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INTRODUCTION

This "Bound Volume" of abstracts, which appears annually, is the official record of the American Society for Mass Spectrometry, containing not only the abstracts of papers presented at the conference, but also reports of workshops and of standing and ad hoc committees, special reports as appropriate, minutes of the annual business meeting and other information of interest to the membership.

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LINKED SCANS

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Introduction

The recent increased use of spectra arising from the collision-induced decomposition of ions both for the characterisation of components in mixtures introduced directly into the mass spectrometer and for the investigation of ion structures has led to the development of several linked scans during the past decade.

In a magnetic deflection instrument of either forward or reverse geometry, the parameters of importance are the accelerating voltage V , the electric sector field strength E and the magnetic sector field strength B . In a linked scan, two of the three parameters are scanned in such a manner that a fixed relationship is maintained between them throughout the scan, the third parameter remaining constant. In the following discussion, unless otherwise stated, it will be assumed that the ions are singly-charged and that the release of internal energy as translational energy of the fragments can be neglected.

Acceleration

An ion of mass m and charge e when subjected to an accelerating voltage V acquires a velocity v and a translational energy given by

$$Ve = mv^2/2 \quad (1)$$

$$v = (2Ve/m)^{1/2} \quad (2)$$

$$mv = (2Vem)^{1/2} \quad (3)$$

From eqn. (3), it is seen that at constant V , the momentum given to ions is proportional to $m^{1/2}$.

The Electric Sector

An ion which has been subjected to an accelerating voltage V will describe a circular path of radius r when subjected to an electric field of strength E perpendicular to the direction of ion motion, where

$$2V/E = r = 2Ve/Ee \quad (4)$$

$$\text{Because } Ve = mv^2/2 \quad (1)$$

$$\text{so that } r = mv^2/Ee \quad (5)$$

$$\text{and } E = mv^2/re \quad (6)$$

From these equations, one can see that

- (i) $E \propto mv^2/2$ so that an electric sector acts as an energy separator
- (ii) $E \propto v^2$ for ions of constant m
- (iii) $E \propto m$ for ions of constant v
- (iv) E is independent of m for ions subjected to a constant accelerating voltage V .

Magnetic Sector

Ions of mass m , charge e and velocity v , when subjected to a magnetic field of strength B perpendicular to the direction of ion motion will describe a circular path of radius R where

$$Bev = mv^2/R \quad (7)$$

$$\text{so that } mv = BRE \quad (8)$$

$$\text{and } B = mv/Re \quad (9)$$

From these equations, one can see that

- (i) $B \propto mv$ so that a magnetic sector is a momentum separator
- (ii) $B \propto v$ for ions of constant m
- (iii) $B \propto m$ for ions of constant v
- (iv) $B \propto m^{1/2}$ for ions subjected to a constant accelerating voltage V .

Normal Operation of a Magnetic Deflection Instrument

After acceleration through an accelerating voltage V , the momentum of an ion is given by eqn. (3) and the momentum of ions transmitted by a magnetic sector of field strength B is given by equation (8). Equating momenta,

$$(2Vem)^{\frac{1}{2}} = BRe \quad (10)$$

$$\text{so that } m/e = B^2 R^2 / 2V \quad (11)$$

With V fixed, a scan of B brings ions of different mass-to-charge ratio to focus at the collector slit whenever eqn. (11) is satisfied. Because the magnetic field acts as a momentum separator and from eqn. (3), momentum $\propto m^{\frac{1}{2}}$ at constant V , the mass scale in the normal mass spectrum is proportional to $m^{\frac{1}{2}}$.

In a single-focussing instrument or in a double-focussing instrument of forward geometry, ions which fragment in the field-free region before the magnetic sector are transmitted by the magnetic sector under appropriate conditions. Let the fragmentation be represented by

$$m_1^+ + m_2^+ + m_n \quad (\text{All have velocity } v_1)$$

Let the daughter ions of momentum $m_2 v_1$ be transmitted when $B = B^*$ such that

$$m_2 v_1 = B^* Re \quad (12)$$

and let m^* be the mass of hypothetical ions formed in the source which are collected at $B = B^*$. Both m_2^+ ions and m^* ions will have the same momentum so that

$$m_2 v_1 = m^* v^* = (2Vem^*)^{\frac{1}{2}} \quad (13)$$

$$m_2^2 v_1^2 = m^{*2} v^{*2} = 2Vem^* \quad (14)$$

But from eqn. (1), $Ve = m_1 v_1^2 / 2$ so that

$$m_2^2 v_1^2 = 2m^* (m_1 v_1^2 / 2) = m^* m_1 v_1^2 \quad (15)$$

$$\text{Hence } m_2^2 / m_1 = m^* \quad (16)$$

Peaks arising from the collection of m_2^+ ions formed in this way are usually of low intensity, often at non-integral mass numbers and are usually broadened by the release of energy during fragmentation.

The V-Scan

In forward geometry instruments, m_2^+ ions formed in the field-free region between the source and electric sector are normally not transmitted since the electric sector is set to transmit ions formed in the source and having energy $V_1 e$ (where V_1 is the normal accelerating voltage) and the energy of the m_2^+ ions is $(m_2/m_1)V_1 e$.

These m_2^+ ions may be collected if B is first adjusted to allow collection of m_2^+ ions formed in the source and the accelerating voltage V is scanned upwards from V_1 . Whenever the relationship

$$V_2/V_1 = m_1/m_2 \quad (17)$$

holds, m_2^+ ions formed from m_1^+ ions will be transmitted when $V = V_2$, since the energy of m_2^+ is $(m_2/m_1)V_2e = V_1e$.

Note that the spectrum is given by collection of m_2^+ ions (constant mass) having energy V_1e (constant energy) so that the spectrum is really a translational energy spectrum of m_1^+ ions which fragment to give m_2^+ ions of energy V_1e .

The E-Scan

In a reverse geometry instrument, m_2^+ ions formed in the field-free region between the electric and magnetic sectors are not normally transmitted since the electric sector field-strength E_1 is set to transmit ions formed in the source and having energy V_1e . The energy of m_2^+ ions formed from m_1^+ ions is $(m_2/m_1)V_1e$ and these may be collected if B is set to allow transmission of m_1^+ ions and the electric sector field-strength is scanned downwards from E_1 . The m_2^+ ions are transmitted whenever the relationship

$$E_1/E_2 = m_1/m_2 \quad (18)$$

holds. In this scan, ions of different masses and energies are collected, giving a translational energy spectrum of m_2^+ formed from a chosen m_1^+ ion.

In both the V-Scan and the E-Scan, energy release broadens the peak, reducing the accuracy with which m_1^+ and m_2^+ , respectively, may be assigned. It was for this reason that the first linked scan was suggested in 1973. Since then, a total of six linked scans have been described, the main features of which are summarised as follows:

Scan	Information obtained	Peak Width
V/E^2	All m_2^+ from given m_1^+	Narrow (a)
B/E	All m_2^+ from given m_1^+	Narrow
B^2/E	All m_1^+ giving chosen m_2^+	Broad
B^2/E	All m_1^+ giving chosen m_2^+	Narrow (b)
$B^2(1-E)/E^2$	All m_2^+ given by loss of chosen m_n	Narrow

(a) Forward Geometry only (b) Reverse Geometry only.

The underlying theory of each of these scans is given below after which the reasons for the different peak widths are discussed.

The V/E^2 Scan

Let V_1 , E_1 and B_1 specify conditions under which m_1^+ ions are collected. Then by rearranging eqn. (11),

$$mV = R^2 B^2 e / 2 = \text{constant for fixed } B \quad (19)$$

$$\text{or } m.Ve = m \times \text{Energy} = \text{constant} \quad (20)$$

Since E is proportional to the energy of the ions transmitted by the electric sector then from (20)

$mE = \text{constant for } m^+ \text{ ions to be transmitted at fixed } B$, irrespective of whether or not m^+ is formed in the ion source. Hence a scan of E is linear in $1/m$.

Suppose that V_2 is the accelerating voltage which must be applied to m_1^+ ions so that after decomposition, m_2^+ ions are transmitted when $E = E_2$. The energy of these ions is $(m_2/m_1)V_2e = (E_1/E_2)V_2e$ and for them to be transmitted by the electric sector, from eqn. (4):

$$2(E_1/E_2)V_2e/r = eE_2 \quad (21)$$

$$\text{or } V_2/E_2^2 = r/2E_1 = \text{constant} \quad (22)$$

Hence if V and E are scanned such that V/E^2 is constant throughout the scan, all m_2^+ ions formed from the chosen m_1^+ ions are collected. The scan is shown schematically in Fig. 1. The disadvantage of this scan is the wide range of V that must be used, causing a large variation in source extraction efficiency.

The B/E Scan

If $m_1^+ + m_2^+ + m_n$ in the field free region between the source and the first sector in an instrument of either forward or reverse geometry and V_1 , E_1 and B_1 specify conditions under which m_1^+ ions are transmitted,

$$\text{Energy of } m_2^+ \text{ ions} = (m_2/m_1)V_1e \quad (23)$$

$$\text{Momentum of } m_2^+ \text{ ions} = (m_2/m_1).m_1v_1 \quad (24)$$

where v_1 = velocity of m_1^+ , m_2^+ and m_n .

In order that m_2^+ ions should be collected, E is adjusted to E_2 such that

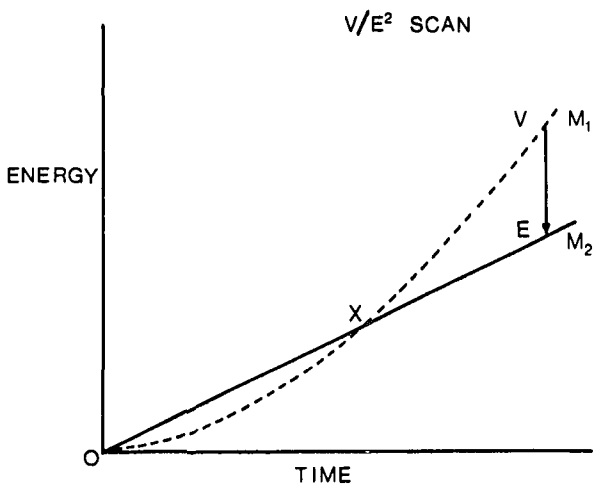


Fig. 1. The V/E^2 Scan indicating the energy loss necessary to allow observation of m_2^+ ions formed from m_1^+ ions between the source and electric sector of a forward geometry instrument.

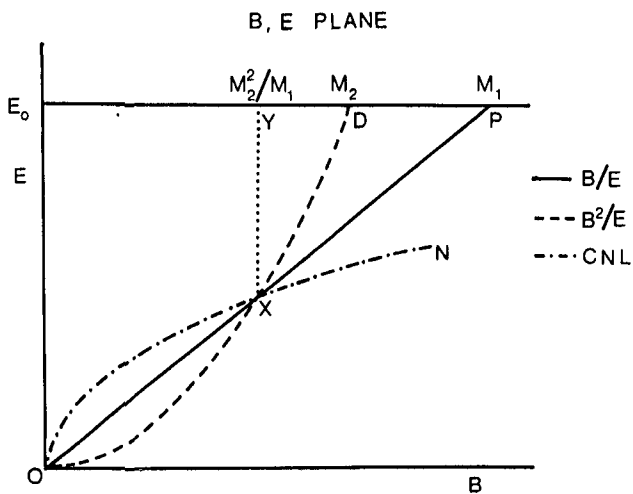


Fig. 2. The B,E plane showing the relationship between the three linked scans used with forward and reverse geometry instruments.

$$E_1/E_2 = m_1/m_2 \quad (E \propto m \text{ at constant velocity}) \quad (25)$$

Similarly B is adjusted to B_2 such that

$$B_1/B_2 = m_1/m_2 \quad (B \propto m \text{ at constant velocity}) \quad (26)$$

From these two equations,

$$B_1/B_2 = E_1/E_2$$

or $B/E = \text{constant}$ determined by the choice of m_1^+ . Hence a scan of B and E such that B/E is constant throughout the scan gives all m_2^+ ions formed from a chosen m_1^+ ion. Since there are no problems of variation of source extraction efficiency, this scan has largely replaced the V/E^2 scan.

The B^2/E Scan

If the same decomposition is considered, eqn. (23) and (24) can be rewritten as

$$\text{Energy of } m_2^+ \text{ ions} = (m_2/m_1)V_1e = m_2v_1^2/2 \quad (27)$$

$$\text{Momentum of } m_2^+ \text{ ions} = (m_2/m_1) \cdot m_1v_1 = m_2v_1 \quad (28)$$

For m_2^+ ions to be collected,

$$\text{Electric Sector: } m_2v_1^2/r = E_2e \quad (29)$$

$$\text{Magnetic Sector: } m_2v_1 = B_2Re \quad (30)$$

When combined, these give

$$B_2^2/E_2 = (m_2/e)(r/R^2) \quad (31)$$

$$= \text{constant determined by choice of } m_2^+.$$

Hence a scan of B and E such that B^2/E remains constant throughout the scan gives all m_1^+ ions which fragment to give a chosen m_2^+ ion.

The Constant Neutral Loss Scan

Whereas the two previous scans allowed one to select m_1^+ or m_2^+ ions for study, this scan allows one to specify m_n and to collect only those m_2^+ ions formed in fragmentations in which the specified neutral mass is lost.

Let V_1 , E_1 and B_1 specify conditions under which m_1^+ ions of velocity v_1 are collected. Eqn. (25) can be rewritten in the form

$$E_2/E_1 = E' = m_2/m_1 = (m_1 - m_n)/m_1 = 1 - m_n/m_1 \quad (32)$$

$$\text{so that } m_1 = m_n/(1 - E') \quad (33)$$

The condition for transmission of m_2^+ ions by the magnetic sector is given

by eqn. (30):

$$m_2 v_1 = B_2 R e \quad (30)$$

Let $m^* = m_2^2/m_1$ = mass of ions formed in the source collected when $B = B_2$.

$$\text{Then} \quad m^* = m_2 (m_2/m_1) = m_2 E' = (m_1 - m_n) E' \quad (34)$$

Substituting for m_1 from eqn. (33) gives

$$m^* = \{m_n/(1-E') - m_n\} E' = m_n E'^2/(1-E') \quad (35)$$

Remembering that $m^*/e = R^2 B_2^2/2V$, this can be rearranged to give

$$\begin{aligned} B_2^2 (1-E')/E'^2 &= (2V_1 e/R^2) m_n \\ &= \text{constant for a chosen value of } m_n \end{aligned} \quad (36)$$

Hence if B and E are scanned such that eqn. (36) is satisfied throughout the scan, m_2^+ ions are collected which have been formed by the loss of the chosen neutral mass m_n .

The relationship between the B/E , B^2/E and Constant Neutral Loss (CNL) scans is illustrated in Fig.2. These three scans can be used on both forward and reverse geometry instruments.

The B^2E Scan

This scan is used on reverse geometry instruments to collect m_2^+ ions of chosen mass from all m_1^+ ions in decompositions which occur between the two sectors. It differs from other scans in that the two sectors are scanned in opposite senses.

Let m_2^+ ions formed in the source be collected under conditions specified by V_1 , E_1 and B_1 where $B_1 = k_1 m_2^{1/2}$ and $E_1 = k_2 V_1$, k_1 and k_2 being constants. Similarly, for m_1^+ ions formed in the source collected at B_2 , $B_2 = k_1 m_1^{1/2}$.

Now suppose $m_1^+ \rightarrow m_2^+ + m_n$ between the sectors and let m_2^+ ions formed in this reaction be transmitted by the electric sector at $E = E_2$. From eqn.

(18),

$$E_2 = (m_2/m_1) E_1 = (m_2/m_1) k_2 V_1 \quad (37)$$

From the above relationships,

$$B_1^2 E_1 = (k_1^2 k_2 V_1) \cdot m_2$$

$$B_2^2 E_2 = k_1^2 m_1 (m_2/m_1) k_2 V_1 = (k_1^2 k_2 V_1) \cdot m_2$$

Hence B^2E is a constant determined by the choice of m_2^+ and independent of m_1^+ . If the instrument is set up so that m_2^+ ions formed in the source are

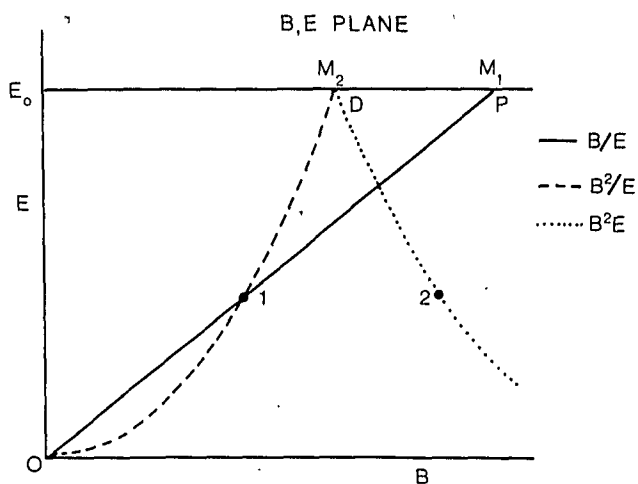


Fig. 3. The B,E plane showing the relationship of the B^2E scan to the B/E and B^2/E scans.

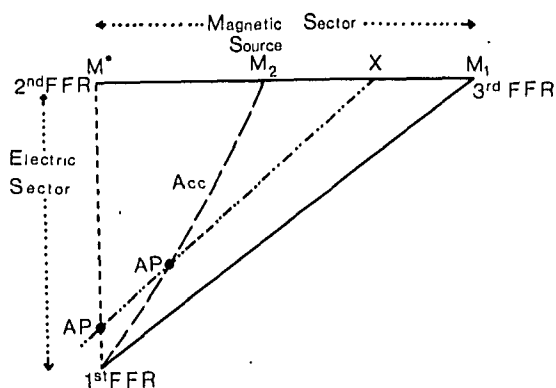


Fig. 4. An indication of the origin of some of the artefact peaks in linked scans carried out on a forward geometry instrument.

collected and B is scanned upwards and E downwards such that B^2/E is constant throughout the scan, m_2^+ ions formed from all m_1^+ in decomposition between the sectors are collected. Fig. 3 illustrates the relationship of this scan to other linked scans.

Resolving Power

All the above equations have assumed that no internal energy is released as translational energy of the fragments so that the velocities of m_2^+ and m_n are the same as that of m_1^+ . When such energy release occurs, it gives rise to a spread in the velocity of m_2^+ and hence in the energy and momentum of m_2^+ . This leads to the broadening of peaks due to m_2^+ ions formed in this way in spectra obtained by the scanning of a single parameter V, E or B. The width and shape of the peaks give information on the magnitude and distribution of the energy release.

In a linked scan, similar broad peaks are observed only if the two sectors are scanned such that m_2^+ ions transmitted by each sector have identical velocities at all times. Such peaks are observed in the B^2/E scan since $B^2 \propto v^2$ and $E \propto v^2$. Consequently, the resolving power with which m_1^+ may be identified is typically 50 - 100. In the B/E scan, the peaks are much narrower since $B \propto v$ and $E \propto v^2$ so that the resolving power with which m_2^+ ions may be identified is typically 200 - 600 although the ability to specify m_1^+ is typically only about 1 part in 300. Both the V/E^2 and constant neutral loss scans give similarly narrow peaks but because the sectors are scanned in opposite senses, the B^2/E scan gives very narrow peaks. Although the resolving power for m_1^+ is typically 3000, that for m_2^+ is normally no more than 100.

Artefact Peaks

Artefact peaks are given by all linked scans but vary with the type of scan and the instrument geometry. They are usually of low intensity and of different shape from normal peaks and often occur at non-integral masses. They normally cause little difficulty especially if an alternative scan is used to check an assignment.

Sources of artefact peaks include

1. Decompositions occurring in the accelerating region.
2. Decompositions within the electric sector.
3. Tails of broad peaks due to energy release given by $(m \pm 1)^+ + m_2^+$ or $m_1^+ + (m_2 \pm 1)^+$.
4. Successive decompositions in the first and second field-free regions.

The origins of artefact peaks of the first two types are illustrated in Fig. 4 for a forward geometry instrument. Any m_2^+ ions formed during acceleration give rise to a continuum of intensity (labelled Acc) and any decompositions occurring within the electric sector give rise to a continuum between the points 1st FFR and 2nd FFR. Usually, if more than a hydrogen atom or molecule is lost, the intensity falls to zero in the centre part of the continuum. Now consider a B/E scan originating at the point X: this will cut the two continua at the points marked AP and give rise to artefact peaks.

Metastable Mapping

From Figures 2 and 3, it is clear that the various linked scans of B and E are essentially different methods of investigating the intensity distribution within the B, E plane. Alternative methods of obtaining this information are the rapid repetitive scanning of E as B is slowly scanned or the rapid repetitive scanning of B as E is decremented. This latter method has recently been used to allow the collection of data by a data system which then stores it for subsequent use. The minicomputer can then simulate B/E, B^2/E and constant neutral loss scans for any values of m_1^+ , m_2^+ and m_n respectively. The origins of various artefact peaks are readily indicated and the spectra given by simulated linked scans closely resemble those given by on-line scans. The application of this technique to the analysis of mixtures and to angle resolved mass spectrometry has recently been described.

This lecture has aimed to describe the underlying principles of the various linked scans and to show the relationship between them. Each may now be regarded as an established technique and it is to be expected that they will find increasing application in mixture analysis and in fundamental

studies of the collision-induced decomposition of ions.

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A BRIGHT FUTURE FOR BASIC RESEARCH: MORE OPPORTUNITIES...AND MORE ACCOUNTABILITY. Remarks of Dr. N. Douglas Pewitt, Assistant Director, Office of Science and Technology Policy, Executive Office of the President, Washington, DC.

Some scientists' interest in a subject peaks when they finally see - in their minds - the grand picture. In my own field of physics, once a theoretician convinces himself that something must exist, he simply assumes that the experimentalists will eventually find it - and he goes on to something else. On the other hand, experimentalists - and I assume this group is largely composed of that unappreciated breed - tend to demand a little more proof before they accept what someone says.

I was thinking of that difference the other day when, for what must have been the nth time, I heard an otherwise astute scientist ask Dr. Keyworth about, and I quote somewhat freely, "the impact on science of all those cuts in federal support for basic research." Now bear in mind that she was asking that question of the man who three months earlier had announced that the administration was requesting a 10-percent rise in basic research for fiscal year 1984, with increases in many science agencies between 15 and 20 percent.

How could there be such a disparity of perceptions about the prospects for science? The answer, I think, is that we in the White House Science Office are like those theoretical physicists - a little ahead of what's happening in the lab. By now, for us, the general shape of the fiscal year 1984 budget is history. We started our work on it more than a year ago, and we're now concentrating on R&D budgets even farther away. But for you real scientists - or honest scientists as Dr. Keyworth would call you - the reality of those FY '84 increases is still at least a half year away.

It's a half year that can be well spent, however. There are changes in store for science, and they're going to take some getting ready for. For that reason, today I'd like to discuss the origins of the strong federal commitment to reasearch - and what the impacts may be on the science community.

Why, though, should growth in funding for science take getting ready for? The answer - and this is important - is that these increases clearly stem from our nation's needs in the 1980's and beyond. These are not the 1960's or 70's. The world has changed, and the rules are changing too.

Can there be any doubt that in the past few years American assumptions about work and about government have changed? Jobs that had been taken for granted for years suddenly disappeared. Social programs, many born in an era of seemingly unbounded American influence, began to overwhelm even the resources of the federal government, and their rate of growth had to be restrained. Not surprisingly, these and other dislocations have brought with them understandable public disillusionment and questioning about the causes and about our future.

