conditions can be established for analytical purposes (with few exceptions, measurements for any given ion within any one XQQ instrument exhibit long-term reproducibility (precision) to within $\pm 10\%$; one laboratory reported this precision for 3 different XQQ instruments (QQQ, BEQQ, EBQQ)).
- ³ The main advantages of the CBRIS database format are:
 - (a) The proposed use of CBRIS in MS/MS is analogous to the use of group frequencies in infrared spectroscopy. That is, the partial cross sections for production of progeny fragment ions can characterize both known and unknown species (so long as the unknown species contain ionic substructures for which the CAD cross sections and product identities are known). Therefore, it may be possible to assign the structure of an unknown species on the basis of the absolute cross sections for the CAD of known ionic substructures.
 - (b) Characterization of an unknown compound by using CBRIS does not require that the compound be in the CBRIS database. By contrast, to characterize an unknown by spectral matching within a "library", the compound must usually be in the "library". In this regard, development of a database of CBRIS for all substructures (or a subset thereof) would be very much more tractable than the development of a library of CAD spectra for all the source compounds that contain all the substructures (or a subset thereof). For example, consider the simplistic analogy where compounds correspond to words, and substructures correspond to letters: more than 500,000 words can be composed with only 26 letters of the alphabet.
- ⁴ Major classes of MS/MS experiments include: (i) Mixture Analysis (quantitation with or without prior separation) and (ii) Structure Elucidation ("isolated" neat compound (prior separation)). For (i), precision within any one XQQ instrument is critical, and the goal is to maximize sensitivity for characteristic daughter ions; "standardized" conditions may be useful, but not necessary (different "standardized" conditions are needed for different substructure classes to compensate for differences in CAD dynamics). For (ii), the goal is to maximize structurally-significant information.

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- (3) R.I. Martinez, Rapid Commun. Mass Spectrom. 3, 127 (1989).

**INSTRUMENTATION AND ION PHYSICS INTEREST GROUP
MEETING AND WORKSHOP REPORT**

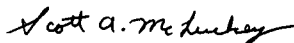
The Instrumentation and Ion Physics Interest Group held a meeting and a workshop on Monday, May 22 from 1:00-2:15 P.M. Roughly one hundred people attended. The meeting portion of the program was used to solicit ideas for symposia, workshops, etc. for next year's program. Few suggestions were made during the meeting since it was held very early in this year's conference. A number of suggestions were submitted, however, as the week wore on. Some of the topics, which may be appropriate for a review lecture, symposium, or workshop included:

- Mobile mass spectrometers
- Ion sources for quantitative mass spectrometry
- High performance detectors
- State-of-the-art in sector technology
- Pressure measurement

Other suggestions were made which were more appropriately classified with other interest groups. All of the suggestions have been forwarded to the Board for consideration.

The workshop portion of the program was entitled "Detectors for Mass Spectrometry". The interest in the topic was reflected in the attendance. Four speakers were asked to participate by giving a brief description of the newest developments in detectors. Ken Schmidt of K+M Electronics described ceramic multipliers that can withstand high temperatures (several hundred °C) and discussed ways to evaluate detector performance. Julius Perel of Phrasor Scientific discussed their work with improving detection efficiency for high mass ions by use of low work function dynode materials and high voltages. Peter Graves of Detector Technology discussed several approaches to improving the lifetime of multipliers including segmented multipliers and multipliers with large area at the output end. He also described an approach to evaluating multiplier performance. John Hooper of Galileo Electro-Optics reviewed the detector requirements for a variety of mass spectrometry applications and described their efforts to produce detectors to meet each set of needs. The presentations were highly informative and generated more discussion than could be accommodated within the allotted time for the meeting. It was evident from some of the feedback from this workshop that many of our members (myself included) understand other aspects of mass spectrometry better than we understand multipliers. For this reason, a session on detectors may be warranted in the near future.

Respectfully submitted,



Scott A. McLuckey
Oak Ridge National Laboratory

Presented at the 37th ASMS Conference on Mass Spectrometry and Allied Topics
May 21-26, 1989, Miami Beach, FL

LASER DESORPTION OF BIOMOLECULES. HOW DOES IT WORK?

A Workshop Sponsored by the Physical Organic Interest Group.

This workshop was organized to consider the mechanisms involved in the laser desorption and ionization of biomolecules. Approximately 100 people attended the workshop which was held Tuesday evening, May 23, 1989.

Prior to the workshop a short business meeting was held of the Physical Organic Interest group. Suggestions for the 1990 meeting for symposia, included laser desorption, ionization, and photodissociation of biomolecules. The attendees were also polled regarding their feelings on the balance of oral versus poster presentations and on the program in general. The level of satisfaction was high on all counts.

Six panelists gave short presentations of various aspects of the laser desorption mass spectrometry of biomolecules.

Franz Hillenkamp considered possible mechanisms which could explain why his technique of matrix (nicotinic acid) assisted UV ($\lambda=266$ nm) laser desorption allows for the desorption and ionization of proteins with molecular weights in excess of 100,000u.

Ronald Beavis also discussed experiments on matrix assisted UV laser desorption and ionization wherein investigations were made of the types of matrices which yield intense spectra and of the sources of spectral peak broadening.

John Amster described experiments performed in a Fourier transform mass spectrometer in which organic molecules were desorbed by UV laser irradiation and were subsequently ionized by either EI or CI.

Kenneth Standing discussed results obtained in a time-of-flight analyzer using low irradiance UV laser pulses to produce ions from peptides in a regime where the ion yield exhibited a slow dependence on the irradiance.

Robert Cotter described results obtained on oligosaccharides and glycoconjugates using infrared laser desorption and ionization wherein the time evolution of the generated ions was investigated.

Peter Williams described experiments in which DNA fragments with molecular weights up to 1,000,000u were laser desorbed from a frozen water matrix and transferred to a catcher foil.

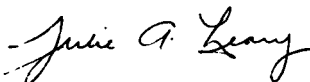
Submitted by:
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YOUNG MASS SPECTROMETRISTS INTEREST GROUP REPORT

This year's topic of discussion at the YMS Interest Group Meeting was on Careers in Mass Spectrometry. Michael Gross, Gary Glish, Barbara Larsen, and Julie Leary each gave a brief presentation and made suggestions to the audience on criteria which should be examined when making career choices. Through audience and speaker participation, several important observations were made: 1) If one really enjoys the job and is motivated, he or she will work the number of hours required to bring satisfactory results i.e.; those in industry have a tendency to work as hard and long as those in academia. 2) In most instances financial rewards were not as important as the type of work and benefits offered. 3) A Post-doctoral position was encouraged if time and finances were not pressing burdens. 4) Of those attending (approximately 200), more than 75% were graduate students and post-doctoral associates.

Suggestions for next year's meeting included how to write specifications for a mass spectrometer, a combined historical group and YMS group meeting, and a tutorial on how to obtain research funding.

Submitted by,

A handwritten signature in cursive script, reading "Julie A. Leary". The signature is written in dark ink and is positioned above the printed name.

Julie A. Leary

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