I've heard some people say that we're at the end of a transition. They claim that the changes were already overdue and appeared suddenly only because we had refused to face reality earlier. Other people claim we're seeing the beginning of significant new directions in American life. They cite problems such as structural unemployment, fierce international industrial competition in technology, and what they see as a shift in the economy from manufacturing to service.

Maybe both points of view are right. In any case, we better recognize that many of the changes are real - they're not some artifact of political ideology that we can legislate away.

Well, what should our response to these changes be? I happen to think we're seeing a natural, even orderly evolution in our society. But not everyone agrees, and I'm afraid that society will find itself stampeded into reacting hurriedly to a perceived crisis rather than reacting wisely to a real condition.

The distinction between a crisis and a condition isn't always obvious. I recently read an article in Forbes Magazine that offered an interesting perspective on one of these supposed American crises: the much-discussed shift from a goods-producing economy to a service economy. The author claimed that we're engaged in a debate about an old situation, because the

U.S. has been increasingly a service economy for the past 40 years. He then went on to argue that thinking about production and service as separate industries misleads us in understanding where our economy is heading.

His thoughts struck a responsive chord, because I can see a parallel to current perceptions about the impact of "high-technology" on our industry. High-technology is a loaded phrase, judging from how often it's invoked these days. But I see it as no more than a way to describe the application of new knowledge to whatever we do. Those people who think of high-technology only in terms of microelectronics and genetic engineering miss an essential point: that perhaps the more important impact may be in how our traditional industries and services can be improved by better technology.

The *Forbes'* writer offered the opinion that even though a Boeing 747 is a tangible manufactured product, we could as well think of it as the means to provide a pure service - transportation. Even though my Chevrolet is not an exotic, high technology product, it is nonetheless the product itself of increasingly high-technology manufacturing techniques. Some people think there's some magic distinction between high-tech and low-tech, or, worse, that in our society the government could direct the development of specific high-tech industries better than the private sector. I invite them to take a more realistic look at how modern commerce evolves.

Finally, to set the stage for a discussion of how the federal government is helping the U.S. industrial capacity to grow, I can't resist quoting a wise passage from that same article. The author, James Cook, says: "In the end, the wealth-producing forces of any economy are its immaterial resources - not oil or gas, copper or iron ore, but rather the intellectual and organizational skills that are the very core of the services."

I want to talk today about those immaterial resources and how government is making an extraordinary commitment to sustaining them.

The federal focus on science that I mentioned earlier is the end result of a year-long effort in the White House to identify actions the federal government could take to strengthen the nation's economy - and particularly our industries.

We quickly concluded that we had to give high priority to producing enough skilled scientific and technical personnel to meet employment demands for coming years. Our current rate of training would fall increasingly short of our needs as the economy picks up steam. One of our major concerns is that shortages of scientists and engineers could actually be a brake on economic expansion because so much of the fastest growth will be in technology intensive fields. And in today's competitive world, if we have to scurry to train new talent, industrial opportunities will pass us by.

Another conclusion was that we had to continue to generate new knowledge in those areas with the most likely impact on new technology and industrial and defense needs. Those two circumstances led fairly directly to our decision to emphasize funding for physical sciences and engineering in universities. As you know, research done in universities also supports and trains graduate students under what are surely the most stimulating intellectual conditions anywhere. What you may not realize is just how many students are supported by federally funded research projects - more than 30,000 graduate students each year.

A third element of our industrial health hinges on a well-known paradox: We have the best basic research capability in the world, but some other countries do a better job than we do of using new knowledge for industrial advances. In light of the size of the federal government's investment - \$15 billion of the taxpayers' money for civilian R&D next year, \$6 billion of which is for basic research - this is a deeply troubling situation. Activity of that magnitude - in today's budget climate - must expect to be scrutinized for how well it meshes with national priorities.

We've made progress in redirecting R&D in the past two years to do just that, but there is still plenty to do. Just think for a moment about our most pressing national needs; then consider that there are still institutions claiming a large portion of those federal R&D resources that don't contribute significantly to training scientific personnel, to improving industrial productivity, or to improving national defense. And in some cases, they aren't particularly at the forefront of scientific research either. What, then, are they doing that's so important? Given the pressure on our federal budget, I

continue to be amazed that so many institutions still feel no qualms about following and protecting their outdated agendas.

Many of you saw an editorial Dr. Keyworth wrote for Science Magazine a few months ago in which he addressed the notion that federal support for R&D is an entitlement, that it's going to come off the top of the budget independent of economic pressures or national priorities. If his message in that article seemed harsh, it's because that attitude is terribly destructive for science and for the nation. The research community has an important role to play in the country's future, but it has to emerge from the never-never land it so comfortably inhabited for the past 20 or so years. It must come to grips with the realities of the 1980's.

So let me make it clear that the increase in support for academic research is not a reward for all the fine things universities have been doing. It's a challenge to the universities to assume a greater role in helping us regain our momentum in world technological leadership.

We are also demanding innovative responses from other research institutions, such as the federal laboratories. Many of those labs, which were established decades ago to deal with specific national problems, are no longer focusing on problems of first-line importance. But they are still valuable resources - good people and good facilities - and we intend to put them to better use.

I admit that is easier to say than to do - and it takes patience, much like steering an ocean liner. We moved the rudder two years ago, and we are finally beginning to sense a change of course. Late this month the White House Science Council will conclude its year-long review of the management and effectiveness of the nation's major federal laboratories. We expect some solid recommendations for improving the efficiency and relevance of research being done there. The study will give us a lever to encourage some needed changes.

Let me describe one example of what I think is an exciting project for how a federal lab can take the initiative for developing better interactions between academic, federal and industrial scientists. This is the Advanced Materials Research Center being established at Lawrence Berkeley Laboratory.

There are two things to realize about the LBL project. One is that this country is in a position to consolidate and expand its leadership in materials science. This center will bring outstanding talent to bear on an area of research with broad potential to affect American industry in coming years. But even more important in the long run is what LBL learns and teaches the rest of us about collaborative research.

Some people have pointed out that there are other research institutions better known for work in materials science - so why has this center been established? The answer is that we have to find ways to better couple the federal labs to current national needs. LBL is uniquely suited - a federal laboratory located on an outstanding university campus, and in a region noted for innovative industry.

LBL is a prototype for mixing different research viewpoints to focus research and expedite the transfer of new knowledge to the marketplace. We ought to be applauding their willingness to venture into this uncharted territory - and we ought to do whatever we can to help them succeed.

The increases in the President's proposed R&D programs for the next year have drawn alot of attention. But its important to bear in mind that our real concern is for quality research, not quantity. Larger numbers of projects, or even larger numbers of scientists and engineers, don't automatically produce leadership.

For that reason, we must permit the best research to be more fully supported so that it can extend its influence. This is the time to do the job with the best tools we have, not a time to dissipate our resources by parceling them out in response to popular demand. It is time for scientific statesmanship.

This statesmanship has an important payoff because it establishes credibility for ongoing support. I don't have to sell the idea of stable funding patterns to you; you know how critical stability is for the operation of experimental facilities. Some members of the science community have been worrying each other that the budget increases for basic research may be short-lived. I'd call that a classic pessimist's outlook, but in any case, let me

reassure you; that has never been our intention. A one-year spike would prove more harmful than useful, because it would introduce instability into the funding pattern.

But how can we achieve stability? How do we make sure we can take full advantage of intellectual opportunities and assure the training of enough new scientists and engineers? And how can we make it predictable enough to permit continuity in long-term projects and in planning for new facilities?

There are no simple answers, though some would have us try to claim a percentage of gross national product as a guaranteed funding level for R&D. Aside from what I predict would be the failure of that mechanism to hold up in the brutal give-and-take of budgetmaking, I've already indicated our distaste for trying to turn R&D into an entitlement.

Why? Because that attitude is deadly to good science. The first entitlement begets more. Pretty soon we have individual disciplines demanding their guaranteed share of the guaranteed pie, then regional demands for portions. Next we'd be divvying up portions between universities, four year colleges and two year colleges. Soon the only criterion that should count - excellence - will be lost in the noise of formula grants, geographic distribution and set-asides.

Moreover, in order to sell and maintain such a funding mechanism, one would have to overstate the benefits to be gained from research. I'm afraid the resulting pressure for tangible results would inevitably divert resources away from basic research and into applied research and development better left to industry.

I think we've shown this year that government does respond enthusiastically to programs, even as esoteric as basic research, that have clear relevance and importance to national objectives. But I have to turn the tables on you at this point. The Administration's plan to maintain this emphasis on basic research - and to create a structure for a strong federal program that will attract bipartisan support for years to come - is not automatic. It's dependent upon cooperation and assistance from the science community itself.

I'm worried about the continued inability - or unwillingness - of scientists to agree among themselves about priorities - or to abide by their decisions when they can agree. I suppose this is an old routine from the White House by now, but our experience in the past two years confirms it. Those disciplines that present well-considered, unified agendas for research have the best chance getting support. Let me put the question to you: In the absence of agreed-upon recommendations, what do we expect the non-scientists who allocate funds to base their decisions on?

I'll give you three choices, none of them good. It may be that funding will be deferred until the community can come to some consensus. Or decisions may be based on non-scientifically relevant factors such as preservation of politically popular facilities. Or disaffected minority viewpoints, which are often the dominant messages transmitted to decision makers, may well carry the day. I come back to the central point: The community has to be willing to establish its own priorities and then stand by them in the public arena.

The coming year is important for the near-future of American basic research. The Administration's proposals have been well-received, and there is every reason to believe that we'll see broad, bi-partisan support for most of the elements of the plan. This favorable reception, if it's supported by the science community and industry too, may set a course for a healthy and beneficial new degree of integration of science and technology in American life.

ION-MOLECULE CHEMISTRY IN EXTRATERRESTIAL ENVIRONMENTS; Alexander Dalgarno, Center for Astrophysics, Harvard College Observatory, Cambridge, MA.

An account will be given of the remarkable array of molecular species which exist in the upper atmospheres of the planets and of Titan, in the comae of comets and in diffuse, dense and shocked interstellar clouds.

The ion-molecule chemistries which are the source of the molecules will be reviewed, and examples of the utility of molecules as diagnostic probes of unusual physical conditions will be presented.

BIOTECHNOLOGY AND MEDICINE OF THE FUTURE
Leroy E. Hood; Department of Biology, California Institute of Technology, Pasadena, CA.

The techniques of biotechnology have revolutionized the study of human development. Indeed, the 1980's have already been termed the "Decade of Biotechnology," in part because of the incredible potential that genetic engineering and allied technologies provide for disciplines concerned with agriculture, energy, chemicals, and especially medicine.

I will describe three distinct techniques of biotechnology - recombinant DNA, microchemical instrumentation, and monoclonal or homogeneous antibodies. I will point out that these biotechnologies are incredibly synergistic and offer enormous opportunities for medicine of the future.

Ion Optics
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Ion Optics

Ion optics is a specialized field of Classical Mechanics, and classical mechanics is a branch of Applied Mathematics. In this paper, the attempt is made to describe some of the arguments and conclusions applicable to mass spectrometry, using as little math as possible.

There is no difference between Ion Optics and Electron Optics. It is only necessary to change the value of the mass, and when positive ions are under consideration, the sign of the charge, to go from one to the other.

Classical Mechanics

The ultimate goal in any classical mechanics problem is to determine the motion as a function of time. In the case of a particle such as an ion, the object is to determine its vector position (three numbers, one for each spatial dimension), $\vec{r}(t)$, at any time, t .

The velocity vector is defined as the time derivative of the position, i.e.,

$$\vec{v} = \frac{d\vec{r}}{dt} \quad (1)$$

and the acceleration vector is given by

$$\vec{a} = \frac{d\vec{v}}{dt} = \frac{d^2\vec{r}}{dt^2} \quad (2)$$

All of the physics in ion optics is embodied in stating Newton's 2d Law of Motion, i.e.,

$$\vec{F} = m\vec{a} = m \frac{d\vec{v}}{dt} \quad (3)$$

Once the force on a particle is specified, Eq. (3) is solved find $\vec{r}(t)$.

In most ion optics problems it is not necessary to find $\vec{r}(t)$. Interest is usually limited to know only the trajectories, and the time it takes for an ion to move along a trajectory is not important. It is convenient to define the speed, u , of the ions as

$$u = |\vec{v}| = (\vec{v} \cdot \vec{v})^{1/2} \quad (4)$$

in which case the velocity vector can be written as

$$\vec{v} = u \frac{d\vec{r}}{ds} \quad (5)$$

where ds is a small displacement along the trajectory and $d\vec{r}/ds$ is the unit vector in the direction of motion.

Motion in Electrostatic Fields

The force in an electric field is the product of the charge, e , of the ion times the electric field strength, $\vec{E}$, which is a vector having both magnitude and direction, i.e.,

$$m \frac{d\vec{v}}{dt} = e\vec{E} \quad (6)$$

In an electrostatic field, where $\vec{E}$ remains constant in time, $\vec{E}$ is the negative gradient of the electrostatic potential, i.e.,

$$E = - \nabla \phi \quad (7)$$

where $\phi(\vec{r})$ and $\vec{E}(\vec{r})$ only.

Combining Eqs. (6) and (7), one quickly arrives at a first integral which states that

$$\frac{1}{2} m \vec{v}^2 + e\phi = W = \text{constant} \quad (8)$$

or

$$K + U = W = \text{constant} \quad (9)$$

where $K = \frac{1}{2} m \vec{v}^2 = \frac{1}{2} m u^2$ is the kinetic energy and $U = e\phi$ is the potential energy. Eqs. (8) and (9) are statements of the conservation of energy, where W is the total energy which remains constant. During the motion any change in kinetic energy is compensated for by an opposite change in potential energy.

By eliminating time from the equations, the differential equation to be solved to determine the trajectory is

$$(W - e\phi) \frac{d^2 \vec{r}}{ds^2} + \frac{d\vec{r}}{ds} \cdot \frac{d(W - e\phi)}{ds} = \nabla(W - e\phi). \quad (10)$$

Profound statement A. Whenever one sees an equation, look not for what is in the equation, but what is not in the equation. In Eq. (10) the mass, m , is noticeably absent. This means that the trajectory doesn't depend on the mass.

This is lovely for mass spectrometry. It says that if one accelerates ions from an ionizer into an ion mass analyzer using electrostatic fields, the trajectories of all ions, irrespective of mass, will be identical if they start from rest at the same position. While the hydrogen ion will move faster than the uranium ion along that trajectory, it will be the same trajectory and both will be identically focussed into the ion mass analyzer without any mass discrimination.

Substituting Eq. (8) into Eq. (10), another form of the trajectory equation can be written down, i.e.,

$$K \frac{d^2 \vec{r}}{ds^2} + \frac{d\vec{r}}{ds} \cdot \frac{dK}{ds} = \nabla K \quad (11)$$

which leads to

Profound Statement B. The only parameter of relevance for trajectories of ions in electrostatic fields is the kinetic energy, K .

This means that electrostatic fields are kinetic energy analyzers. On the plus side, this means that electrostatic sectors will disperse ions on the basis of their kinetic energies. On the minus side, it implies that ions which start with different kinetic energies will not follow the same trajectories which means if you are trying to focus ions to a sharp point or line image an energy spread will lead to "chromatic aberration" in the lens system.

Motion in Magnetic Fields

The motion of an ion of mass, m , and charge, e , moving in a magnetic field with a field strength vector, $\vec{B}$, is given by Newton's second law by

$$m \frac{d\vec{v}}{dt} = e (\vec{v} \times \vec{B}) \quad (12)$$

where the multiplication process is the "vector cross product". This means that the force and therefore $d\vec{v}/dt$ is perpendicular to both $\vec{v}$ and $\vec{B}$.

Since $d\vec{v}/dt$ is perpendicular to $\vec{v}$, the first integral is

$$K = \frac{1}{2} m v^2 = \text{Constant}. \quad (13)$$

The force changes only the direction of motion and not the speed. Since constant speed and a force perpendicular to the direction of motion are the hallmarks of uniform circular motion, we can equate the scalar magnitude of the force to the expression for the force providing uniform circular motion and find the radius of the circle. It is

$$R = \frac{mu}{eB} = \frac{\sqrt{2mK}}{eB} \quad (14)$$

where $u = |\vec{v}|$. The product of mass times speed is the magnitude of the "momentum".

Profound Statement C. Magnetic fields are momentum analyzers, and separate ions of different momenta.

A magnetic sector mass spectrometer works by accelerating ions of all masses to the same kinetic energy $K (= \frac{1}{2} m u^2)$ by an electrostatic field. The ions are then passed through a magnetic field where their momenta are measured. The mass is obtained by eliminating the speed u between the values of $\frac{1}{2} m u^2$ and mu . Figure 1 shows the trajectories in a 180° magnetic sector instrument emphasizing that the radii of ions of different mass in the same magnetic field are proportional to the square root of m .

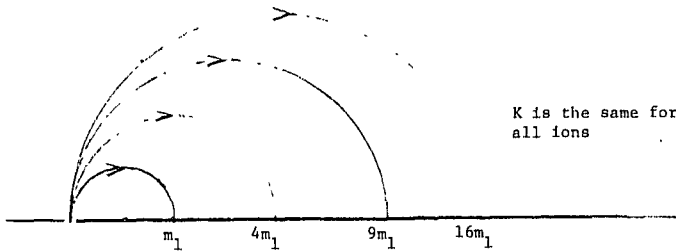


Fig. 1. Trajectories in a 180° Sector

The above comments about uniform circular motion apply only if the motion is in a plane perpendicular to the direction of the magnetic field. Eq. (12) implies that there is no component of force in the direction of the magnetic field, and the component of velocity in that direction has a constant value. If that component is not zero (circular motion), then the ion trajectory is helical, and the overall motion of the ion is along the direction of the magnetic field. This effect is used in confining particles by magnetic fields in controlled thermonuclear research.

The time that it takes for an ion to execute one full circle (or a full traverse around a magnetic field line in case of helical motion) is of interest. It is

$$T_L = \frac{2\pi m}{eB} \quad (15)$$

More frequently $1/T_L$ is talked about, this being the "Larmor frequency",

$$f_L = \frac{eB}{2\pi m}. \quad (16)$$

Profound Statement A'. There is nothing about the ion except its charge and mass appearing in Eq. (16). This means that the Larmor frequency is independent of the speed, momentum, kinetic energy, direction of motion, or anything else beside the m/e of the particle. Fig. 2 shows some trajectories of ions moving in the plane of the paper. If they all start from point A at the same time, they will all come back to the point A at the same time. Since frequency is the most accurately and precisely measureable quantity in all of physics, measurement of masses and mass differences by measurement of Larmor frequencies is uniquely powerful.

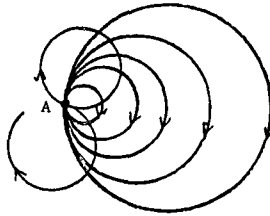


Fig. 2

Direction-Focussing

If ions of the same mass, charge and kinetic energy all pass through a point S, but have different directions of motion they are said to be direction-focussed if their trajectories subsequently pass through another point, I. Fig. 3 shows four trajectories in a 180° sector magnetic mass spectrometer.

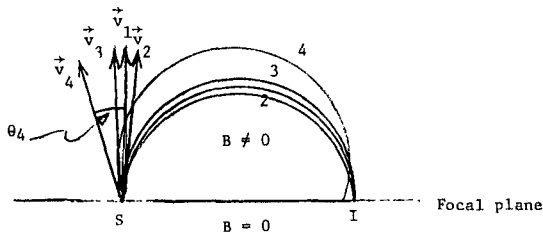


Fig. 3

It is seen that: (1) Trajectories 1, 2 and 3, starting from source point S, are focussed at the image point, I. (Actually the image will be a line parallel to the direction of the magnetic field, since the ions will probably have a component of velocity that is perpendicular to the paper); (2) if the angle is too large, e.g., θ_4 , trajectory 4 does not cross the central trajectory 1 at the image point, I.

It is said that the 180° sector shows "first-order" direction focussing. We'll come back to define this more precisely a little later.

Very few mass spectrometers use the 180° sector nowadays. Usually the angle is less. The notions of focussing still hold however; the only thing different is that outside the magnetic field the trajectories are straight lines rather than arcs of circles. Fig. 4 shows such a sector.

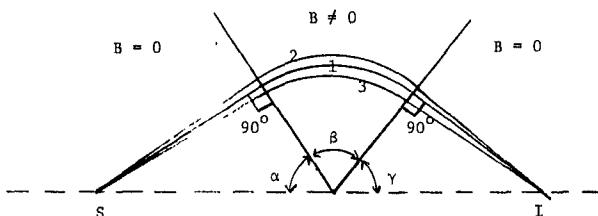


Fig. 4. Trajectories in Magnetic Sector of less than 180° .

It turns out that if the central trajectory enters the magnetic field normal to the field edge, the image point I will be along a straight line connecting the source point and the center of curvature of the central trajectory. Or, $\alpha + \beta + \gamma = 180^\circ$, in order to get first order focussing. Again the image will not be a point, but rather a line that is parallel to the direction of the magnetic field in the magnet.

Fringe Fields.

Unfortunately, magnetic fields do not have sharp boundaries. Fringe fields extend beyond the edge of the iron of the magnet and the field doesn't build up to its full strength until one is well inside the region between the pole faces. Fig. 5 is a sketch of the situation, drawn with enormous artistic license, but giving the general idea.

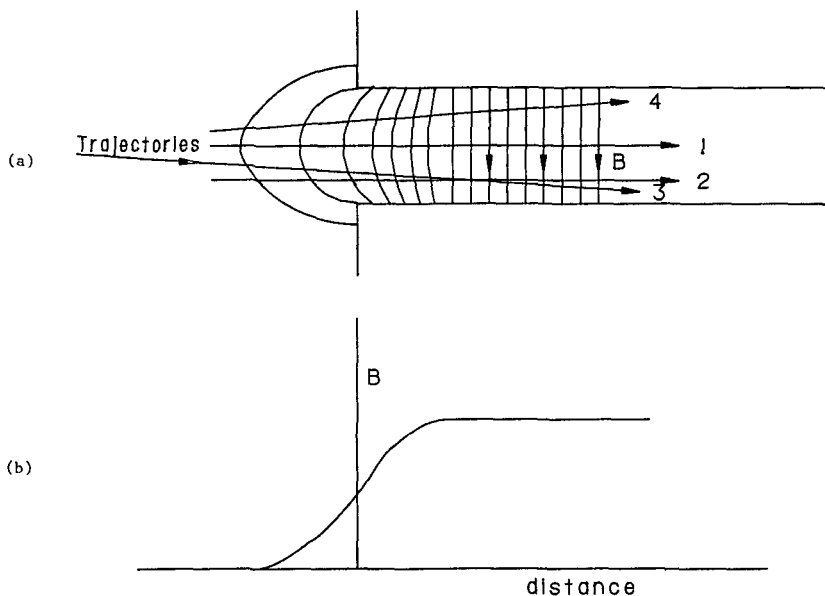


Fig. 5. (a) Magnetic field lines near edge of the magnet. Closely spaced lines indicate strong field.
 (b) Magnitude of magnetic field with distance from the edge of the magnet.

An effective length of a trajectory in a magnetic field can be related to the angle through which the direction of motion is changed by passing through the magnet. It turns out that the effective length of an ion trajectory is slightly greater than the true length of the trajectory while between the pole faces. Further, the effective length of a trajectory near a pole face (such as 2) need not be the same length as a central trajectory (such as 1). The image can be slightly curved rather than being a straight line. Hexapole lenses (Electrostatic) can be used to straighten out the image.

Nowadays magnets shaped so that the ions enter the magnet perpendicular to the edge are getting increasingly less common. The reason for this is to get some focussing of the ions in the direction of the magnetic field and make the image at least a shorter line if not a point image. If the major motion is in the X-Y plane, and the magnetic field points in the Z direction, one gets some Z-axis focussing. Fig. 6 illustrates this.

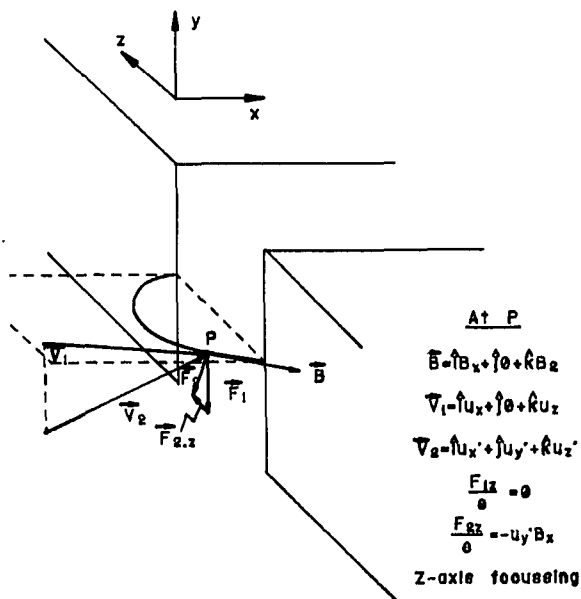
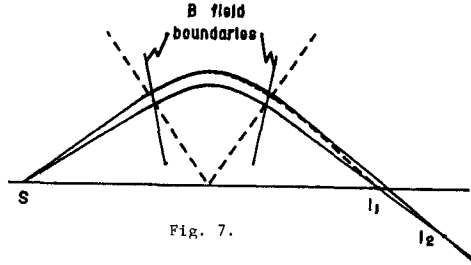


Fig. 6. Z-axis Focussing by Magnetic Fringe Fields

The curved line represents a single fringe field line. An ion with velocity $\vec{v}_1$ approaches the magnet at a point near one of the pole faces. It does so perpendicularly to the edge of the magnet and has no component of velocity in the Y-direction. From Eq. (12) there is no component of force in the Z-direction.

Another ion approaches the same point but with a velocity $\vec{v}_2$, which does have a component in the Y-direction. Eq. (12) says that this one will see a force in the Z-direction that will tend to push it back toward the central plane between the pole faces.

It is increasingly common to see magnets shaped as shown in Fig. 7.



Instead of having the magnetic field edges perpendicular to the central trajectory as in Fig. 4 (dashed lines) the field edges are as shown by the solid lines. This gets some Z-axis focussing. The price one pays is relocation of the image position. Since the upper trajectory in the figure goes a shorter distance in the field than the central trajectory, and the lower trajectory goes a longer distance, the image position is moved from I_1 to I_2 .

Direction Focussing Re-visited.

The word "first-order" focussing has already been used. The easiest way to explain this and higher order focussing is to turn to an electrostatic example, namely deflection of ions in a uniform electrostatic field between two parallel plates. Although he had never heard of ions or electrons, the problem was actually solved by Galileo. The problem is identical to that of the range of a projectile moving in a uniform gravitational field.

Fig. 8 shows the trajectory of an ion launched with a speed v_0 at an angle, α , with respect to the lower plate.

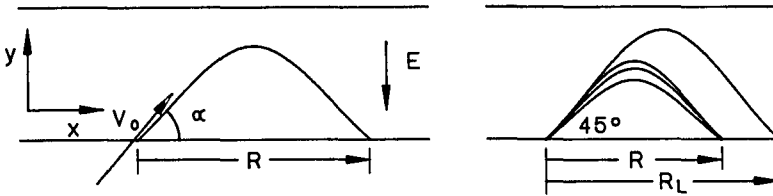


Fig. 8. Focussing in a Parallel-plate Energy Analyzer

The equations of motion in the X and Y directions are

$$\frac{d^2 y}{dt^2} = -\frac{eE}{m} \quad \text{and} \quad \frac{d^2 x}{dt^2} = 0 \quad (17)$$

Eliminating t , and solving for the trajectory we have

$$y = \tan \alpha \cdot x - \frac{eE}{4K} \sec^2 \alpha \cdot x^2 \quad (18)$$

which is a parabola. The range, R , of the projectile is defined as the non-zero value of x when $y = 0$. From Eq. (8) it is given by

$$R = \frac{2K \sin(2\alpha)}{eE} \quad (19)$$

Galileo was interested in choosing the angle such that R would be maximum. This occurs when $dR/d\alpha = 0$. Doing this to Eq. (19) requires that $\cos(2\alpha) = 0$ or $\alpha = 45^\circ$.

We are more interested in another consequence of $dR/d\alpha = 0$, which also states that if you change α slightly, you get no change in range, i.e., trajectories over a small range of angle about 45° will all have the same range, R . This is what "focus" means. In the right hand drawing in Fig. 8, this focussing is depicted.

Eq. (19) says that the range is proportional to kinetic energy, K , and the device can be (and often is) used as an energy analyzer. It also says that if the range is fixed (with a detector being fixed at R), the kinetic energy of ions arriving at the detector is proportional to the electric field strength, E . This is the way these devices are usually used.

The parallel plate analyzer is said to be a first-order focussing device, because it is only the first order derivative of R with respect to α that vanished. The second order derivative

$$\frac{d^2R}{d\alpha^2} = -\frac{8K \sin(2\alpha)}{eE} \quad (20)$$

is not zero at 45° .

Other commonly used energy separators that give only a first order focus include the 127° sector that uses two segments of concentric circular cylinders. Most electric sectors on double focussing mass spectrometers have an angle less than 127° . The focussing properties of these small sectors can be treated similarly to the matter of going from a 180° magnetic sector to a smaller angle (see Figs. 3 and 4, above).

One can achieve higher order focussing. The parallel plate device that has zero first and second derivatives of R with respect to angle (and therefore called a "second-order focussing" device) has three plates as shown in Fig. 9A.

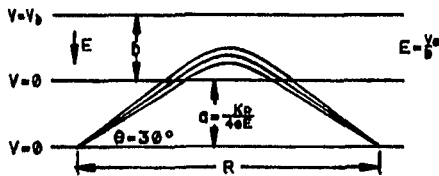


Fig. 9A. Second-order Parallel Plate Analyzer

Between the lower two plates there is no electric field and the trajectories are straight lines. In passing through the middle plate the ion enters a downwardly directed electric field. It passes through the middle plate and focusses at the lower plate. Solving the trajectory equations for the three regions, requiring that the position and direction of motion are continuous as the ion passes through the middle plate, yields the result that both first and second order derivatives of R are zero when the angle $\theta = 30^\circ$. The range is given by

$$R = 2.60 \frac{K}{eE}. \quad (21)$$

The thing that having second order focussing gets one is a broader range of angles that the initial bundle of ions can start out with and still end up being well focussed.

I don't know anybody who uses a second order focussing parallel plate analyzer. I know lots of people who use another second-ordering focussing device though, namely the Cylindrical Mirror Analyzer. It is shown in Fig. 9B.

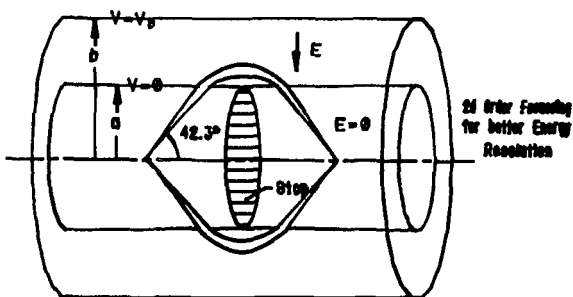


Fig. 9B. Cylindrical Mirror Analyzer

If ions of kinetic energy K are launched from a point on the axis at an angle of 42.3° with respect to the axis, they will follow trajectories as shown and focus at a point on the axis on the other side of a stop (to prevent ions from going on straight line paths to the focal point). Both the first and second derivatives of the position with respect to angle vanish at $\theta = 42.3^\circ$. The relationship between the kinetic energy, the geometrical parameters shown and the voltage, V_0 , applied to the outer cylinder when the inner cylinder is grounded is

$$K = \frac{2.6196 \text{ eV}}{2 \ln(b/a)} b \quad (22).$$

CMA's are used primarily for determining electron energies (as in Auger analysis). While they could be used as energy filters in SIMS, having such a large angle of entrance into the mass analyzer as 42.3° is pretty hard to accommodate.

Focussing Electrostatic Lenses

When one says "optics" one usually thinks of light optics and focussing or defocussing lenses made of shaped glass and usually cylindrically symmetrical. Two ion optical analogs are shown in Fig. 10.

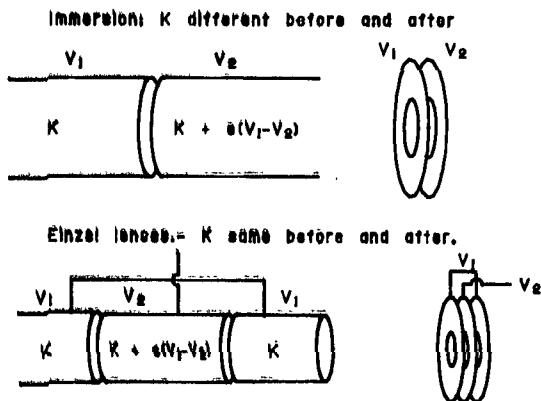


Fig. 10

The upper is an "immersion" lens, named by analogy to immersion lenses used in microscopy, which are characterized by the speed of light being different in different media. In the ion optics case, the speed (and therefore kinetic energy) is different on either side of the gap. Both cylinders and apertures are shown.

The "einzel" or single lens, which is the analog of a glass lens where the light has the same speed on either side of the lens, contains three elements, the outer two being held at the same potential and the middle element has another potential.

There are three inescapable unhappy features of electron or ion optical lenses as compared to light optical lenses:

1. All ion optical lenses display "chromatic aberration", meaning that ions with different initial energies will focus at different points. Being electrostatic, ions with different energies follow different trajectories.

In mass spectrometry there is always a spread of initial energies from three causes. The least significant is the thermal energy spread of the neutral molecules before having an electron removed. Depending on the ion source temperature the thermal energy spread is typically 0.025 to 0.05 eV.

The second source of energy spread occurs in dissociative ionization where the ion is a fragment ion of a larger molecule. On being ionized, the molecular ion will make a vertical transition to a repulsive molecular ion state and then dissociate leaving the excess energy as kinetic energy in the two fragments. This energy is typically between a few tenths of an eV up to 2 or 3 eV in some cases. Both the above causes of energy spread are God-made.

The third and usually greatest energy spread is man-made in his quest to get larger signals, by putting on an electric field in the ionizer to push or pull ions out in the direction of the mass analyzer. The ion formed near the repeller in an ionizer starts at a point with higher potential energy than one made near the exit aperture and retains a higher total energy, and therefore a higher kinetic energy when both ions are moving in regions at the same potential. This energy spread can run from several to many eV.

The usual cure for chromatic aberration is a brute force one: Give the ions a large acceleration voltage compared to whatever energy spread they have. The more k_{av} , the better.

Of course energy spreads and chromatic aberration can be serious problems in

measuring fragmentation patterns. In reviewing the literature some years ago, I found that the probability of making 0^+ from 0_2 had been measured at everywhere between 3% and 16%. R. T. Brackmann and I then designed an experiment to gather up all the ions and make a proper measurement of the probability. It turned out that 33% of the ions made by electron impact of 0_2 were 0^+ . I suspect that this kind of discrepancy attends many, and maybe most, of the fragmentation patterns shown in mass spectral libraries.

2. Electrostatic lenses are always convergent, never divergent as light optic lenses often are. To get divergence in an optical lens one uses a "cross-over" which means one focusses the beam to a point, after which the trajectories diverge. The small spot on a TV tube is an image of a cross-over made near the cathode of the electrode gun.

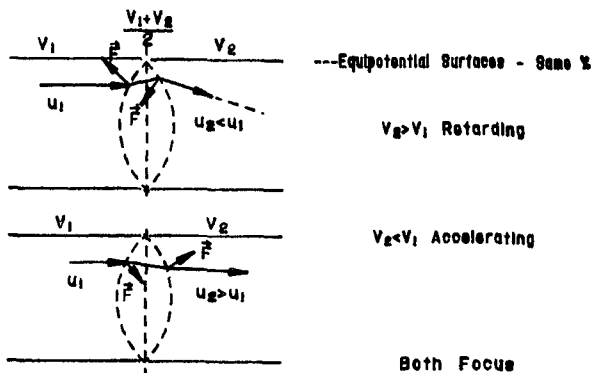


Fig. 11A

Figure 11A helps to understand the convergence theorem. In the upper figure an ion in an immersion lens is to be retarded. A single equipotential surface is shown on either side of the median plane. As the fast ion comes in, the force it sees (which is perpendicular to the equipotential surface) slows it down and pushes it outward. Because of its high speed its outward motion is relatively small. The equipotential surface on the right also slows the ion but pushes it inward. Because the ion is going more slowly on the right, the inward force effects are greater than the outward force effects on the left, and the net deflection is inward toward the axis.

In the case of an ion being accelerated as in the lower figure, the force at the left equipotential accelerates the ion toward the right and also pushes it toward the axis. At the right the ion continues to be accelerated to the right and is also accelerated outwardly. Because the ion is faster on the right, the outward force is not as effective as the inward force on the left and the net deflection is again inward toward the axis.

3. All electrostatic lenses have spherical aberration. Fig. 11B depicts this, in the case of an einzel lens. Ions that are initially far away from the axis cross the axis at different points than those near the axis.

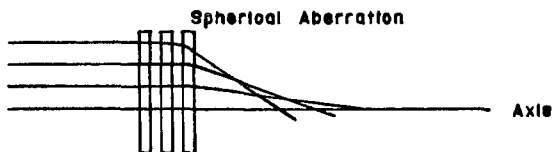


Fig. 11B

Spherical aberration is contended with by using paraxial (near the axis) trajectories. Put in a beam stop to exclude the ions initially far from the axis, or make the diameter of the lens elements large compared to the beam diameter, and keep the directions of motions of the ions very nearly parallel to the axis.

Using paraxial rays has an additional advantage. Namely the trajectory equation in the cylindrical coordinates can be approximated by the "paraxial ray equation" which is linear and therefore enormously more easily solved than the full-blown trajectory equation.

Focussing Magnetic Lenses

If a particle is moving at a speed of a few percent or so of the speed of light, substituting magnetic lenses for electrostatic lenses becomes attractive. Television tubes and nuclear accelerators all use magnetic lenses, primarily I believe, to avoid the nuisance and cost of high-voltage techniques as compared to using magnetic fields. But they are dealing with particles on only one value of m/e .

Because magnetic lenses would focus on the basis of the momentum of an ion, they would give mass discrimination if the ions all had the same energy. In addition, ions in mass spectrometers move slowly and the cost of magnetic lenses to produce the huge fields required would probably be prohibitive.

Strong Focussing - Quadrupole Lenses

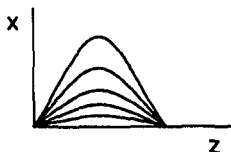
In a modern nuclear accelerator, ions achieve their extremely high energies (billions of eV) by being given many small energy kicks as they move along their trajectories. After each kick, the beam must be refocussed. As energies increased in the post-World War II period, spherical aberration was getting to be a terrible problem. Miniscule losses of beam current at each refocussing became intolerable as the number of refocussing operations increased. (99% raised to the 10,000-th power = zero). Strong focussing had to be invented.

Strong focussing starts with the question of how one can get perfect direction focussing. An obvious way is to have the displacement from the axis, x , of a particle moving in the Z direction be given by

$$x = A \sin(kz) \quad (23)$$

where k is a constant.

If $x = 0$ at $z = 0$, it will again be zero at $z = \pi/k$, irrespective of the value of A . An ion with a small A travelling near the axis will cross it at the same point as an ion starting out almost perpendicularly to the axis, as shown in Fig. 12A.



Perfect Direction Focussing

Fig. 12A

Working backwards with the equation of motion, one wishes to have a field (we'll use an electric field here rather than magnetic) in which $E \propto -x$. Since $\vec{E} = -\nabla\phi$, we want $\phi \propto x^2$.

Static fields (both electric and magnetic) must satisfy Laplace's equation which means that the only potential with x^2 appearing also has y^2 in it, and the potential is given by

$$\phi(x,y) = \frac{U}{r_0^2} (x^2 - y^2) \quad (25)$$

This field can be produced between the sheets of a hyperbolic cylinder, as shown in Fig. 12B where $\pm U$ are voltages as shown and r_0 is the inscribed radius as indicated, the shortest distance from the axis to any surface.

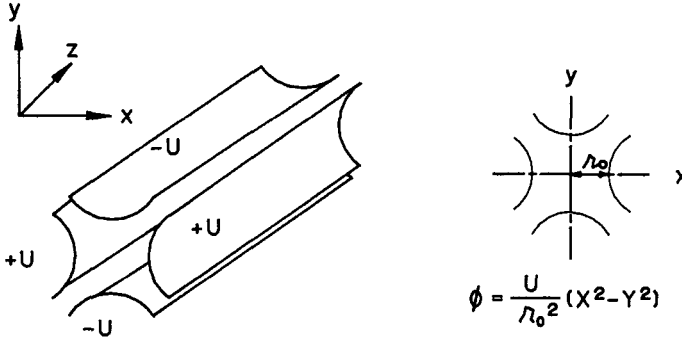


Fig. 12B. Hyperbolic Cylinder

This meets all the requirements for perfect direction focussing in the X direction, as planned.

But what goes on in the Y-direction is disaster. The potential given in Eq. (25) leaves the Y-direction motion given by

$$y = A \sinh(kz) = \frac{A}{2} (e^{kz} - e^{-kz}), \quad (26)$$

While perfectly focussing in the X direction, the Y direction motion is exponentiating away from the axis and one gets a long line image.

The cure recognized by our nuclear accelerator friends was to use not one, but two quadrupole lenses in tandem with the polarities reversed. Fig. 13 shows a quadrupole lens pair.

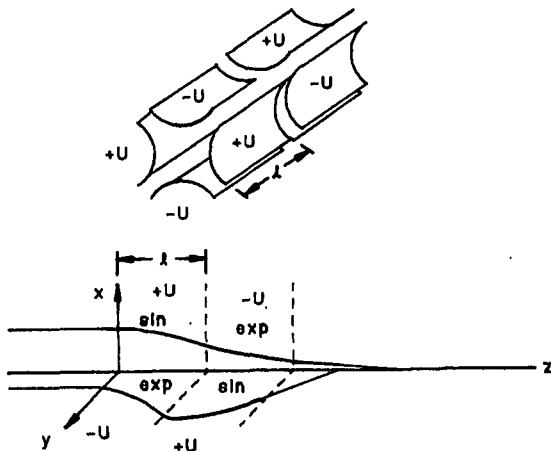


Fig. 13. Quadrupole Lens Pair

Looking first at an ion travelling in the Y-Z plane, entering from the left it follows an exponential trajectory in the first lens. When it enters the second lens, however, its trajectory becomes a section of a sine curve which turns it around and directs it back toward the axis. When it leaves the second lens it follows a straight-line trajectory and crosses the axis.

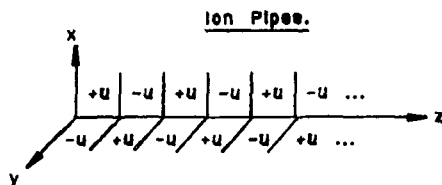
In the X-direction, its being in an exponential trajectory region in the second lens is no problem. Because it is heading toward the axis following its sinusoidal motion in the first lens, it follows a trajectory where x is exponentially decreasing. After leaving the second lens, it too follows a straight line trajectory until it crosses the axis.

It should be noted in the drawing that the axis-crossing points of the two trajectories are not coincident. The particular lens shown in the figure is astigmatic. Indeed a wonderful thing about quadrupole lens pairs is that they can be configured to produce a wide range of astigmatic imaging as well as point-to-point focussing. They can be used to convert the line image in a magnetic mass spectrometer into a point image and I am surprised that commercial manufacturers of such instruments do not make more use of quadrupole lens pairs for beam shaping and Z-axis focussing.

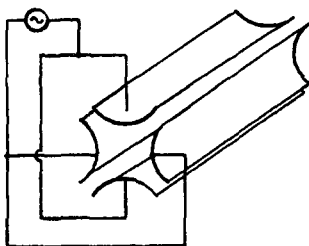
The quadrupole lens pair also pretty much allows one to ignore traditional rules about placement of ion source and detector with respect to the center of curvature, at least in low resolution work such as takes place in atomic collision physics. The rule of thumb nowadays is to put the magnet and source anywhere that's convenient and with one or two quadrupole lens pairs along the beam path, you can put almost any shape of image almost anywhere you want it.

Ion Pipes

If 2 quadrupole lenses are so great, would not 4 or 40 or 400 be even better? This is the beginning for ion pipes. Fig. 14 shows two concepts.



Equivalent to



Stable trajectories if $\frac{m}{e} \geq \frac{2u}{(3.76) \times 10^8}$

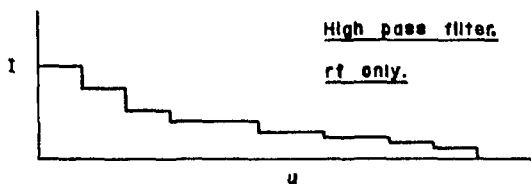


Fig. 14. Ion Pipes

In the first, quadrupole lenses with alternating polarity of dc potentials are placed end to end. An equivalent (and much more easily built arrangement) is to have a hyperbolic cylinder to which an ac voltage is applied.

In either case, solving the equations of motion yields the result that if an ion has an m/e value greater than a certain amount, the motion will be oscillatory about the axis and the ion will be piped down the structure; if the ion has a smaller m/e value, its trajectory will be unstable and it will move out toward the surfaces of the structure and be taken out. The ion pipe is thus a "high-mass" filter, which rejects low masses.

With the continuous structure and a sinusoidal rf voltage, an ion will be on a stable trajectory if

$$\frac{M}{Z} > 0.0535 \frac{V}{f^2 r_0^2} \quad (27)$$

where M is the mass in AMU, Z is the number of electronic charges on the ion, V is the amplitude of the rf voltage in volts, f is the frequency in MHz of the rf voltage and r_0 is the inscribed radius (see Fig. 12B) in cm.

If one increases the value of V the transmitted ion current is as shown in the lower of the drawings of Fig. 14. One by one the light ions drop out. Clearly one can make a mass analysis device by using only rf on the quadrupole structure. One needs only to take the derivative of the transmitted ion current with respect to V to get what looks like a conventional mass spectrum. In practice, the steps downward are not quite sharp enough in most of the mass filters I have used to allow good enough resolution, when only rf voltages are used.

Probably the most extensive use these days of ion pipes is in the middle quadrupole in triple-quadrupole MS/MS instruments. Ions colliding with a collision gas produce fragments which are piped on to the third mass quadrupole. One uses rather weak rf voltages to ensure that lighter fragment ions are not rejected.

Velocity Focussing.

The matter of energy spread has been briefly touched on in connection with chromatic aberration in electrostatic lenses. The energy spread and accompanying momentum spread must also be contended with in the mass analyzer that a beam is focussed into. The most common way to do this is by using spatially separated electric and magnetic sector fields. These are the "double-focussing" (direction and velocity focussing) instruments.

Fig. 15 shows one such instrument design, from Reference 2.

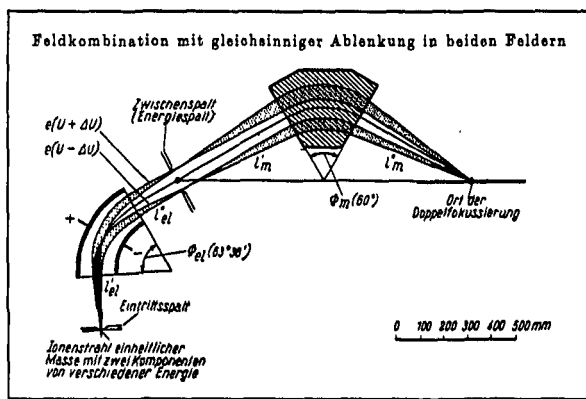


Fig. 15. Field combination with similar deflection in both fields. (Eintrittsschlitz = entrance slit; Ort der Doppelfokussierung = position of double focussing).

The 63° electric sector direction-focusses ions from the source to a line image at the Zwischenraum. This line image is then the source for direction focussing by the magnetic sector into the detector. Note that this is an old design, where all the old fashioned rules about where you locate slits and centers of curvature are strictly obeyed.

For velocity focussing, the faster ions are separated from the slower ions in the electric sector, the faster ones not being able to turn the corner as well during the time they are in the field. Because of their direction, they enter the magnet higher up and

therefore stay in the magnetic field longer than the slower ions, and get curved more. The result is that ions with different velocities end up at the same detector.

There must be about as many designs for double focussing instruments as there have been people who seriously attempted to design one. In some the trajectory is C-shaped, i.e., the two fields deflect the ions in the same direction; there are S-shaped trajectories in which ions are deflected in opposite directions by the two fields. One can put the electric sector first or the magnetic sector first, in the "forward" and "reverse" geometries. Adding shaped pole edges and toroidal electric sectors for Z-axis focussing, putting in quadrupole lens pairs to produce desired compensating astigmatism, putting in hexapole and other lenses to straighten our curved line images etc are among the embellishments found on commercial instruments today. Any manufacturer will be pleased to point out the virtues of his particular optics, and his competitors will point out the vices.

Perfect Point-to-Line Focussing

Separated field instruments give focussing only up to some order in the direction and velocity, depending on design. When one superposes perpendicularly crossed electric and magnetic fields, one has perfect focussing in both direction and magnitude.

When both fields are present, the equation for motion becomes governed by the Lorentz force, and

$$m \frac{d\vec{v}}{dt} = e (\vec{E} + (\vec{v} \times \vec{B})). \quad (28)$$

In solving the trajectory equation one transforms to a coordinate frame moving perpendicular to both fields with a drift speed

$$v_d = \frac{E}{B} \quad (29)$$

(in MKS units).

The equation of motion in the moving coordinate frame is Eq. (2), i.e., uniform circular motion at the Larmor frequency occurs. The trajectories of uniform circular motion in a frame moving at a constant speed are cycloids. Since the Larmor frequency depends neither on velocity or direction, all the ions of one m/e value meet each other after one Larmor period at a point which is v_d times T , from where they last met: Fig. 16 shows several trajectories of different initial conditions for two masses. Since the Larmor period is proportional to m/e the mass scale is linear. Sometimes called a "trochotron" one such instrument was built at the Bureau of Standards about 35 years ago.

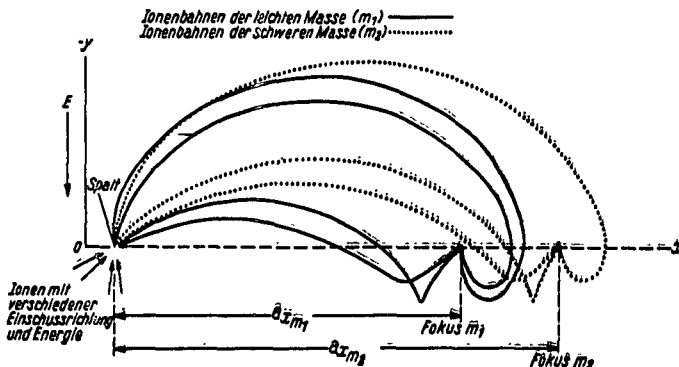


Fig. 15. Cycloidal trajectories in superposed E and B fields. The direction of the B field is perpendicular to the paper.

Wien Filters

Superposed electric and magnetic fields are also used in the Wien velocity filter, which is probably the mass selecting device with the highest transmission of beam. The fields are perpendicular to each other and again Eq. (29) applies.

In the Wien filter the fields are chosen to make $dv/dt = 0$. Only an ion with a speed $v = E/B$ passes through undeflected. From the energy given the ion and the speed determined by E and B , one obtains ions of a single mass. One can see qualitatively how it works by noting that the electric field gives a force on the ion directed toward, say, the left. The magnetic field produces a velocity-dependent force toward the right. When the forces are equal there is no deflection. A lighter ion with its higher speed sees more magnetic field force and is deflected toward the right. A heavier ion with its lower speed sees less force from the magnetic field and yields to the electric field, being deflected to the left. Wien filters are often used to mass select an ion beam for use in atomic physics.

Time-Varying Fields.

Everything said so far has referred to static fields, i.e., they do not change in time. We now consider time-varying fields.

Nothing much need be said about changing magnetic fields as far as mass spectrometry goes. They can't be changed any more rapidly than needed for scanning a mass spectrum due to inductance of the magnets. Furthermore, the change of magnetic field is so miniscule over the time that an ion remains in a magnetic field, it is for all intents and purposes a static field. Still further, all that a changing magnetic field would do is to induce an electric field which would be added to whatever electric field is present from electric sources,

Electric fields can be changed rapidly enough to be strongly felt within the time that an ion is in the field. Several types of mass spectrometers employ time-varying fields.

A. Time of Flight Instruments

The simplest time-varying field instrument is the TOF mass spectrometer. One applies a very short single pulse electric field to the ions. If the ion stays entirely within the field region for the entire pulse it gets a momentum given by

$$mv = eEt, \quad (30)$$

where t is the time that the field is turned on. More often the pulse field is used as a gate to admit a pulse of ion current into a large static accelerating field. In either case, after leaving the field the ion drifts in a field-free region to a detector a distance, L , away. The time at which a pulse of current arrives is recorded. An ion with a kinetic energy, K , has a speed $v = \sqrt{2K/m}$. The time of arrival of the ions following the electric field pulse is

$$t = \frac{L}{v} = \frac{L}{\sqrt{2K}} \sqrt{m} \quad (31)$$

Ions accelerated to the same kinetic energy arrive at different times if they have different masses.

TOF instruments, like most others, are afflicted with a chromatic aberration problem. Here an energy spread smears time resolution and therefore mass resolution. A partial cure is to use an electrostatic mirror field at the end which retards, stops and reaccelerates an ion back into a detector located near the source. In effect, the ion after traversing L rolls up a potential hill and rolls back down. A fast ion has to go further up the potential hill and this takes more time than a slower ion spends in the mirror field; the faster ion travels further overall than the slower ion. While the faster ion reaches the mirror field ahead of the slower ion, after the reflection, the faster ion is playing catch-up and it reaches the detector simultaneously with the slower ion.

It can be shown that in a uniform mirror field with field strength, E , the time for an ion to reach a detector located at the source position is

$$t = \frac{2L}{v} + \frac{2mv}{eE} \quad (32)$$

Taking the derivative and setting $dt/dv = 0$ for first order velocity focussing,

$$v^2 = \frac{eEL}{m} \quad \text{or } K = \frac{1}{2} eEL \quad (33)$$

is the relation between K , E and L . Of course, to reflect an ion the potential hill must be high enough that it can convert all the kinetic energy to potential energy and actually stop the ion. In the case of a uniform field, the length of the mirror field must be one-half the length, L , of the drift space.

B. Periodic Field Instruments - Linear Accelerators

Fig. 17 shows a linear accelerator type of mass spectrometer which is really a scaled down version of a nuclear linear accelerator.

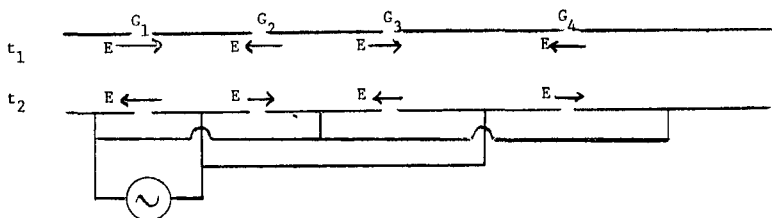


Fig. 4.

A sinusoidal potential of the form $V = V_0 \sin(2\pi ft)$ is applied, and the potentials on adjacent cylinders are 180° out of phase.

An ion, approaching from the left, sees no electric field while within the first cylinder. When it reaches the gap, G_1 , at the time t_1 , it gets an energy kick from the field and then moves into the field-free region within the second cylinder. By the time it reaches the gap G_2 , at time t_2 , the voltages have changed by 180° and the ion gets another energy kick. The process is repeated each time an ion of the correct m/e reaches a gap and the chosen ion gains energy. Those of other m/e values do not reach the gaps at the correct phase of the alternating voltage and do not pick up the maximum energy. Energy separation at the detector is made to distinguish the chosen ion from all others.

In this particular example, the energy kicks are given every 180° of the oscillating voltage, and the lengths of the cylinders increase with distance. In other devices, the frequency is raised so that an ion stays within a field-free region for several cycles and reaches a gap at the time it has the maximum field across it, in phase with the alternating voltage.

Alternative to using cylinders, grids have been used to define the gaps and regions that will be field-free. Care must be taken with locating gaps and field-free distances. If the field-free distances are all of the same length, an ion with half the velocity (4 times the mass of an ion of interest) will get the energy kicks intended for the ion of interest. By choosing the distances to be different, these "harmonic ions" can be effectively eliminated.

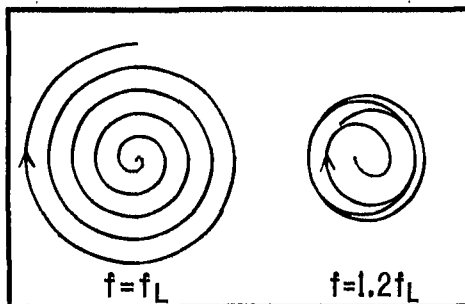
C. Ion Cyclotron Resonance

The trouble with linear-accelerator-type devices is that they can get long. So we

steal another idea from the nuclear accelerator people, E.O. Lawrence in this case, namely combining a magnetic field with a crossed oscillating electric field, and give the ions energy kicks while they are travelling on circular orbits in the magnetic field.

If the magnetic field is in the Z-direction and the electric field is in the Y-direction and oscillates at the Larmor frequency ($f_L = eB/2\pi m$), there is a component of the electric field in the direction of the ion motion^L except at the two instants when the ion travels exactly in the X-direction. An ion whose Larmor frequency coincides with the applied frequency thus constantly gains energy from the field (the kicks are not sharp kicks but gentle, almost continuous shoves). As it gains energy its Larmor radius increases and the motion is that of a spiral, as shown in Fig. 18, on the left.

Fig. 18. Ion trajectories in ICR.



The ion and the field are said to be in resonance and in phase.

If the ion's Larmor frequency is different from the applied frequency the motion is as shown in the right hand figure, this particular trajectory being that for the applied frequency being 20% higher than the Larmor frequency. For a while the ion spirals outwardly, but the ion and the applied field soon get out of phase. The outwardly spiralling ion stops and it begins to spiral inward. In the course of time its trajectory puffs in and out. The nearer the applied frequency is to the Larmor frequency, the greater the maximum distance from the center is.

Three methods are used to detect the ions in an ICR device. In "omegatrons" one puts an ion current detector far away from the center and intercepts the ion current far out on the spiral. Another method uses the fact that the selected ion is constantly gaining energy at the expense of the oscillator providing the electric field, whereas on the average a non-resonant ion does not. One simply measures the power drain on the oscillator.

The third method is not to use an alternating field, but instead to shock the ions with an electrical pulse, get them all oscillating at their Larmor frequencies and then "listen" to them ring. As an ion comes near a metal surface it induces a charge on that surface. The induced charge moves to the surface from ground. Having it move through a resistor or inductor provides a voltage signal. The voltage signal is quite complex in time since ions of all different Larmor frequencies contribute to it. Fourier analysis of the voltage signal is made to determine the masses and abundances of the ions.

Resolution in an ICR device is effectively equal to the number of Larmor periods the ion completes. Collisions with residual gas molecules interrupts the phase relationship needed and one requires long path lengths (good vacuum) and short Larmor periods (strong magnetic fields) for good resolution. The advent of superconducting magnets makes ICR very attractive. And ICR's old vice of gas collisions limiting its capability becomes a virtue for purposes of MS/MS.

D. Quadrupole Mass Spectrometers

The most commonly used time-varying field instrument is the quadrupole mass spectrometer. The most common form is the hyperbolic cylinder ion pipe shown in Fig. 14. The only difference is that both dc and radiofrequency fields in series are applied to the surfaces.

Fig. 19 shows this and two other configurations.

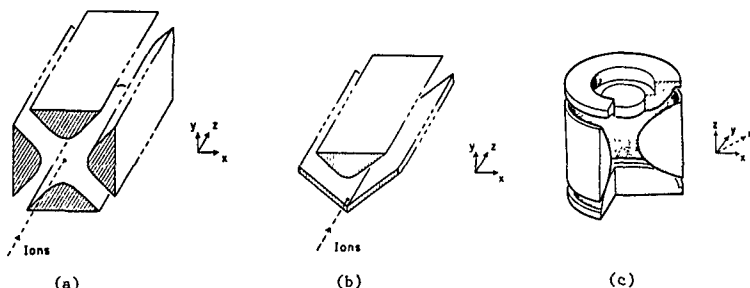


Fig. 19

(a) is the quadrupole mass filter, which is often made using circular rods rather than hyperbolic surfaces because of the relative ease of machining to the very high tolerances required. (b) is a monopole, where the V-shaped piece is grounded and physically replaces the mathematical potential surfaces with potentials equal to zero between adjacent poles in a quadrupole. (c) is a hyperboloid of revolution and is used as a three-dimensional ion trap.

With both dc and rf fields applied, the equations of motion can be reduced to the Mathieu equation, solutions for which were obtained centuries ago by our astronomer friends. The principal feature of the solutions is that the trajectories are either stable or unstable (as in the case of the rf ion pipe), but that with the dc potential added the instruments pass a limited band of masses (rather than all masses above some selected value.)

The width of the band of masses passed depends on the values of U and V, the dc voltage and amplitude of the rf voltage, respectively, and can be adjusted to transmit a single mass, thus making it a mass spectrometer. Fig. 20 shows the "stability" diagram for the quadrupole mass filter.

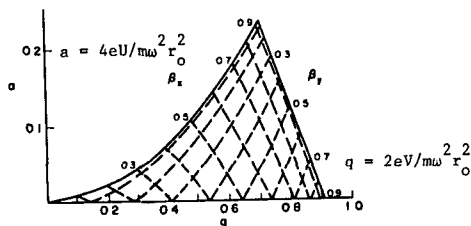


Fig. 20

Trajectories for which the values of "a" and "q" are in the triangular cross-hatched area are stable; those outside the triangle are unstable. One chooses the value of a (U) and q (V) such as to operate very close to the sharp peak of the stability diagram, to select ions of a given mass, and then changes U and V simultaneously to select other ions.

The curvature of the left-hand side of the triangle is to be noted, in connection with a fringe-field problem of quadrupoles. Since the rf and dc potentials are placed on the same surfaces, the ratio of U/V remains constant in the fringe fields, and the values of both fall off as one moves away from the end of the quadrupole. This places an ion on an unstable trajectory as it attempts to enter the quadrupole through the fringe fields and makes injections of ions difficult. The solution to the problem was first recognized by

W. M. Brubaker who added a very short quadrupole on which rf voltage only was applied at the end of the analyzing quadrupole. This effectively lets an ion see the rf fringe fields before it sees the dc fringe fields, and the values of a and q seen by the ion remained within the triangular area while in the fringe fields. This author found another solution which was to pass the ions through a leaky dielectric tube as they entered the mass filter. The tube acts as a conductor to dc fields and therefore excludes them from the interior of the tube, but it acts as a dielectric to the rf fields and allows them to penetrate to the interior of the tube.

Quadrupoles are very forgiving devices, in comparison with most mass spectrometers. They accept a fairly broad energy spread on the ions; all that is necessary is to make the quadrupole long enough or the frequency of the rf high enough to allow the ion to see enough rf oscillations to know whether it is to be passed or rejected. One can be relatively careless about the position and direction of entry of the ions into the quadrupole. In a sense they have both direction and velocity focussing. There is no limit to the mass range (Extranuclear has delivered one unit with a mass range of 100,000 AMU and extendable). The limitation on quadrupoles is mass resolution (to around 6000 FWHM definition) which is set by the accuracy to which the mass filter itself can be machined and assembled. No doubt, this will improve with improved machining techniques.

Production of Electric Fields

Eq. (7) states that an electrostatic field is the gradient of an electrostatic potential, i.e., $E = -\nabla\phi$. Virtually all electron and ion optical devices in the past have used this fact and the fact that electrically conducting metal surfaces are equipotential surfaces, to produce electric fields around and between metallic lens elements. Some of the basic facts about producing electric fields in this way are:

- a. Boundaries are good conductors, i.e., metals, to which voltages are applied.
- b. Boundaries are equipotential surfaces.
- c. No currents flow in the boundaries.
- d. Static surface charges appear on the boundaries.
- e. The direction of E is perpendicular to the boundary at every point on the boundary.

There is another way of producing electric fields. It follows directly from Ohm's law,

$$\vec{J} = \sigma \vec{E}$$

where J is the current density (amperes/m²) and σ is the electrical conductivity of the medium through which the current flows. It follows from Maxwell's fourth equation that an electric field parallel to a surface and immediately inside a material is identical to the electric field immediately outside the surface.* The electric fields at boundaries can define the fields throughout a region of space just as well as potentials can.

This permits producing electric fields in vacuum by producing currents in bounding surfaces. Basic properties of this approach are:

- a. Boundaries are poor conductors, i.e., leaky dielectrics, with voltages applied at the edges to produce currents within materials of the boundaries.
- b. Boundaries follow E field lines.
- c. Currents flow in the boundaries.
- d. No static charges appear on the boundaries.
- e. The direction of E is parallel to the boundary at every point on the boundary.

With leaky dielectric lens elements the geometrical configuration changes and often performance is improved in ion optical systems. Figure 20 gives three examples of both methods to produce the same fields.

* provided that there is no magnetic field that is changing with time present.

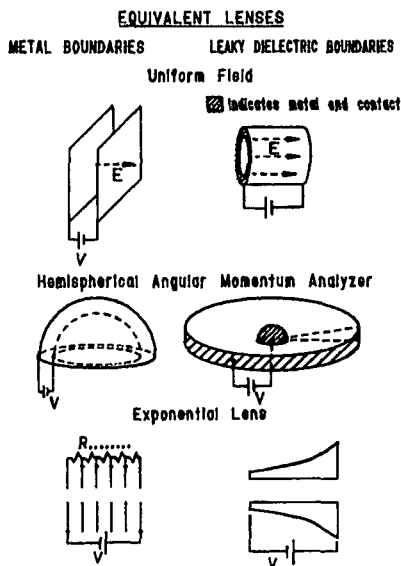


Fig. 20

The first is a uniform field. Conventional optics calls for two parallel plates whose dimensions are large compared to their separation. The field becomes non-uniform near the edges. The leaky dielectric approach uses a simple cylinder with current running along its length. The field is uniform throughout the cylinder all the way to the inner wall. Such a field has been used in drift tube measurements at Extranuclear.

The second is a hemispherical angular momentum analyzer (which is also a first-order energy analyzer). Instead of two concentric metal hemispheres between which the ions move, the leaky dielectric equivalent is a single flat disc with a small metal hemisphere at the center. The disc is linearly tapered from the center to the outer perimeter. This is the sphericEL energy filter that is sold by Extranuclear for energy filtering in SIMS. Not only does it eliminate the bulky metallic hemispheres which can get in the way in an application, but it allows a broader angular divergence. Fringe field effects are greatly improved over the conventional devices.

The third example is the well-known exponential lens used to simultaneously retard and focus ions with paraxial trajectories. In place of having a series of metal aperture plates with a chain of resistors whose values are chosen to produce an exponential field along the axis, one simply shapes the wall thickness of a leaky dielectric tube appropriately.

Because electric currents flow constantly in these devices energy is dissipated in them. One seeks to keep the currents low to minimize heating, and hence makes them from "leaky" dielectric materials. As long as any ion currents which strike them are small in comparison with the currents flowing within the material, they work fine. The development of synthetic materials with good uniformity of conductivity and conductivity in the desired range (typically 10^4 to 10^7 ohm-cm) makes possible the increased use of leaky dielectric devices for the production of electrostatic fields. I suspect that they will increasingly be found in mass spectrometry in future years.

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A TANDEM QUADRUPOLE-FOURIER TRANSFORM MASS SPECTROMETER

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Quadrupole mass filters and magnetic/electrostatic sectors are the most common types of analyzers now used for mass spectrometry. Quadrupole mass filters consist of four parallel rods with appropriate rf and dc voltages applied to pass only ions within a narrow mass range. Sector instruments utilize magnetic and electric fields to disperse ions of different mass-to-charge ratios. These instruments are called scanning spectrometers because a mass spectrum is obtained by bringing ions of different mass-to-charge ratios sequentially to focus at the detector. At any given instant only a narrow range (around 0.1 %) of the mass spectrum is detected, and those ions not focused on the detector simply strike the walls of the apparatus and are lost. This is a very inefficient process which ultimately limits the detection sensitivity of the spectrometer. Another problem with quadrupole and sector instruments is that they require mechanically complex, precision machined components to achieve maximum mass resolution.

Fourier transform mass spectrometry (FT-MS) utilizes new technology and operating principles which overcome many of these limitations. FT-MS instruments are far simpler mechanically than conventional mass spectrometers, and advanced electronic techniques are utilized to achieve unmatched stability, mass resolution and mass measurement accuracy. Rapid switching under computer control is possible between various modes of operation such as positive or negative ions, electron impact ionization, low pressure chemical ionization, and laser multiphoton ionization.

In spite of the many advantageous features of FT-MS, the commercially available instruments have not been widely accepted for analytical applications. One difficulty is that the general performance of the spectrometer, its mass resolution and detection sensitivity, degrades to serious levels if the pressure in the analyzer cell exceeds about 10^{-6} torr. This makes it difficult to interface a FT-MS instrument to previously developed sample handling and separation techniques such as fast atom bombardment, gas chromatography, and liquid chromatography because all of these present a large gas load to the spectrometer. Another problem is that the analyzer cell can be overloaded with ions, making it difficult to detect minor components.

One approach to overcome these problems is to separate the ion production and ion detection regions. A spectrometer of this type, called a tandem quadrupole-Fourier transform mass spectrometer, has recently been developed and is shown in Fig. 1. Positive and negative ions are produced in a standard ion source of a Finnigan MAT quadrupole mass

spectrometer. The ions are focused into a beam and injected into a quadrupole mass filter. By adjusting the rf and dc voltages applied to the quadrupole rods, unwanted ions can be rejected and ions of interest can be transmitted. Next the ions are accelerated into a superconducting solenoid magnet, trapped in a modified ICR analyzer cell, and detected by FT-MS. The vacuum manifold is evacuated by high-capacity turbomolecular pumps to decrease the pressure caused by the sample inlet.

It appears that tandem quadrupole/FT-MS combines many of the best features of both instruments. Highly developed chromatographic techniques and sample ionization methods which are currently available on quadrupole mass spectrometers may be combined readily with the ultra-high mass resolution available with FT-MS detection. High mass resolution is possible because the FT-MS analyzer cell is maintained at low pressure by several stages of differential pumping. Another advantage is that the FT-MS analyzer cell is not overloaded with extraneous ions because the quadrupole rods reject ions which are not of interest. Ion space charge effects are thereby avoided and the full dynamic range of the image current detector can be used for the ions of importance. Also, ions in the FT-MS analyzer cell can be stored for several minutes since the pressure is so low, and this permits a sequence of experiments to be performed on the same set of ions.

Progress with FT-MS has been very rapid in the last two years since the first commercial instrument was introduced. By today's standards FT-MS requires sophisticated electronics and expensive computer hardware. But these costs are decreasing rapidly, and it is safe to say that the unique analytical capabilities of FT-MS will become more widely available in the coming years.

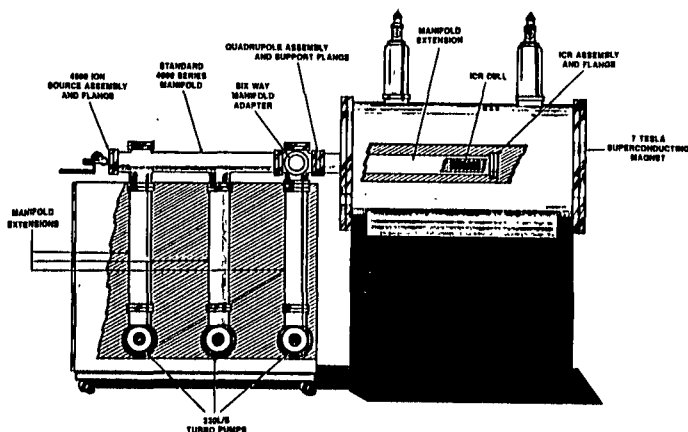


Fig. 1: Tandem quadrupole-Fourier transform mass spectrometer.

A MOLECULAR BEAM/QUADRUPOLE MASS SPECTROMETER SYSTEM
WITH SYNCHRONIZED BEAM MODULATION AND
DIGITAL WAVEFORM ANALYSIS

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This paper discusses the physical and performance characteristics of the subject molecular beam/mass spectrometer (MB/MS) system, and includes application of the system to a low-pressure (1 to 30 torr) microwave-discharge flow reactor (DFR). The DFR was used to form methylperoxy radical and enable study of its subsequent destruction in the presence/absence of NO_x . Recently, the system has been used to study decomposition and oxidation of highly reactive pilot fuels, e.g., silane (SiH_4), which can aid ignition and flameholding in subscale supersonic ramjet combustors being studied at the Langley Research Center.

The present 4-stage differentially-pumped, modulated-MB/MS system is illustrated in Fig. 1. It represents the result of a series of mechanical design modifications to an earlier MB system developed by Midwest Research Institute; originally it was a highly efficient atmospheric-pressure sampler, and was coupled to a Bendix-TOF MS. The DFR exits just below the sampling orifice, which protrudes into the mechanically pumped fourth stage. A system flow chart, Fig. 2, illustrates (a) control of the beam chopper synchronization by four repetitive analog scanners (PROMIMS), and (b) coupling and synchronization of the four sequential analog outputs to a digital processing oscilloscope/minicomputer (DPO).

The system shown in Fig. 2 allows near-simultaneous monitoring of four masses (48 msec/set), with the capability for long-term digital averaging (up to $\sim 10,000$ repetitions) of the time-multiplexed, modulated molecular beam (84 Hz), analog outputs. The chopper control interface subsystem was developed so that channel completion pulses from the sequential analog controllers (12 msec dwell time for each PROMIM) are initially shaped and combined to produce a 42 Hz square-wave, which serves as a driver for the beam chopping circuitry. Next, phase detection and control circuits are combined with a voltage controlled oscillator for powering the beam chopper motor; this results in stable long-term (e.g., >500 s) chopper synchronization when the four analog outputs are processed sequentially and repetitively. Phase is maintained (typically ± 2 -degrees) such that each 12-ms ion current output is split equally between conditions of molecular beam off and on, typically with ~ 1 ms transition time. An important benefit of the present chopper synchronization is that it allows extensive digital waveform averaging with substantial improvement in signal/noise ratio. The 84 Hz chopper modulation rate, which is a non-multiple of 60 Hz and is fast enough to avoid pressure oscillations in the ion source region, also permits attainment of 100-percent real-time sampling efficiency with near-maximum DPO sampling/averaging rate.

The 4-channel programmable amplifier in Fig. 2 was developed to provide a wide range of independent signal gain and baseline shift control for each mass output. In addition, 2-ms signal blanking circuits were included in order to eliminate channel switching transients which adversely affect the DPO autoscale function. Pixel averaging along the eight predefined sampling sections (darkened blocks) of the DPO waveform, shown in Fig. 3, followed by subtraction of averaged ion-source background (MB off) signals, provides differential (MB only) outputs from which species ratios and statistics are calculated.

Results obtained during the DFR study of methane oxidation kinetics are used to illustrate the performance of the overall system. For example, the Fig. 3 data, which were obtained at 1.5 torr with 20 eV ionization energy and a standard ion source, support a lower detection limit of about 1-ppmv (5×10^6 molecules/cc) at masses which do not contain major background species. In summary, the presently developed MB/MS system, including controlled MB modulation and digital waveform analysis, has led to very substantial improvements in attainable signal/noise ratio, detection limit, and accuracy over prior systems.

Fig. 1- THREE-STAGE MODULATED MOL. BEAM/MS SYSTEM WITH DISCHARGE FLOW REACTOR

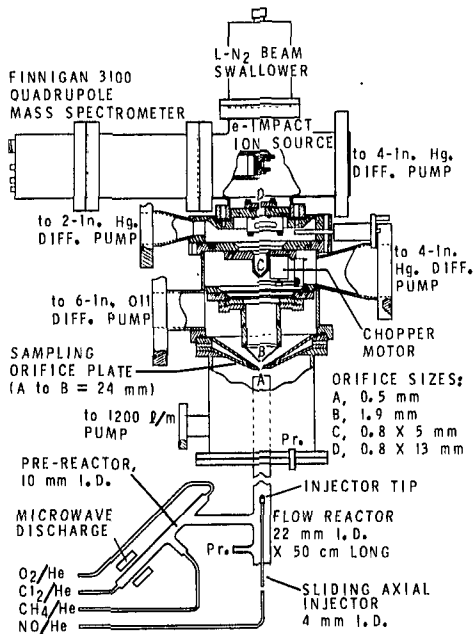


Fig. 3- TYPICAL SINGLE-RUN OUTPUT FROM DPO DATA PROCESSING SYSTEM.

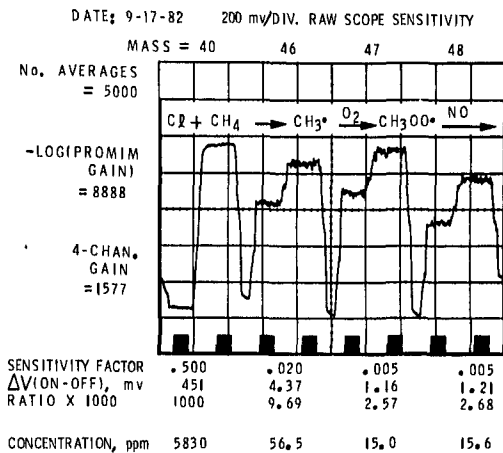
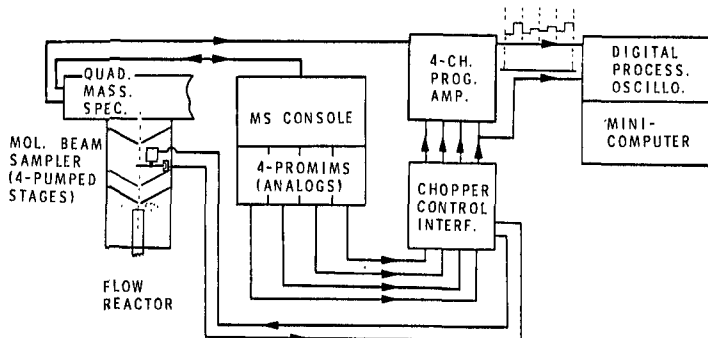


Fig. 2- CONTROL OF MODULATED MOLECULAR BEAM/MS SYSTEM AND DIGITAL AVERAGING OF REPETITIVE 4-MASS SIGNAL



RECENT IMPROVEMENTS IN ION TRAP TECHNOLOGY.

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Finnigan MAT Corporation, San Jose, California 95134.

The ion trap mass spectrometer has been characterized by many investigators over the past ten years. Hyperbolic electric fields are used for mass separation in the ion trap and the basic equations that describe the ion trap are similar to the equations for the quadrupole mass analyzer. To date, mass range, resolution, and complexity of operation have limited the ion trap from being used as an analytical instrument.

Figure 1 is a line drawing of the ion trap detector (ITD). Sample enters the ion storage region from the GC via a heated fused silica transfer line. The sample is then ionized by electron impact (EI) and all ions within the mass range of interest are stored. The ITD is then scanned through the mass range causing the ions to be ejected from the ion storage region sequentially from low to high mass. Finally, the ejected ions are detected by a conventional electron multiplier. The resultant output from the electron multiplier is a mass spectrum indicative of the sample introduced into the ion storage region. The ITD is interfaced to the IBM PC based data system where complete data storage occurs.

Figure 2 is an example of a capillary column GC analysis of 5 ng each of a phenol mixture to determine if chlorocresol is present. The ITD was scanned from mass 50 to mass 300 every 1 second. Step 1 is the gas chromatogram obtained from the analysis. Step 2 shows a chromatogram of m/z 142, which is a characteristic mass of chlorinated methylphenols. As can be seen, at scan number 544 a peak appears. The next step (3) is to obtain a mass spectrum of this scan and then finally in step 4, compare the unknown spectrum to a reference spectrum. In this example the NBS (National Bureau of Standards) compilation of mass spectra library is used.

An important aspect of mass spectrometry is minimum sample detectability. Figure 3 shows the detection limit comparison between the flame ionization detector (FID) and the ITD. These data are from splitless injection capillary column GC analysis of 100 pg of naphthalene. The top trace is the FID chromatogram and shows a signal-to-noise ratio (S/N) of approximately 10:1. The bottom trace is a portion of the mass spectrum of naphthalene obtained from the ITD in a normal mass scanning mode; the S/N for m/z 128, which is the ion characteristic of molecular weight, is approximately 12:1. As can be seen, the detection limit for both detectors is approximately the same under the above conditions.

The ITD is designed to operate with mass resolution adequate to only separate adjacent masses. Figure 4 shows the actual mass peak profiles of perfluorotributylamine for low mass m/z 69 (a) and its naturally occurring carbon 13 isotope at mass m/z 70 and for high mass m/z 502 (b) and its naturally occurring carbon 13 isotope m/z 503.

In conclusion, the ITD is an inexpensive mass spectrometer that offers good performance and several operational advantages. From the performance standpoint the ITD has: comparable sample detection to conventional GC detectors, and similar resolution and mass range when compared to a quadrupole mass spectrometer. Among the operational advantages are: simple, reliable design, and minimal tuning or adjustments.

Finnigan MAT Ion Trap Detector

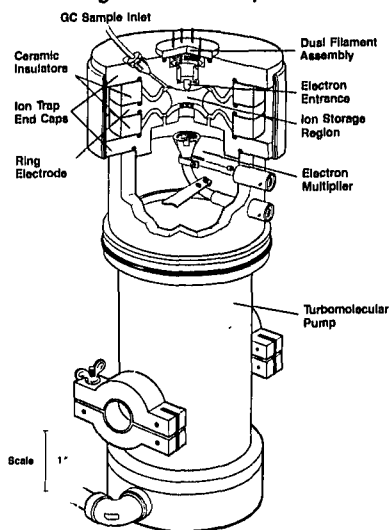


Figure 1

Procedure for Compound Identification Utilizing the ITD

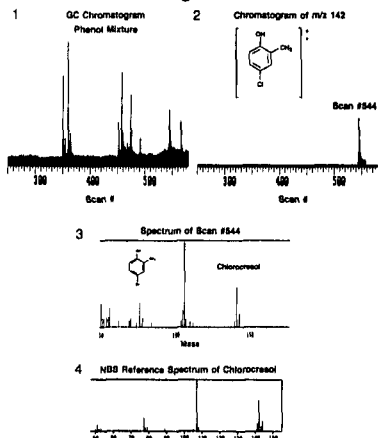


Figure 2

Detection Limit For FID vs. ITD

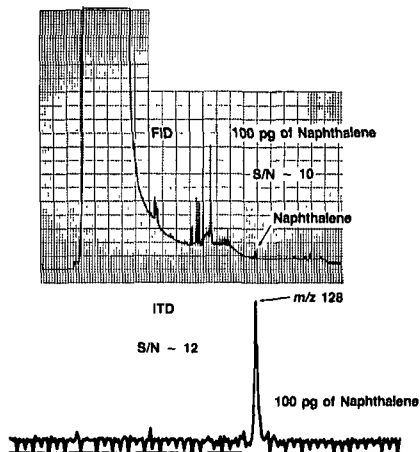


Figure 3

ITD Performance at m/z 69 (a), and m/z 502 (b) from FC-43

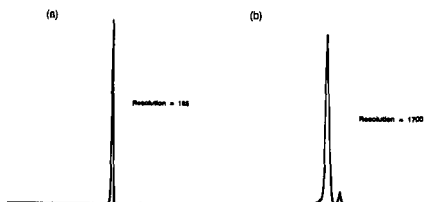


Figure 4

NEUTRALIZATION-REIONIZATION MASS SPECTROMETRY; P. O. Danis, P. J. Todd, G. I. Gellene, J. C. Ray, B. K. Carpenter, and F. W. McLafferty, Chemistry Dept., Cornell Univ., Ithaca, NY 14853

NRMS is a new mass spectrometric technique for neutrals formed from ions (Durup, et al., Chem. Phys., 1977, 25, 245;; McLafferty, JACS, 1980, 102, 3360) which appears to be useful for studying neutral products of gaseous ionic reactions and properties of highly unstable molecules. A precursor ion for the desired neutral is mass analyzed, and neutral species are produced by metastable or collisionally activated dissociation (CAD), charge exchange, etc. Residual ions are stripped from the resulting beam of fast neutrals by a defocusing ion lens, and the beam is reionized (and dissociated) by collision with a neutral target gas. The Cornell tandem double-focusing mass spectrometer is particularly convenient for NRMS; parent ions from reionization of neutrals of a specific mass can be selected by the second electrostatic analyzer before CAD and mass analysis in magnet-II.

Relatively high intensity neutral beams can be generated by the method of Porter and Gellene. (G. I. Gellene and R. F. Porter, Acct. Chem. Res., 1983, submitted for publication). The ion beam leaving MS-I exit slit interacts with metal vapor generated in a microfurnace; charge exchange efficiency is maximized by matching the ionization energies of the metal and ion. The CAD mass spectra of neutrals are often closely similar to those of the corresponding ions except that low energy products from neutrals are much less abundant. Not too surprisingly, the CAD mass spectra of neutral acetone is quite similar to the ordinary electron ionization mass spectrum of this compound.

Unique data can be obtained from unstable neutral species such as ND_4 and cyclobutadiene which are difficult to prepare by other methods. C_4H_4^+ ions from a cyclobutadiene-adduct, separated in MS-I, neutralized by the metal vapor, and reionized by CAD yield a mass spectrum of neutral cyclobutadiene. The effect of isotopic labeling and different metals on the ion yield and CAD spectrum is currently under investigation, as such data could provide evidence of the structure and ionization energy of cyclobutadiene.

The MM 1: A TRULY MOBILE MASS SPECTROMETER

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The mobile mass spectrometer MM 1 transports the analytical laboratory to the analysis location, in contrast to the normal practise of transporting the samples to the stationary laboratory.

Examination of the chemicals involved in the bursting of or leakage from tanks in truck or train accidents, detection of leakages in chemical plants, rapid surveillance of refuse dumps and even detection of chemical warfare gases are typical examples of the tasks for which the MM 1 mounted in different types of vehicles, is suitable. The MM 1 can also be employed as a stationary detector, e.g. for process or environmental control in a chemical factory, involving many monitoring points.

The MM 1 can continuously monitor up to 22 organic pollutants in the air and by use of the surface sampler up to 12 on the surface, during one measurement. In the air the detection limits are in the low ppb range, on the surface in the low nanogram/cm² range. The sophisticated software virtually eliminates false alarms due to interfering ions from other substances.

Compared with the current sophisticated hardware (from tandem ms-ms to very special ionisation methods) being presented at this conference, the MM 1's mass spectrometer is rather classical in concept, consisting of double filament electron impact source, a hyperbolic one piece glass quadrupole, and a conventional Cu-Be multiplier. A little more unusual is the use of an 80 l/s ion getter pump to maintain the vacuum in the UHV system.

The rest of the equipment is however rather unusual. This includes, in particular, the membrane inlet system, the flexible samplers, the electronics and the software controlling system. Nearly all the components of the mass spectrometer and the whole electronics were developed from scratch to achieve the four following unusual goals:-

- 1) Resistance to extreme environmental conditions, e.g. temperature and humidity extremes, shock and radiation
- 2) Easy and fast operation by the operator after minimal training
- 3) Minimum ambiguity in the analytical results
- 4) Fast reporting of instrumental failures.

This is reflected in the first goal, that the apparatus must work in the temperature range from -10°C to 50°C, be shock and vibration proof up to 2 g and radiation proof up to 300 rem. Versions of the MM 1 protected against NFMP (narrow band electromagnetic pulses) are also available.

The MM 1 can be mounted in off road vehicles and be operated while driving over rough terrain. Mounting of the MM 1 in armoured vehicles with air filtration units to protect the operators has been successfully carried out. This enables the operators to use the air and surface samplers from inside the vehicle, without being exposed to a hostile external environment. In the mobile version the MM 1 may be connected with special vehicle orientation units. This allows the printing out of the vehicle's exact position on the analysis report. The second goal, simple operation, is achieved by microprocessor control, video display and intelligent push button operation. The

complete monitoring task can be carried out by the use of only four push-buttons. These monitoring tasks include air monitoring, surface monitoring, with display of concentration v. time curves for individual ions, as well as the occasional acquisition of spectra either from the air or the surface. The latter allows compound identification by a small, built-in library, or printout of the spectrum in list form. The complete monitoring procedure uses data stored in an exchangeable PROM, so that the MM 1 can be rapidly adapted to changing analytical tasks. In this PROM the data for up to 60 compounds can be stored.

The third goal, no false alarms, is achieved by a sophisticated algorithm controlling the monitoring procedures. For each substance four characteristic ions, of known abundance, are monitored by a SIM procedure. Substances, e.g. diesel fuel, which could give rise to false alarms and are frequently present, are included in the monitoring procedures. A system of individual interference levels protects against false alarms in such cases where a high concentration of compound generates interfering ions over the mass range of interest.

A continuously operational supervision program can detect more than 80 instrumental errors or malfunctions. All the errors are counted, serious errors are displayed on the video screen. Additionally a check program for all the essential functions of the apparatus can be manually started. Built-in service programs allow the measurement of 30 analogue values (temperatures, voltages, currents) and about 100 digital switching states.

Although specially developed for pollution monitoring, the MM 1 is capable of other analytical measurements. Special programs allow the acquisition and easy data handling of a series of spectra, monitoring of selected ions with individual alarm or warning levels as well as the display of concentration v. time curves for four ions. With the help of these programs the chemist can develop an analytical procedure tailor-made for his own problem and create, within the instrument, his own data PROMS for subsequent use.

VELOCITY COMPACTION IN A TIME-OF-FLIGHT MASS SPECTROMETER

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Despite the unique combination of efficient heavy-mass ion transmission, high sensitivity and rapid time response, current limitations in mass resolution severely restrict the usefulness of Time-of-Flight Mass Spectrometers in many laboratory situations. A concept for improving the resolution performance of a pulsed-beam time-of-flight mass spectrometer (TOFMS) is described whereby the velocities of a plurality of iso-mass ions are equalized (velocity compaction) by subjecting a transiting ion bunch, partially separated into iso-mass ion packets, to a specific time-dependent and monotonically time-varying acceleration force field. Concurrently, space compaction or space focussing is achieved through a speeding up of the retarded ions (relative to the advanced ions) in a given iso-mass ion packet. For low constant voltage ion extraction, the condition for which the slower ion of a given mass will attain the same velocity as the faster one is given by the relation

$$\Delta V(t)/\Delta t = (V/l) \sqrt{2qV_x/m} \quad 1)$$

where the extraction voltage V_x is negligible compared to V , the applied voltage value at the time of passage of ions of charge to mass ratio q/m over accelerating region of length l . In eq. 1), $\Delta V/\Delta t$ is the time rate at which the voltage is to be increased over the accelerating region. Moreover, if the voltage $V(t)$ is varied according to the relation

$$V = V_{ref} t^r/c \quad 2)$$

then velocity compaction will apply equally to all mass groups. Here V_{ref} is the voltage applied at the time ions of some reference mass enter the varying acceleration region and c and r are adjustable constants which depend on the extraction voltage V_x and the distance between center of ion formation and extraction grid, and the lengths of a post extraction drift region and the varying accelerating region.

Computer simulations of the flight trajectories (arrival times) were made and incorporated an ion source temperature (Maxwell-Boltzmann) distribution of velocities. A mass resolution of 5000 ($M/\Delta M$)_{fwhm} is indicated at mass 1000 amu. A Bendix Model 12 Time-of-Flight mass spectrometer was modified¹ for a laboratory demonstration of this new ion focussing principle. Results of a successful demonstration of a single stage version of a velocity compaction TOFMS are consistent with the computer simulation results over the range of lower masses that were experimentally studied. A value of $M/\Delta M$ _{fwhm} equal to 1176 was obtained at mass 173 amu. In summary, when the acceleration field is properly contoured in both space and time velocity compaction and space compaction simultaneously are achieved for a wide range of iso-mass ion packets and the mass resolution for heavier mass ions is particularly improved while the inherent efficiency of this instrument for heavy mass ion transmission is retained. A two stage version, capable of much higher mass resolution, is proposed for future study.

1) M. Luis Muga, "An Improved Time-of-Flight Mass Spectrometer", International Application Publication Number WO 83/00258, 20 January 1983, Patent Cooperation Treaty, World Intellectual Property Organization, Geneva, Switzerland,

APPLICATION OF A NEW TIME-OF-FLIGHT SIMS

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Sputtering of organic molecules results in the emission of parent-like secondary ions as $(M\pm H)^{\pm}$ e.g., provided the molecules are in an appropriate chemical environment (for a review see /1/). Yields in the 10 % range can be obtained from amino acids, e.g. These yields and typical damage cross sections of some 10^{-14} cm² result in a probability of some percent for a surface molecule to be transformed into a parent-like secondary ion during sputtering. A considerable decrease of this probability is expected for larger molecules.

A complete utilization and efficient analysis of the generated secondary ions is crucial in organic and inorganic trace analysis, for the undisturbed analysis of surfaces that are very sensitive to ion bombardment and especially for secondary ion mass spectrometry of very large organic molecules. These requirements demand a high transmission mass spectrometer detecting all secondary ions simultaneously after their e/m analysis. Additional properties of such an instrument should be a high mass range, a high resolution and energy and angle focussing.

Time-of-flight (TOF) instruments are characterized by high transmission, high mass range, simultaneous detection of all masses, but a relative low mass resolution. An optimum application of this type of mass spectrometer as an extremely sensitive SIMS instrument has to consider some main properties of secondary ions: their angular and energy distribution, their origin from an extended surface area, the large fraction of metastable ions, and the sputtering yield of the sample.

A TOF-instrument for secondary ion mass spectrometry may be of a straight forward construction or it may be more sophisticated and adapted to these special needs of secondary ion analysis. In our laboratory we have developed and operate now a TOF-instrument that meets some important requirements. It is equipped with a pulsed, mass separated primary ion source that enables the removal of a monolayer on 0.05 cm² in some minutes, an energy and angle focussing time-of-flight path, and a post-acceleration combined with an ion-electron-photon

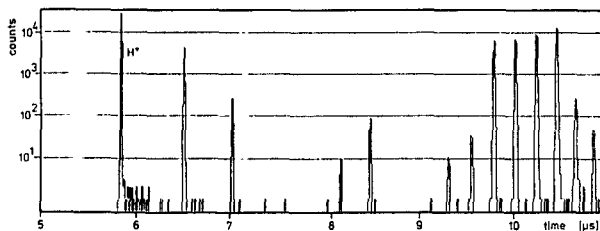


Fig. 1: Positive spectrum in the low mass range. Total number of primary ions: $8 \cdot 10^7$

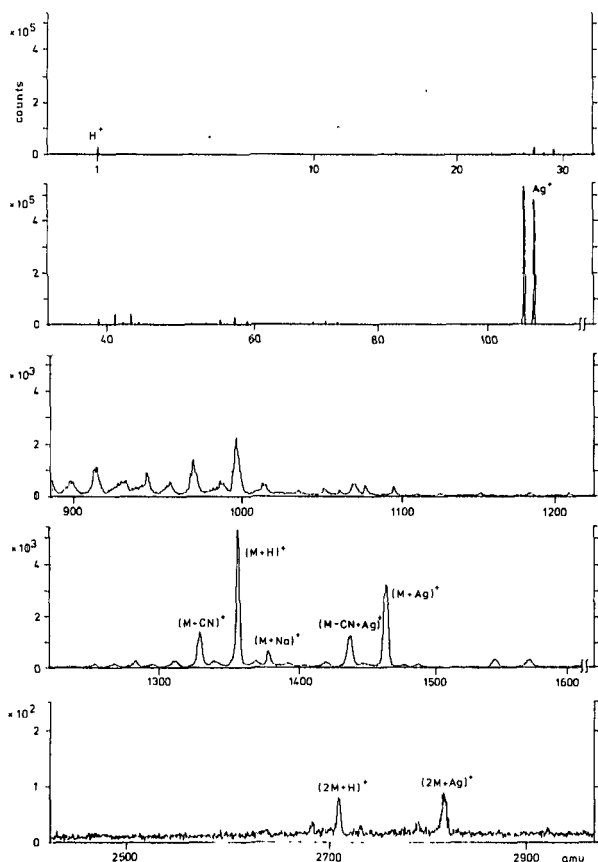


Fig. 2: Positive spectrum of vitamin B12. Total number of primary ions: $3 \cdot 10^8$

converter, providing a stable and efficient detection of large molecular ions up to several 1000 amu. The instrument can be operated for positive and negative ions. The positive and negative secondary ion emission from various biomolecules as vitamin B12, gramicidin D, bradykinin, folic acid analogues etc., and other solid surfaces as anion covered Ag and teflon have been investigated in the mass range 1 - 4000 amu. A mass resolution of about $m/\Delta m = 750$ was obtained routinely. The biomolecules were deposited as a mono- or submonolayer in a target area of about 0.05 cm^2 . This corresponds to typical amounts of sample material in the pmol range. For a mass spectrum as it is shown in Fig. 2, about 1 % of the sample material is sputtered.

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BEQQ: PERFORMANCE OF NEW INSTRUMENTATION FOR MS/MS

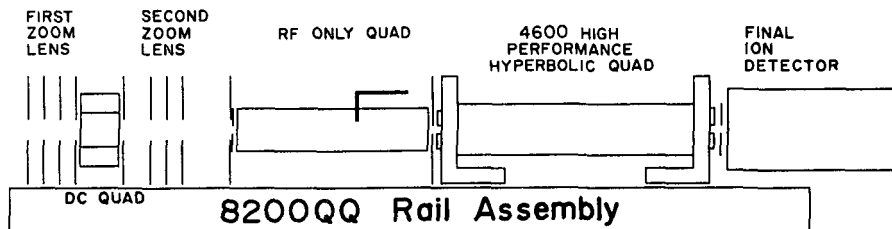
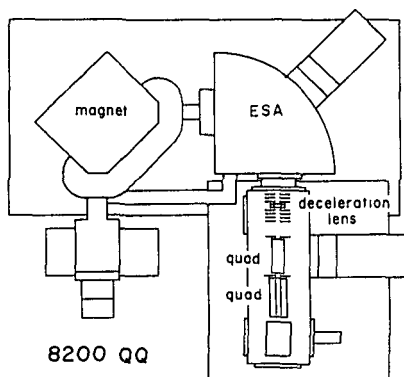
A. E. Schoen and P. Dobberstein, Finnigan MAT, 355 River Oaks Parkway, San Jose, CA 95134; J. W. Amy, J. D. Ciupek, and R. G. Cooks; Purdue University, West Lafayette, IN 47907.

A high resolution double focussing BE mass spectrometer is combined with a quadrupole collision cell and a high performance quadrupole mass analyzer to yield a hybrid BEQQ instrument(1). Based on a Finnigan MAT 8200 double focussing mass spectrometer, a lens system is added to decelerate and shape the ion beam prior to its entry into a highly efficient, variable energy quadrupole collision cell. The daughter ions emerging from this cell are then mass analyzed by a Finnigan MAT 4600 series quadrupole. The BE section of the instrument provides high parent resolution for MS/MS while the double quadrupole accessory provides unit mass daughter resolution to 1800 daltons. All MS/MS scan modes consistent with BE geometry are enabled as well as new linked scan modes involving the added quadrupole section. A variety of scans are available to record all daughters from a selected parent ion, all ions undergoing a specified neutral loss and all parent ions producing a specific daughter.

The QQ accessory is constructed on an alignment rail which permits axial adjustment and interchangeability of the ion optics. This rail is mounted in a vacuum manifold which allows easy access to all interior parts. A key element of the design is a dual zoom deceleration lens system. The first section of the lens translates the ion beam from the exit slit of the 8200 to a DC quadrupole lens and adjusts the beam's energy to 200 volts. This permits both 3 keV ions as well as daughters from prior CAD, which will have less kinetic energy, to be sampled by the following elements. The DC quadrupole shapes the ion beam and the final zoom lens decelerates the 200 eV ion beam to the desired collision energy required by the RF only quadrupole collision cell.

A range of collision energies is available in four regions of the instrument for positive, negative and negative to positive ion/molecule reactions. Variable low energy collisions in the collision quadrupole allow the use of energy resolved mass spectrometry (ERMS) for the determination of isomeric ion structures based upon the resultant breakdown curves. Addition of a needle collision cell to the source enables angle resolved mass spectrometry (ARMS) for 3 keV collisions in the first field free region.

The applicability of this instrumentation for mixture analysis is demonstrated by the separation of 1-methylfluorene and phenazine by the sector region of the instrument and the recording of the constituent daughter spectra. At a resolution of 6000 (10% valley), the daughter spectrum of each constituent of a 1:1 mixture is almost entirely free of any interfering peaks from its isobar. The use of parent scans to reduce the complexity of mixture analysis is demonstrated by pyrolysis of DNA. The quadrupole section is set to pass mass 136⁺ which is characteristic of protonated adenine. The pyrolysis of salmon testes



DNA in the CI ion source produces a large number of breakdown products. As the BE section scans these products, only those which produce mass 136 upon CAD will be observed. The simplified spectrum shows only a few candidates for subsequent structural elucidation to demonstrate the adenine contribution. Since there can be many peaks at each integral mass, this procedure can be used at moderately high resolution to further increase the specificity of the assay.

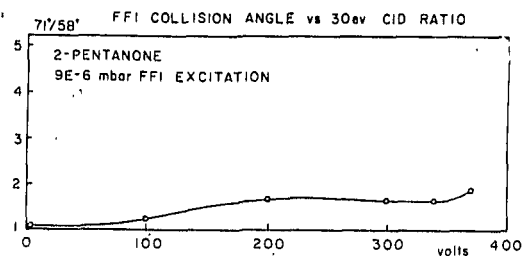
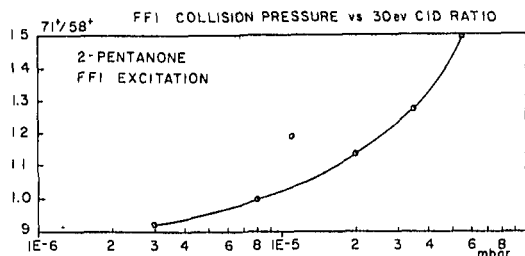
The use of multiple collisions permits a new degree of detail to be achieved in the characterization of gaseous ions. We report a simple new procedure in which CAD in the quadrupole collision region is employed to characterize the products of non-dissociative inelastic collisions in the first field free region. The first collision occurs at high translational energy and by selection of the direction of the scattered beam, energy transfer is controlled. The ability to manipulate internal energies separately from the collisional dissociation step allows the presence of isomeric forms of an ion to be recognized. This follows directly from the fact that changes in internal energy which are not accompanied by structural changes produce minimal effects on the CAD spectrum. Internal energy changes which shift the distribution of isomers should be recognizable by marked changes in the CAD spectra.

These capabilities are illustrated for the case of 2-pentanone, which upon ionization by electron impact, yields predominantly the keto ion although a striking dependence of the CAD spectrum upon source temperature has been interpreted as reflecting contributions from the enol structure(2). A 30 eV CAD spectrum of ionized 2-pentanone will produce a $71^+/58^+$ ratio of .9 at 250 degrees C. When the parent ion of 3-ethyl-2-pentanone is subjected to 3 keV collision in the first field free region and the 86^+ ion produced in that collision is subjected to 30 eV CAD, the $71^+/58^+$ ratio observed is 10. This ratio is attributed to a predominantly enol structure. If non-dissociative collisions of 86^+ from 2-pentanone in the first field free region are sampled as a function of collision gas pressure, the

$71^+/58^+$ ratio is observed to increase indicating a net increase in the fraction of ions undergoing collisions yet surviving those collisions in an altered state. As the collision angle is increased from zero to four degrees, the amount of energy transferred in the sampled collisions increase. The subsequent 30 eV CAD spectrum shows a minimal response in the $71^+/58^+$ ratio over a wide range of internal energies until a threshold for isomerization is reached. At this point the ratio rapidly increases with angle indicating that the enol structure has been generated in the original collision.

There is a large array of other complex experiments which can be accessed using this type of instrument. Some indications of these capabilities have been given previously,(3,4) while others, including methods for obtaining pure doubly charged spectra, procedures for simultaneous photo and collisional dissociation, and improved performance in linked scans by using the energy and mass filtering characteristics of the double quadrupole section, will be described in work in preparation.

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Features of a Thermal Desorption Molecule Microscope with Nanometer Resolution
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Molecule microscopy depends on the spatial variations of emissions of neutral molecules from the sample and produce images that reveal variations in the number of molecules present on the surface of (or, in some cases, coming through) the sample. These molecules can have been part of the sample, or may have been applied as a surface stain. There being no useful lens for neutral molecules, spatial resolution must be obtained as follows. An aperture can be scanned above a sample from which molecules are evaporating so that only molecules from one region of the sample can enter a detector, as determined by the geometry of the straight line molecular paths. Or the aperture can be at the end of a micropipette equipped with a permeable plug, useful when the sample is in liquid solution as with surviving tissue. Finally, the sample can be kept at such a temperature that negligible evaporation of molecules occurs except where local stimulation of evaporation by heating, electron stimulated desorption (ESD) or other means takes place in response to a localized scannable pulsed source of energy, such as a focussed beam of electrons. We have done some work with all three systems: aperture or "pinhole," micropipette² and scanning desorption^{3,4} but it is the last, the scanning desorption molecule microscope (SDMM) which has the possibility of nanometer resolution.

SDMM with its surface specific and essentially chemical contrast mechanism has many applications in which the localization of small molecules on sample surfaces can be used to give otherwise unobtainable information. Studies of the distribution of water on biological surfaces can give information about the sites where it binds and help elucidate the various specific recognition processes that take place at these surfaces. Water plays a role in corrosion initiation and failure in metallurgy, and in catalytic reactions in chemistry. We expect the SDMM to be useful wherever the irregular nature of samples does not permit diffraction studies of the adsorbate.

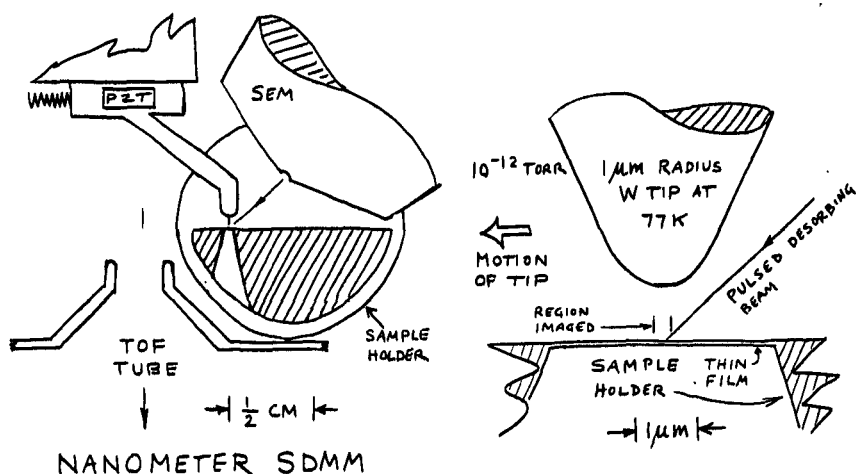
To establish the contrast mechanism for SDMM with thermal desorption as it might be used in biology we have studied the desorption of water from protein, carbohydrate and lipid⁵. As a frozen thin sample is raised from 100K to 800K three kinds of peak in the rate of desorption of water are found--peaks around 120K from the sublimation of ice in zero order, sample specific BET peaks in the range from 220K to 500K and pyrolysis peaks above 500K⁶. As long as the sample is not pyrolysed it can be retained. We have also investigated the ESD of water and ethanol from the same samples and find cross-sections around 10^{-16}cm^2 ³.

Consider a monolayer of water adsorbed on a surface--a 2nm spot will have about 40 molecules and they must provide the signal that eventually generates one element of the picture produced by the SDMM. Initially we plan to have 10^4 elements in the picture, each 2nm by 2nm with 3 to 5 shades of gray (attainable if the background is less than 3 counts per element) and requiring 10^2 seconds exposure.

Although thermal desorption is feasible for 10nm resolution with thin samples on thin substrates, because the spread in heat is comparable to the substrate thickness, we choose to use ESD to attain 2nm resolution. The sample will be on a thin (10nm) substrate to minimize the effects of "bloom." For a desorbing pulse 10^{-6} seconds long and with ESD cross-sections of 10^{-16}cm^2 currents of 10^{-12}A must be delivered to a 2nm spot, well within the current state of the art. The desorbed neutrals will impinge on a cold tungsten tip of $1\mu\text{m}$ radius and stick. The tip oscillates at 100Hz (eventually under servo control to ca $0.1\mu\text{m}$) from a position $1\mu\text{m}$ above the sample to a position at the entrance of a time of flight (TOF) mass spectrometer. When the tip is above the sample it collects a pulse of desorbed molecules. When it is above the TOF tube a short (10^{-9}sec) positive high voltage (400kV) pulse is applied to the tip which field desorbs and ionizes the molecules with high efficiency and sends the ions down the TOF tube for detection with appropriate fast electronics.

How low a pressure must be maintained in the vacuum system? If less than 1 molecule is to hit the detector tip during 1 cycle of the desorption-detection process, the pressure should be in the vicinity of 10^{-9} Pa, readily attainable--most easily by cryopumping in this case.

The self-explanatory figures show schematically the arrangement that we are currently assembling to scales where cm and μm details are revealed.



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AN EXB ELECTRON CAPTURE ION SOURCE

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An electron capture ion source using crossed electric (E) and magnetic (B) fields has been modeled, constructed and tested. Electrons are accelerated by E, but never reach the drawout electrode due to B. This is the same E and B arrangement used in the cycloidal mass spectrometer, with the electron traversing a repeating cycloidal orbit. A serious drawback to the cycloidal mass spectrometer is the velocity retardation during portions of the cycloidal trajectory. This effect is used to advantage as an electron capture ion source, since low energy electrons are much more efficiently captured than high energy electrons. Electron temperatures of less than 200°C can be achieved with E and B fields commonly encountered in ion sources without using a high pressure system as in chemical ionization. The ion source has been tested with SF_6 , and demonstrated high sensitivity.

Fast atom bombardment (FAB) mass spectrometry employing high field magnet and data system technologies can be used to examine the structures of a variety of high molecular weight (>1000) peptides. Twenty peptides, ranging in weights from 1000 to 5700, were examined under positive ionization FAB conditions. Characterization of individual peptides requires identification of both the polyisotopic molecular protonated envelope and significant fragment ions. Partial spectra displaying both of these properties are presented. Contributions of the polyisotopic elements to the molecular protonated ion envelope become important in the interpretation of the data at high mass and will be discussed. Additionally, the types of fragment ions include those that indicated the amino acid sequence of the peptide, those that identify disulfide bonds, and those that suggest reaction between the peptide and the sample matrix.

Example of spectra for peptides, including neuropeptides, hormones, immunopotentiating peptides, virus peptides, peptides resulting from protein hydrolysis, and peptides from uremic serum ("Middle Molecules"), will be presented.

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A two pronged software strategy to systematically generate and identify possible metabolites for a given compound has been developed as an aid to the interpretation of pharmacodynamics results. The first step utilizes computer techniques for parent structure perception and application of biotransformations to generate possible metabolites. The unbiased nature of this program avoids elimination of candidate metabolite structures from consideration. In the second step the generated structures are assigned probabilities for being the actual metabolites of the parent compounds based on the experimentally observed chromatography and mass spectrometry results. The power and potential of this approach will be illustrated with specific examples.

CHARACTERISATION OF TEN ANTIBIOTICS OF THE QUINOMYCIN GROUP BY
FIELD DESORPTION MASS SPECTROMETRY

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Quinoxaline antibiotics are derived from various species of *Streptomyces*. The history of their discovery and their biological properties have been thoroughly reviewed by Katagiri et al.¹ The basic skeleton of the quinoxaline antibiotics is characterised by two quinoxaline-2-carboxamide chromophores linked to a cross-bridged dilactone cyclic peptide. In one family of quinoxaline antibiotics, the quinomycins, the cross-bridge is a dithioacetal, as is the case in the antibiotic echinomycin (Figure 1), whereas in the other family, the triostins, the cross-bridge is a disulphide.

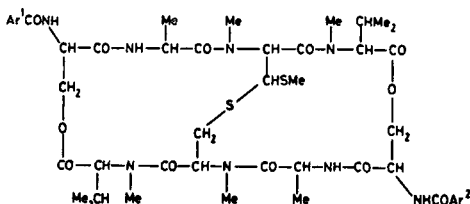


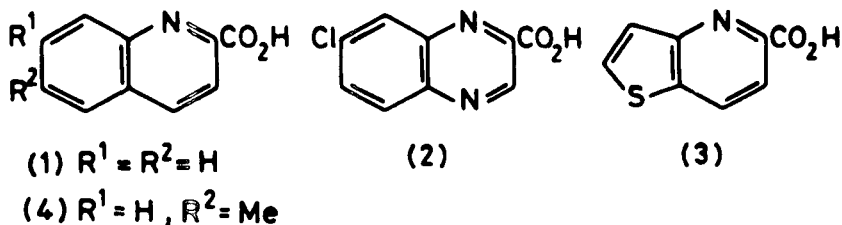
FIGURE.

Waring and Wakelin² have proposed a model of bifunctional intercalation for the DNA echinomycin complex. More recently, Lee and Waring³ have compared the binding properties of different naturally occurring, semi-synthetic and synthetic derivatives of the quinoxaline antibiotics.

Pulse-labelling studies with the triostin-producer *Streptomyces* S-2-210-L have shown that all the constituent amino-acids have L-precursors, and that L-tryptophan and quinoxaline-2-carboxylic acid can be used as precursors for the chromophoric moieties.^{4,5} Addition of quinoxaline-2-carboxylic acid to the culture medium of *Streptomyces* sp. 732 and *Streptomyces* S-2-210-L led to the production of new derivatives with quinoline chromophores replacing the quinoxaline rings.⁶ The production of new derivatives of echinomycin with quinazolin 4-one-3-acetyl residues has also been reported.^{7,8} We now report the characterisation by field desorption mass spectrometry (F.D.M.S.) of 10 antibiotics of the quinomycin group which have been produced by directed biosynthesis; 8 of these antibiotics are new.

The new derivatives have been biosynthesised in order to study the role of the chromophores in intercalation of the antibiotics into DNA. Their molecular weights were determined by F.D.M.S. since conventional electron impact (E.I.) fails to produce molecular ions from these antibiotics. Full details of the preparation and isolation of the antibiotics will be reported elsewhere,⁹ but briefly, 1 litre cultures of *Streptomyces echinatus* A8331 were grown in a maltose minimal medium,⁶ and supplemented at inoculation time with 0.75 mmol of one of the various analogues (1)-(4) (Figure 2).

The mycelium was collected after 10 days of fermentation, and extracted with chloroform and acetone. In the cases of the substrates (2)-(4), the culture filtrate was additionally extracted with ethyl acetate. The combined extracts were partially purified by preparative t.l.c., and the pure antibiotics isolated by preparative h.p.l.c.



The antibiotics thus obtained were subjected to field desorption mass spectrometry^{10,11}. Determination of the molecular weights makes it possible to distinguish between the antibiotics with one or two of the substituted chromophores. Because of the asymmetry of the molecule (Figure 1), there are in each case potentially two antibiotics which contain one substituted chromophore and one quinoxaline (Ari ≠ Ar²). These antibiotics have the same molecular weights and are not distinguished in the present study, but they were separated in the extract of the fermentation supplemented with 6-methyl-quinoline-2-carboxylic acid.

Molecular weights were determined using a Kratos MS 50 mass spectrometer equipped with a combined E.I./F.D. source. The field desorption wires were activated with benzonitrile. The mass marker was calibrated with tris-(perfluoroheptyl)triazine using the instrument in the E.I. mode. The sample was dissolved in CHCl₃ and applied to the wire with a syringe. Desorbed samples could be cationised with Li⁺^{12,13} by applying a saturated methanolic solution of lithium chloride to the wire, prior to the application of the sample. The source temperature was 125°C above ambient and desorption occurred at an emitter current of 18-20 mA. Our F.D. sample size was typically 10-15 µg.

In general, the above procedure gave rise to both M⁺ and (M+Li)⁺ ions, thus permitting an unequivocal determination of the molecular weights (Table). In the case of 2QCl it was not possible to observe M⁺ and (M+Li)⁺ at the same time, but in a spectrum taken without cationisation, the ion (M+H)⁺ was observed. It is interesting to note that the addition of analogue-precursor (4) to cultures of *S. echinatus* led to the production of a 2QnM derivative of triostin A - as confirmed by NMR analysis.¹⁴

The present results attest to the power of field desorption mass spectrometry in the determination of molecular weights of relatively involatile compounds of biological interest. This is especially true when the compounds are available in only small quantities, as may be the case in directed biosynthesis or mutasynthesis.

TABLE: Substrates added to cultures of *Streptomyces echinatus* A8331, and molecular weights of identified products.

Substrate	Antibiotic acronym (mol. wt.) ^a	Antibiotic acronym (mol. wt.) ^b
(1)	1QN (1099)	2QN (1098)
(2)	1QCl (1134)	2QCl (1168)
(3)	1TP (1105)	2TP (1110)
(4)	1QnM (1113)	2QnM (1126)

^aThe molecular weights in column 2 are for the structures with one substituted chromophore and one quinoxaline substituent. ^bThe molecular weights in column 3 are for the structures with two substituted chromophores.

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NEW APPLICATIONS OF MASS SPECTROMETRY TO CIS-DICHLOROPLATINUM
AMINO ACID COMPLEXES

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A number of pure enantiomeric cis-dichloroplatinum (II) amino acid complexes have been synthesized for possible use as anticancer chemotherapeutic agents.¹ Those complexes isolated as potassium salts (PtKCl_2 Gly [1], PtKCl_2 Tyr [2], and PtKCl_2 Phe [3]), are water soluble. Those which contain methionine (PtCl_2 Met-OMe [4], PtCl_2 Met-NH₂ [5], PtCl_2 Leu-Met-OMe [6], and PtCl_2 Leu-Met-NH₂ [7]) are non-ionic and water insoluble. Mass spectral analysis by conventional EI and CI methods was unsuccessful. However, analysis by field desorption (FD), fast atom bombardment (FAB), and direct chemical ionization (DCI) yielded results which were both interesting and informative.

Generally, FD mass spectrometry of the potassium salts (compounds 1-3) gave spectra which consist of one ion cluster with an m/z value corresponding to the molecular weight of the complex plus an additional potassium ion. The weak fluctuating ion beams obtained from these compounds by FD made it difficult to obtain good ion statistics and accurate isotope patterns.

Positive ion FAB mass spectrometry of the potassium salts gave no spectra. Negative ion FAB gave strong spectra with good isotope patterns. Both M-K and M-H ions were prominent along with several fragment ions. However, the spectra were complicated by several higher mass ions corresponding to the successive additions of small neutral species such as water, HCl, and glycerol (from the sample matrix).

Only one non-ionic complex (compound 4) was studied using FD. One cluster of ions was evident corresponding to the molecular ion. The production of an M^+ ion is consistent with vaporization of the complex followed by subsequent field ionization in the gas phase near the tips of the emitter needles.

FAB analysis of the non-ionic complexes was generally unsuccessful. Either no spectra were obtained (compounds 4 and 5) or the spectra were not representative of the known composition of the sample. Positive ion spectra of compounds 6 and 7 in thioglycerol contained ions derived from the peptide portion of the complex. There were no ion clusters which would indicate the presence of platinum or chlorine. In the negative FAB spectra just the opposite was true. There were clusters of platinum and chlorine extending to $m/z \sim 2,000$, but no evidence of the dipeptide portion.

DCI using isobutane was successful with the dipeptide complexes 6 and 7. Ions corresponding to MH^+-HCl and $\text{MH}^+-\text{PtCl}_2$ were evident for both 6 and 7; an MH^+ ion was obtained for 6.

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ANALYSIS OF COMPLEX MIXTURES FOR TRACE INVOLATILE SPECIES BY LC/MS/MS.

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Introduction

Tandem mass spectrometry has attracted considerable attention in recent years as a technique which allows the direct analysis of crude samples for the presence of trace species. However, many analytical situations involve matrices which are simply too complex to analyze directly, either because of ionization suppression or because of the presence of interfering species. We have recently shown the benefits of a fast chemical prepreparation and cleanup by Thin Layer Chromatography before analysis by Direct Insertion Probe/MS/MS, for trace levels of drugs in equine urine samples (1). The TLC step provides a sensitive prescreening which requires only "positives" to be analyzed further, and the MS/MS provides a fast and highly specific identification of the compounds of interest in what can still be a relatively complex extract. Of even greater potential utility, however, is the combination of LC/MS/MS. The power of this technique in the analysis of real samples was first shown in an application involving the detection of sulfadimethoxine in a raw equine urine extract (2). Since that work was performed, a new LC/MS interface has been developed for the TAGA® 6000 Triple Quadrupole System. The interface allows the entire flow of up to 1 ml/min from a standard wide bore column to be introduced without solvent stripping or splitting, into the Atmospheric Pressure Chemical Ionization Source of the TAGA®. Many of the perceived difficulties with other LC/MS interfaces are eliminated, allowing a relatively wide degree of flexibility in choosing chromatographic conditions. With the introduction of this simple and reliable interface, applications ranging from the identification of unknown peaks in a UV chromatogram, to the quantitative determination of impurities in industrial materials are now possible. Several recent applications are described in this paper.

Experimental

The LC/MS interface couples directly to the APCi source of the TAGA® 6000. The eluent from the LC column (or detector) is carried (without splitting) by narrow bore tubing into the interface, where the liquid is nebulized by a high velocity gas jet. The droplets of solvent and solute are vaporized in a heated quartz lined spray chamber and transfer line, and the vaporized mixture of solvent and solute molecules carried by the nebulizing gas into the ionization region of the source. Chemical ionization at atmospheric pressure generates primarily quasi-molecular ions with minimal fragmentation, so that a single MS scan of ions from the source provides molecular weight information from very simple spectra. The parent ions can then be selected by Q1 and fragmented by 0 to 130V collisions in Q2, generating structurally characteristic daughter ions for analysis in Q3. The gentle vaporization/ionization conditions minimize undesirable thermal or collisional decomposition in the source, and provide good molecular weight information even from involatile and thermally labile molecules.

Analysis of unknown mixtures can be accomplished by injecting the sample into the column even under conditions where full separation is not achieved. An LC/MS run is performed by scanning Q1 in a resolving mode while Q2 and Q3 are in the RF only (total ion) mode, and the data examined to identify the molecular weight and elution time of the components. For further specific identification, even in the presence of high levels of coeluting species, a second run (LC/MS/MS) can be performed in which several of the ions of interest are selected and fragmented to generate daughter ion spectra. Combinations of full scan single MS and CID of selection ions at defined times can also be performed, in addition to SIM (with one MS) or SIM/SIM runs in which low levels of specific components are sought.

Results

Figure 1 shows the single MS spectrum of methaqualone from an injection of 6 ng onto a C18 micro-Bondapak column, with 50/50 acetonitrile/1% acetic acid at ml/min. Figure 2 shows a full scan LC/MS/MS spectrum (daughter ion spectrum) from an injection of 30 ng of methaqualone on-column. The full scan LC/MS spectrum shows a strong m/z 251 parent ion (along with some unsubtracted background peaks). An LC/MS/MS run in a SIM/SIM mode readily detects 6 ng of methaqualone with an S/N of 5/1.

The technique has been applied to a mixture of seven water soluble vitamins, of widely varying concentrations. Amounts injected in the mixture range from 50 µg of sodium ascorbate (composing 98% of the mixture) to 40 ng of riboflavin. Both basic and acidic components are present, and full separation is not achieved under the chromatographic conditions selected. In each case except folic acid, the parent ion MH⁺ dominates.

Figure 3 shows the EICP of the m/z 328 parent ion of butorphanol, detected in the urine extract of a racehorse after administration. The single MS spectrum shows the presence of the m/z 328 parent ion. Below is shown the daughter ion spectrum of 328 acquired during the LC/MS/MS run, compared to the daughter ion spectrum of authentic butorphanol. A good match is obtained allowing positive confirmation of the presence of the drug in the urine extract, in a situation in which other coeluting components are present.

Many biochemical problems require the use of largely aqueous mobile phases for the elution of highly polar compounds. The use of up to 100% water poses no problem in the LC/MS interface. Adenine, cytosine and methylcytosine are easily detected in a mixture of 20 ng each, with a mobile phase of 10/90 methanol/water. In addition, involatile modifiers can be used if necessary without impairing performance of the interface or ion source except for a requirement of a simple 5 minute cleaning step after 1 or 2 days. For example, hexane sulfonic acid has been used as an ion pairing reagent for both basic drugs and basic vitamins. The LC/MS spectra are still dominated by MH^+ ions.

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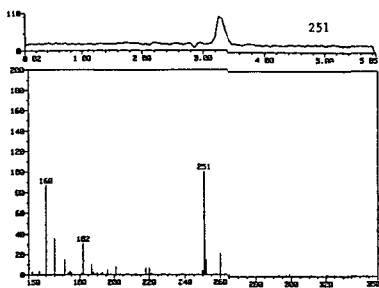


FIGURE 1. Full scan LC/MS spectrum of 6 ng of methaqualone.

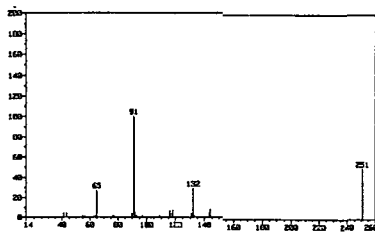


FIGURE 2. CID spectrum from 30 ng of methaqualone on-column.

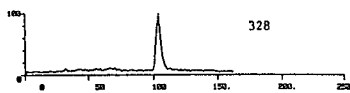


FIGURE 3. Extracted ion current profile of m/z 328 from equine urine extract.

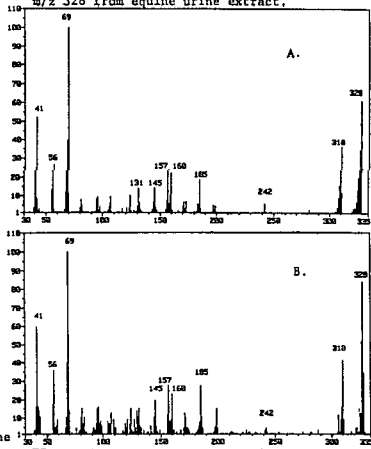


FIGURE 4. A. CID spectrum of m/z 328 from butorphanol. B. CID spectrum of m/z 328 from urine extract.

LAMMA : CALIBRATION AND APPLICATION FOR NEPHROTOXICOLOGY STUDIES

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Laser microprobe mass spectrometry (LAMMA) is a recent elemental and molecular micro-analysis technique in which a powerful laser shot perforates a thin specimen and the generated ions are measured by time-of-flight mass spectrometry. Because of its advantageous absolute and relative sensitivity, LAMMA offers a promising alternative for microanalysis down to the subcellular level, in biomedical applications.

The potential of LAMMA was explored while examining two important nephrotoxicological problems.

Cisplatin used alone or in combination with other drugs has recently emerged as an effective agent in cancer chemotherapy¹. This antineoplastic compound however, exhibits a marked nephrotoxicity. In this context, it is interesting to localize the heavy metal at a cellular or even ultrastructural level. Renal biopsies were taken of a dog that had been intravenously administered the antitumor drug cisplatin. By means of LAMMA, platinum was detected in proximal tubular cells in the dog kidney (Fig. 1). Although platinum was unambiguously proven to be present in the cells, and not in the extracellular renal tissue, further subcellular tracing appeared unfeasible because the low concentration and high ionization potential of platinum necessitated using a high laser power, resulting in large section perforations ($\sim 6 \mu\text{m}$) and a limited spatial resolution.

Also using LAMMA, subcellular localization of aluminium was established in a liver and bone biopsy of a dialysis patient with vitamin D-resistant osteomalacia². Aluminium was persistently detected together with iron in electron-dense structures, compatible with lysosomes, within the liver cells (parenchymal as well as Kupffer cells, Fig. 2). No evidence was found for aluminium accumulation in the nucleus, cytoplasm and intercellular space. In the bone sections aluminium was found to be present at the osteoid/calcified-bone boundary.

In order to quantify the LAMMA-signals of e.g. platinum and aluminium from biological tissue specimens, a new standard system for LAMMA-purposes was introduced; it is based on loaded ion-chelating resin beads, present in the section to be analysed after coembedding with the tissue and ultra-microtome cutting. A typical calibration graph is represented in Fig. 3. With such targets the characteristics of the LAMMA microanalysis were optimized and the influence of the instrumental parameters on the LAMMA spectra and laser-induced specimen perforations were studied.

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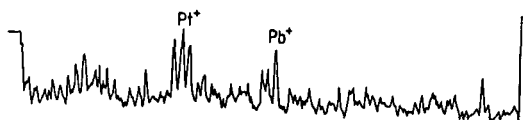


Figure 1 : LAMMA mass spectrum originating in a proximal tubular cell of dog kidney after cisplatin treatment. A platinum signal is clearly present (lead was either originally present or contaminated).

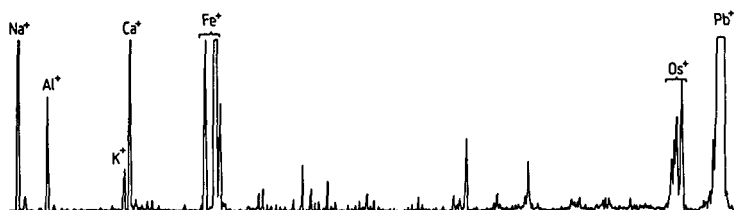


Figure 2 : LAMMA mass spectrum originating in an electron-dense subcellular structure, compatible with a lysosome of a liver cell from a dialysis patient, clearly showing aluminium accumulation together with iron (osmium and lead signals are due to the fixation and the staining procedure, respectively).

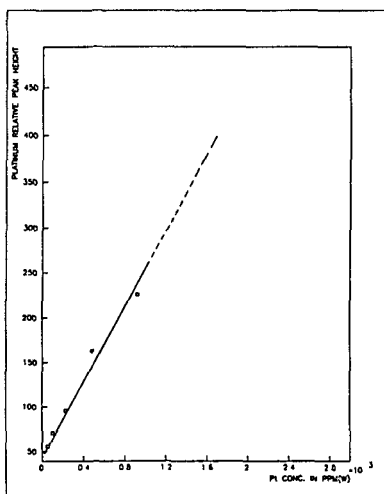


Figure 3 : Calibration plot for LAMMA analysis of platinum standards (showing satisfactory linearity up to some 2000 ppm).

DETERMINATION OF CHEMICAL EQUILIBRIUM CONSTANTS USING FAB/MS

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The equilibrium constants for certain types of chemical reactions, such as the dissociation of a proton from a weak acid, can be determined using fast atom bombardment mass spectrometry. If one considers the simple case of an acid HA dissociating to give the conjugate base A⁻, then for dilute solutions where the pK_a of the acid is between 3 and 10, the Henderson-Hasselbalch equation holds. This equation states that the pK_a of an acid is equal to the pH plus the log of the [A⁻]/[HA] molar ratio. Using FAB, this ratio can be obtained from the ion intensities of the molecular species as follows:

$$\begin{aligned} [\text{HA}] &= I(\text{HA}) + \text{H}^+ + I(\text{HA}) + \text{Na}^+ \\ [\text{A}^-] &= I(\text{A}^- \text{Na}^+) + \text{H}^+ + I(\text{A}^- \text{Na}^+) + \text{Na}^+ \end{aligned}$$

This treatment requires that the pH of the solution has been brought to within 1 unit of the expected pK_a of the acid using NaOH. When no excess salt is present, then I(HA)+Na⁺ is negligible, and the value of pK_a (ie, the pK' of an acid in 50% glycerol/water) can be readily determined. For a variety of acids, acid strengths can be directly related by these values.

Correction for the effect of the glycerol/water matrix can be calculated by use of the Born equation or can be determined experimentally. In the case of simple acids, the pK (in 50% glycerol) > pK (water). Similarly, the effect of ionic strength can be calculated using the equations of Debye and Hückel, or can be determined experimentally.

Using these methods, fifteen acids of several charge types were measured and the results compared to the values previously reported in the literature. The correlation was excellent, with the average deviation being ±0.04 pK units. A few examples are;

acid	measured pK _a	literature pK _a
acetic	4.82	4.76
hexanoic	4.87	4.88
sulfanilic	3.23	3.17
tris(hydroxymethyl)methylaminopropionylsulfonate	8.44	8.4
gly-gly (pK ₁)	3.12	3.15
fructose 6-Phosphate (pK ₂)	6.05	6.11

The value of this approach lies in its application to systems where the analysis of the acid and its conjugate base are difficult whether due to the complex nature of the acid itself or due to a mixture with other components, making potentiometric and spectrophotometric measurements impossible. Further, the basic principles used in determining pK_a can be used for the measurement of a wide variety of chemical equilibria in chemical and biological systems.

IDENTIFICATION OF RAT LIVER MICROSOMAL METABOLITES AND XANTHINE OXIDASE
DNA-ADDUCT OF 1-NITROPYRENE IN VITRO

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Recently, a large number of nitro PAHs have been implicated as the components responsible for the so-called direct-acting mutagenesis observed in diesel exhaust particulates (1,2) and in extracts of Xerox toner (3). Hence, it was of interest to investigate the metabolic transformation and possible formation of metabolically activated intermediates as well as covalent adducts of the chemically active intermediates with DNA. It is important to understand how this class of compounds may exert their biological effects.

Studies of the metabolism of 1-nitropyrene were carried out using rat liver microsomes previously induced with 3-methylcholanthrene or phenobarbital. Also, incubation of 1-nitropyrene with purified mammalian nitroreductase, xanthine oxidase in the presence of native calf thymus DNA was performed to investigate covalent adducts with the DNA.

The metabolites were separated by high performance liquid chromatography (HPLC) and identified by high resolution mass spectrometry (HRMS), as well as high field nuclear magnetic resonance (NMR). Eight metabolites were found (see Fig. 1, HPLC chromatogram of metabolites); six of them were identified as 1-aminopyrene, N-acetyl-1-aminopyrene, N-formyl-1-aminopyrene, 1-aminopyrene-4,5-epoxide (or 1-aminopyrene-5-quinone), 1-nitropyrene-4,5-epoxide, 1-nitropyrene-4,5-diol (see Table 1). Fig. 2 shows their mass spectra. Covalent adducts were formed by incubation of 1-nitropyrene with calf thymus DNA in the presence of xanthine oxidase. The 1-nitropyrene-DNA adducts were separated by Sephadex LH-20 and HPLC after sequential purification and enzymatic hydrolysis. The major adduct was identified as N-(deoxyguanosin-8-yl)-1-aminopyrene by FAB negative ion mass spectrometry and EI HRMS for the permethylated compound. Fig. 3 shows the FAB and EI HRMS spectra of this adduct. This structure agrees with that reported by Howard et al. (5) using NMR.

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METABOLITES OF 1-NITROPYRENE FORMED IN RAT LIVER MICROSOLES

Peak Number (in HPLC chromatogram)	Molecular ion (M ⁺)	Composition	Suggested Structure
4	217.0883	C ₁₂ H ₁₁ N	<chem>c1ccc2c(c1)ccc(cc2)[N+](=O)[O-]</chem>
3	243.0941	C ₁₃ H ₁₃ NO	<chem>c1ccc2c(c1)ccc(cc2)C(=O)O</chem>
2	234.0874	C ₁₂ H ₁₁ NO	<chem>c1ccc2c(c1)ccc(cc2)C(=O)C</chem>
7	233.0840	C ₁₂ H ₁₁ NO	<chem>c1ccc2c(c1)ccc(cc2)C(=O)N</chem>
8	263.0975	C ₁₄ H ₁₃ NO ₂	<chem>c1ccc2c(c1)ccc(cc2)C(=O)O[N+](=O)[O-]</chem>
1	291.0646	C ₁₄ H ₁₁ NO ₃	<chem>c1ccc2c(c1)ccc(cc2)C(=O)O[N+](=O)[O-]</chem>
5	247.0821	C ₁₃ H ₁₃ NO ₂	<chem>c1ccc2c(c1)ccc(cc2)C(=O)O[N+](=O)[O-]</chem>

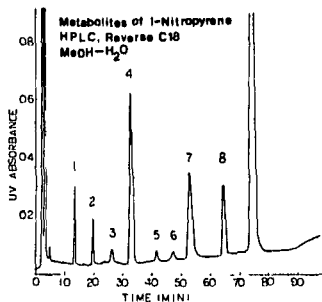


Figure 1. HPLC Chromatogram of 1-Nitropyrene Metabolites

Figure 2. Mass Spectra of 1-Nitropyrene Metabolites

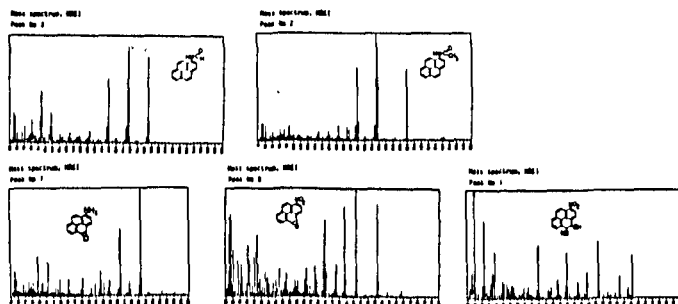
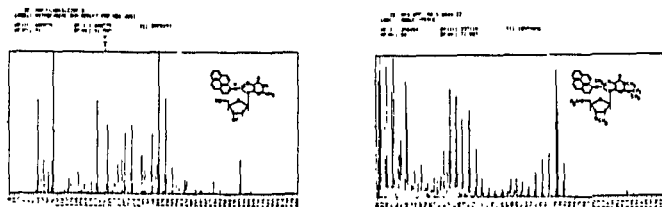


Figure 3. Mass Spectra of 1-Nitropyrene-DNA Adduct



IDENTIFICATION AND DETERMINATION OF DIHYDROXY-NITROSAMINES BY GCMS OF t-BUTYL-DIMETHYLSILYL ETHER DERIVATIVES

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The carcinogen, N-nitrosodiethanolamine (NDELA) has been found at relatively high levels in some cosmetics (up to 49 ppm, 1), metal working fluids (up to 3%, 2) and in tobacco products (up to 7 ppm, 3). N-Nitrosobis(2-hydroxypropyl)amine (BHP) is a potent pancreatic carcinogen in hamsters and induces colon, esophagus, prostate, and thyroid tumors in rats (4). The presence of BHP has not been previously reported in environmental samples or manufactured products but we have found 0.25 to 1 ppm in commercial samples of the precursor amines, diisopropanolamine and triisopropanolamine.

Results of a typical analysis of triisopropanolamine are shown in Table 1. The amine (5 g) was dissolved in an aqueous solution of 15% ammonium sulfamate in 12% sulfuric acid to inhibit artifactual nitrosamine formation. The solution was saturated with ammonium sulfate and extracted with ethyl acetate on a Celite 560 column. After concentration of the extract, the t-butyldimethylsilyl (TBDMS) ether was prepared by reaction with t-butyldimethylsilylchlorosilane-imidazole reagent (Applied Science Co.) at 60°C for 30 min. Quantitative determination was by GC on a 1% Carbowax 20M-TPA column at 150°C with Thermal Energy Analyzer (TEA) detection. Addition of 500 ppb of BHP to this sample indicated that recovery was greater than 90%. Ultraviolet irradiation of the initial amine solution destroyed the nitrosamine, and analysis of this sample indicated that the BHP found was not produced during the analytical procedure.

The TEA is highly specific for N-nitroso compounds, but it is essential that the presence of nitrosamines in environmental and commercial samples be confirmed by an independent method. We normally employ GCMS using high resolution ($M/\Delta M=10,000$) selected ion monitoring for this purpose.

Underivatized NDELA and BHBP can be determined by GC, but extensive adsorption increases sample requirements to 1 μ g or more. GC of nanogram quantities requires a less polar derivative. We have evaluated GC behavior and mass spectra of acetates; trifluoroacetates; methyl-, trimethylsilyl (TMS)- and TBDMS-ethers of NDELA, BHP, and other hydroxy-nitrosamines. Mass spectra of TMS and TBDMS derivatives of NDELA are compared in Figure 1. The intense (M-57) peak at m/z 305 representing loss of a t-butyl group is typical of TBDMS derivatives. Figure 2 compares TMS and TBDMS derivatives of BHP. The (M-57) peaks constitute 14% and 11% of total ionization for the TBDMS derivatives of NDELA and BHP, respectively, providing sufficient sensitivity for GCMS determination of nanogram quantities using high resolution selected ion monitoring. We monitor the $C_{12}H_{29}N_2Si_2O_3$ peak at m/z 305.1715 for determination of NDELA and the $C_{14}H_{33}N_2Si_2O_3$ peak at m/z 333.2030 for BHP. These ions contain all the heteroatoms of the original compounds, thereby increasing the specificity of the analysis.

Our criterion for confirmation requires that the MS analysis confirm the identity of the GC peak and that the amount determined by GCMS is within 20% of the quantity found by GC-TEA. The high resolution MS procedure provides a detection limit of approximately 0.2 ng, using a capillary column, and is comparable to TEA detection limits.

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Table 1. Determination of BHP in Triisopropanolamine

BHP Added (µg/kg)	BHP Found (µg/kg)
0	250
0	240
500	740
500	680
UV Irradiated (366 nm, 4 hr)	Not Detectable (<70)

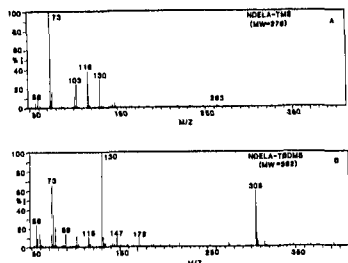


Figure 1. Electron impact mass spectra of (A) NDELA-bis-tri-methylsilyl ether and (B) NDELA-bis-t-butyl-dimethylsilyl ether.

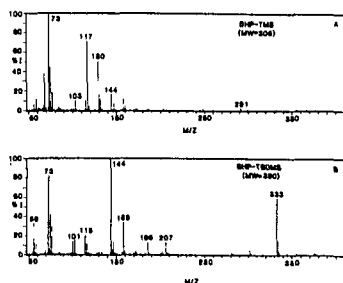


Figure 2. Electron impact mass spectra of (A) BHP-bis-tri-methylsilyl ether and (B) BHP-bis-t-butyl-dimethyl silyl ether.

ATTACHMENT OF MUTAGENS AND CARCINOGENS TO NUCLEOSIDES AND
PROTEINS.

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In protecting against cancer the main question
remains how carcinogens and mutagens are attached to proteins
and or to nucleosides.

In order to adress this question, we have first
investigated the linkage between α_2M globulin + methyl naphto
benzacridin by EI and C.I. spectroscopy.

The group of angular benzacridines represent an
especially interesting family of heterocyclics because it con-
tains numerous carcinogenic compounds. The mass spectrograms
of these aromatic heterocyclic substances are known to give
significant peaks corresponding to multiple charged ions.

In some instances, a doubly charged ion is higher than a
singly charge ion.

α_2M globuline: an overview.

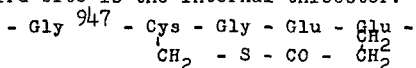
In inflammatory states proteolytic enzymes may
be activated in the blood or released from cells into the cir-
culation. At least seven different plasma proteins have been
demonstrated to inhibit these protein cleaving enzymes in
vitro. Of these, α_2M globulin is unique in that it forms com-
plexes with many proteases.

It was demonstrated by Schramm that α_2M globulin exists in open
and closed form.

With aid of electron microscopy we demonstrated that the closed
form exists in the plasma and the open form in serum. We con-
verted the closed form to the open form by trypsin digestion.
The α_2M globulin contains three reactive site. The primary
site is cleaved by proteases in the bait region of α_2M glo-
bulin and the cleavage is located on the sequence Arg⁶⁶⁰-Val-
Gly-Phe-Tyr-Glu.

It is a second site for the attachment of the blood protein
factor XIII and a third site is the internal thioester.

The thioester.



The E.I. spectra of MNBA labeled α_2M globulin is dominated by the peaks of MNBA, M/z 343, $M - 14$ 329, $M + 28$ 315, and $M/2$ 171.5. In the low weight fragments we found m/z 42, 56, 58, 70, 84 and the height weight fragments $M+ 42$, $M+56$, $M +89$ and $M+ 146$.

The C.I. spectrum exhibits $M +57$ 400, $M+ 43$ 386 and $M+ 91$ coming from the thioester.

The thioester plays a crucial role in maintaining the nature of the protein conformation.

C.I. spectrum of C_3 and C_{3b} proteins of the complement.

Initiation of the alternative pathway of complement activation involves the random attachment of C_{3b} of all biological particles in contact with plasma. The process requires continuous proteolytic generation of metastable C_{3b} . The responsible protease arises as a result of conformational changes in C_3 which occur upon thioester hydrolysis. It was found that nucleophilic scission of the single bound intra thioester chain is present in these molecule in the absence of any peptide bound cleavage. We note in the spectrum of labeled C_{3b} a protein that contains a thioester appreciable by the presence of $M+ 32$ the S^+ peak.

There are quite different peaks of attachment of immunoglobulin IgG and IgM. The presence in the spectra of m/z 130 and m/z 107 indicate the non covalent binding of tryptophan and tyrosin.

The attachment point are probably the same in α_2M globulin + methyl naphtho benzacridine and the complement protein and MNBA as the α_2M globulin -enzyme.

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MOB 10 | HYDROXYL RADICAL LOSS AND REARRANGEMENTS IN THE MASS SPECTRA OF DIALKYLNITROSAMINES. N. Sundaram, J. L. Cone and P. P. Roller, Analytical Chemistry Section, LCM, Nat'l Cancer Inst., NIH, Bldg 37/3R-27, Bethesda, MD 20205.

The analysis, chemical reactivity and spectral characteristics of carcinogenic N-nitrosodialkylamines are of both practical and theoretical interest. One of the abundant characteristic ions of acyclic dialkyl nitrosamines corresponds to the loss of OH radical from the molecular ion, a fragmentation requiring hydrogen abstraction by the oxygen atom of the nitrosamino moiety from the alkyl sidechain. We have examined this and other fragmentation pathways of methyl-butyl-, -hexyl and -octyl nitrosamines using ^2H and ^{15}N labelled analogs, metastable studies and high resolution measurements. Our studies indicate, for example, that the C-2 and C-3 hydrogens are involved to an equal extent in the case of methylbutyl nitrosamine, whereas for the methylhexyl analog the C-2 hydrogen, which would be expected to transfer to the oxygen through a favoured 6-membered transition state, is not involved in the OH loss (<5%). We are finding, that ions that require intramolecular hydrogen transfer become prominent at low ionization energy, indicating that such rearrangements are energetically favourable. Analogy to the electrochemical oxidation of dialkyl nitrosamines will also be made.

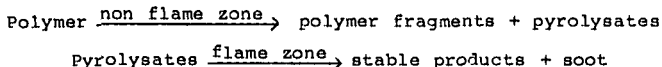
MOC 8 | SOME ASPECTS OF THE STRUCTURE OF PETROLEUM ASPHALTENES; Roy J. Pancirov and James G. Speight, Exxon Research and Engineering Company, P. O. Box 121, Linden, NJ 07036.

An integrated technique has been developed for the study of the thermal chemistry of petroleum fractions - particularly the asphaltenes. The procedure involves the integrated use of a pyroprobe/gas chromatographic/mass spectrometric technique which offers information about the structural-types and distribution within the volatile products from the thermal decomposition of asphaltenes. The technique offers itself as an attractive on-line analytical method for the study of structural types that occur in asphaltenes as well as a technique for studying the parameters that can influence asphaltene decomposition. The concept of deducing "average" structures of asphaltenes is briefly discussed in terms of the observance of the lower molecular weight species in the volatile products of the thermal decomposition.

Characterization of Aerosols from Fires

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The combustion of a polymeric material in a flame is a complex process involving many sequential parallel physical and chemical processes. A simplified mechanism for the process can be postulated as



The important aspect of the mechanism for this study is the fact that polymer fragments are produced. Even though the fragments pass through the flame, they are present in the aerosol material as lower molecular weight species of the original polymer. By removing the volatile compounds from the aerosol, the polymeric material can be used to fingerprint the fuel source.

Aerosol material was individually generated in a cup furnace inside a plexiglass chamber from 10 common polymeric materials (Table 1). A nonflaming mode was used in all decompositions. The aerosols were collected on a glass fiber filter. After removing the volatile compounds by vacuum treatment at 60°C for 48 hours, the remaining material on the top layer of the filter was coated onto a 510°C Curie-point wire. Pyrolysis of the samples was done on an Extranuclear Curie-point pyrolysis mass spectrometer (Py-MS). Pyrolysis time was 5 seconds with a rise time of 100 ms. Forty spectra were summed together for each pyrolysis mass spectrum. Multivariate statistical analysis (Pattern recognition) was conducted on the Py-MS data using principal component/factor analysis with graphical rotation, distance calculations and non-linear mapping. Most of the computer routines used were in the ARTHUR package (1).

Figure 1 shows the non-linear map of the pyrolysis data for the 10 aerosols. The map indicates that aerosols from the 10 materials are structurally different. Comparison of the Py-MS spectra to those of the original polymers show a great deal of similarity. These observations were consistent with the fact that polymer fragments survived the combustion process (2).

Twenty mixtures (2 unique sets) each containing up to 4 components were formulated at various compositions from the 10 original polymers. Analysis of the pyrolysis data from these 20 mixtures by multivariate statistics showed that each mixture produced a unique Py-MS pattern. The spectrum for each mixture was first visually analyzed using the mass spectral peaks characteristic of the components of the mixture. Next, principal components factor analysis was done. In order to extract the chemical information, a series of graphical rotations were performed on the first six factors. Figure 2 represents the spectrum generated by rotation of factor 1 by 135° with respect to factor 2. The upper part of this spectrum corresponds to polyurethane GM-29. The spectrum of the original GM-29 is shown in Figure 3. Similar data was extracted for most species contained in the mixtures. In general, this approach was used to predict the components in the mixtures with 88 percent correctness.

A least squares approach was also applied to the pyrolysis data for quantitation (3). The program used reference spectra for a best fit to the mixture mass spectrum. The results of the least squares analysis showed that some materials such as PVC in relation to the amount in the mixture produces large quantities of polymeric aerosol. Others are affected by accompanying materials in the mixture and show no predictable trend. Future work in this area will include flaming combustion studies as well as a series of blind tests.

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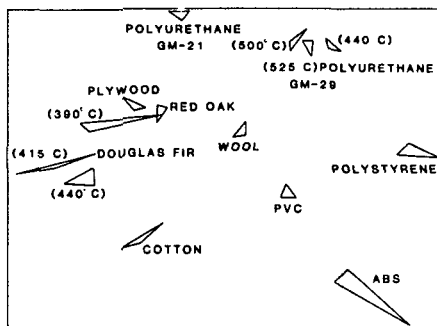


Figure 1. Non-linear Map of Various Aerosol Materials.

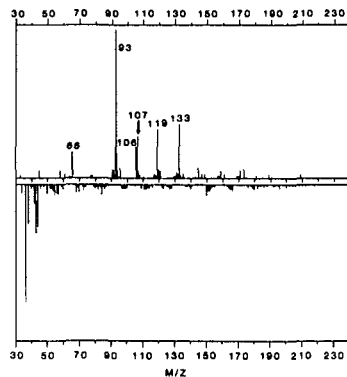


Figure 2. Factor Spectra of GM-29.

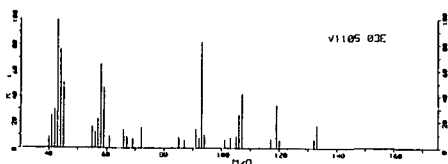


Figure 3. Py-Mass Spectrum of Nonflaming Particulate Material from Urethane GM-29 Conditioned with Vacuum.

Table 1. Materials used in nonflaming combustion.

<u>Material</u>	<u>Combustion Temperature (°C)</u>
Douglas Fir	440
Plywood	455
Red Oak	455
Cotton	500
Wool	625
Polyurethane GM-21	370
Polyurethane GM-29	525
Polystyrene	465
Polyvinylchloride	575
ABS	550

POLYMER CHARACTERIZATION USING THERMOGRAVIMETRY COUPLED TO TRIPLE QUADRUPOLE TANDEM MASS SPECTROMETRY (TGA/MS/MS), Bori Shushan, SCIEX®, 55 Glen Cameron Road, Thornhill, Ontario, Canada, L3T 1P2 and R. Bruce Prime, IBM Corp., San Jose, CA.

Thermogravimetric Analysis (TGA) has become an indispensable tool in the routine characterization of polymeric materials. However, the complexity of thermal degradative processes and the great variety of additives which can be present in polymer formulations requires the combination of TGA with other analytical techniques before a reasonably clear picture of the chemistry involved can be drawn. Of all the available combinations, that of TGA-mass spectrometry (MS) appears to hold the greatest promise for the rapid, on-line analysis of gases evolving from the TGA apparatus. Although TGA/MS combinations have been used successfully for over a decade^{1,2} the technique still suffers from the inability to unambiguously identify chemicals co-evolving during the thermo-degradation of polymers. With the recent introduction of triple-quadrupole tandem mass spectrometers (MS/MS)³ real time structural identification of single components within complex evolved-gas mixtures can be carried out rapidly under computer control. This paper will describe the coupling of TGA with MS/MS for the identification of individual compounds produced during the thermolytic decomposition of a representative polymer and how this information can be used to elucidate degradative pathways.

EXPERIMENTAL

A schematic of the TGA/MS/MS interface is shown in Figure 1. The TAGA® 6000 triple quadrupole mass spectrometer is fitted with an atmospheric pressure chemical ionization (APCI) ion source. This ion-source uses air as the CI reagent gas permitting the routine use of oxidative purge gases within the coupled TGA apparatus. Using this interface, several commercially available TGA instruments can readily be coupled to the TAGA® 6000 MS/MS including the Perkin-Elmer TGS-2 (top and middle, Figure 1) and DuPont 951 (bottom, Figure 1). In both cases a ball-and-socket joint connects the TGA to the all-glass transfer line permitting easy coupling and decoupling.

After passing through a short section of wide-bore capillary a portion of the TGA effluent is carried into the APCI ion source by a high velocity stream of carrier/reagent gas. The amount of TGA effluent allowed into the ion source is accurately controlled by a micrometer 'dump' valve connected in series with a device which measures the pressure on either side of the capillary restriction (this pressure is directly proportional to the flow across the capillary). The relatively high gas velocities as well as the fact that the whole interface assembly is heated (250-350°C) avoids condensation of less volatile materials on the interface walls. Chemicals entering the ion source from the transfer line are converted to their quasi-molecular or molecular ions via well characterized APCI reactions⁴. The ions are then focussed into the high vacuum analyzer portion for MS and/or MS/MS analysis.

To illustrate the basic operation of the TGA/MS/MS system a 12.4 mg sample of cellulose acetate was heated in a DuPont 951 TGA from 100 to 600°C at 20°C/min using air as purge gas (200 ml/min) where 1.3% of the TGA effluent was rapidly transferred to the mass spectrometer ion source by a 2 l/min flow of preheated carrier/reagent gas.

RESULTS AND DISCUSSION

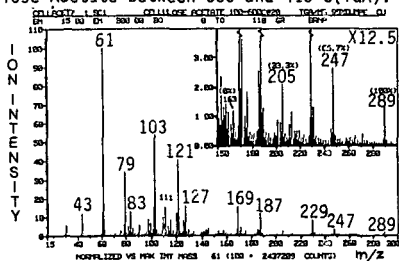
In the single MS mode the first of three axially aligned quadrupoles is automatically and repetitively scanned and the mass spectra recorded using the SCIEX® MS/MS data system. Figure 2 shows the average of mass spectra recorded between the 360 and 410°C (the temperature range of maximum weight loss). The complexity of this spectrum underscores one of the limitations of conventional TGA/MS. Since each peak corresponds to the protonated molecular ion of a neutral evolved during thermolysis, only molecular weight information is available. Structural assignment on the basis of molecular weight is, however, questionable. In order to unambiguously identify a component without foregoing direct TGA-mass spectral integrity, MS/MS techniques must be employed.

Figure 3 (top) shows the Reconstructed Ion Thermogram for the 169⁺ ion (notice how closely this RIT resembles the DTG trace). Below the thermogram is the MS/MS daughter ion spectrum of the 169⁺ ion. In order to generate this spectrum the first quadrupole (Q1) was set to filter 169⁺ ions only. The ions were induced to fragment in the second quadrupole by collisions with a neutral gas (Argon) at a pressure of ca. 10⁻⁴ torr. While fragmentation proceeded, the third quadrupole (Q3) was scanned between 10 and 171 amu to yield this spectrum of fragment ions originating from the 169⁺ precursor. The whole process takes a few seconds under computer control and the resulting fragmentation pattern can be used to elucidate the precursor ion's structure (see structures Figure 3). Many such daughter ion spectra can be obtained sequentially and repetitively during a single TGA run providing complete structural information on the evolved compounds. A summary of results for the TGA/MS/MS analysis of cellulose acetate is provided in Table 1. Based on these results a scheme for the thermolytic breakdown of cellulose acetate is proposed which proceeds through the excited rearrangement product (A) (see Scheme 1). Using the ion intensities of the acetylated analogues of (A) (at m/z 163⁺, 205⁺, 247⁺ and 289⁺ obtained from inset of Figure 2) the %-acetylation can be estimated using equation (1). This is possible since, in a microscopic sense, the m/z 289⁺ ion,

CONCLUSIONS

REFERENCES

- FIGURE 2:** Averaged APCI-mass spectra of Cellulose Acetate between 360 and 410°C(TGA).

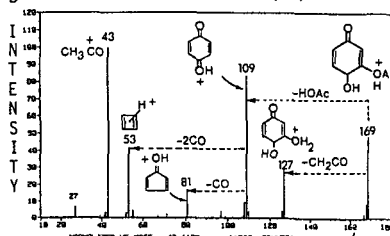

$$\left[\begin{array}{c} \text{H} \quad \text{OR} \\ | \quad | \\ \text{---} \text{C} \quad \text{C} \text{---} \\ | \quad | \\ \text{H} \quad \text{OR} \end{array} \right]_n \xrightarrow{\Delta} \begin{array}{c} \text{O} \\ || \\ \text{RO} \text{---} \text{C} \quad \text{C} \text{---} \text{OH} \\ | \quad | \\ \text{H} \quad \text{OR} \end{array} \quad \longrightarrow \text{OTHER PRODUCTS} \quad (\text{see Table 1})$$

CELLULOSE ACETATE
R = -H, -COCH₃

ION	STRUCTURE	ION	STRUCTURE ^a
61 ⁺	<chem>CC(=O)O</chem>	169 ⁺	
103 ⁺	<chem>CC(=O)OCC(=O)C</chem>	187 ⁺	
109 ⁺		229 ⁺	
127 ⁺		247 ⁺	
a. of neutral evolved from TGA.			

$$\% \text{ ACETYLATION} = \frac{(I_{205} \times 33.3) + (I_{247} \times 66.7) + (I_{289} \times 100)}{I_{163} + I_{205} + I_{247} + I_{289}} = 55.7\%$$

The diagram illustrates the TAGA 6000 MS/MS system. It includes three views of the main unit: a top view and two side views. Key components labeled include the Carrier Flow inlet, TOGA Oven, Interface Oven, Wide Bore Capillary, Microvalve Dump Valve, Heated Transfer Line, Pressure Sensor, Dupont 181 Oven, and Quartz Tube. An APG Ion Source is shown connected to the main unit.



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Pyrolysis Studies of Chlorinated Poly(vinyl chloride)

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The thermal decomposition of chlorinated PVC has been studied by pyrolysis-gas chromatography (Py-GC), Py-GC-mass spectrometry (Py-GC-MS), and Curie point pyrolysis-mass spectrometry (Py-MS). We have examined a series of chlorinated PVC's that have also been characterized by ^{13}C NMR.¹ Our primary objective was to determine if correlations could be made between the NMR and pyrolysis data. A secondary objective was to determine if Curie point Py-MS in combination with pattern recognition techniques could differentiate CPVC samples with varying chlorine contents.

In our study five of the seven NMR samples¹ were analyzed by pyrolysis-gas chromatography (Py-GC). NMR characterization data along with chlorobenzene pyrolyzate yields are given in Table I. Chlorobenzene correlation with %Cl by ^{13}C NMR is nearly linear (correlation coefficient .988), but a better linear correlation is obtained by plotting chlorobenzene yield versus mole-% (111) + (020) structures from NMR (correlation coefficient .999). This linear correlation is consistent with established PVC decomposition pathways,²⁻⁴ since either (111) or (020) structures can lead to chlorobenzene formation.

Six of the NMR samples¹ were also analyzed by Curie point Py-MS using an Extra-nuclear 5000-1 system (The University of Utah). Computerized processing of the Py-MS data involved the use of spectrum calibration and normalization procedures described elsewhere,⁵ as well as multivariate statistical analysis routines available in the Statistical Package for the Social Studies (SPSS).⁶ Multivariate analysis consisted of principal component analysis of the complete Py-MS data matrix followed by canonical variate analysis of the correlations between the nine most significant (eigenvalues > 1.0) principal components (factors) and the chlorine content of the CPVC samples as determined by ^{13}C NMR.¹ Finally, the canonical variate "spectrum" was calculated according to a procedure described by Windig *et al.*⁷ The canonical variate spectrum (Figure 1) enables one to visualize the ion signals most strongly correlating with changes in the chlorine content. The positive spectrum is dominated by chlorobenzene signals (m/z 112-114), but several other chloro-aromatic and aromatic hydrocarbon signals are apparent as well. Several hydrocarbon peaks are also observed on the negative side of the canonical variate spectrum. These compounds are either unsaturated aliphatics or else aromatics with appreciable aliphatic character.

In short it was found that numerous Py-MS fragments are strongly correlated with increases in the chlorine content of the polymer. These preliminary results suggest that Py-MS with computerized statistical analysis holds considerable potential for characterizing the composition and studying thermal decomposition mechanisms in chlorine-containing polymers.

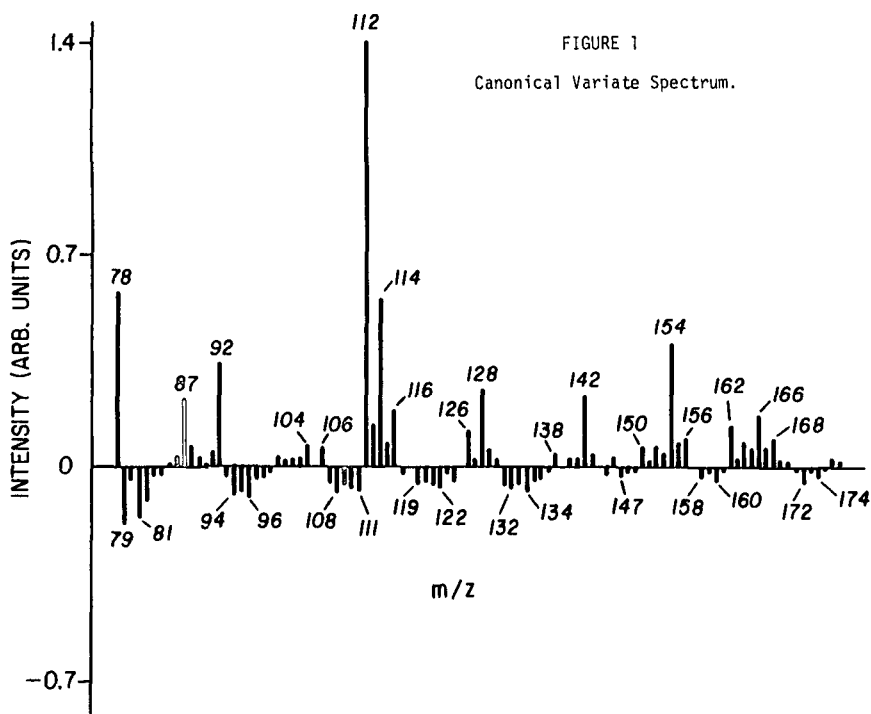
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TABLE I.

Chlorobenzene Pyrolyzate Yield and Other Characterization Data for Chlorinated PVC's.

Sample No.	% Cl ^a	% Cl ^b (NMR)	mol % ^b (111)	mol % ^b (020)	wt.-% ^c Chlorobenzene
1	56.2	56.8	0.0	0.0	0. ^d
2	56.7	57.3	1.1	0.0	0.081 ± 0.012
3	57.8	57.7	1.7	0.0	0.15 ± 0.04
4	58.3	58.7	3.3	0.5	nd ^e
5	58.0	59.5	4.0	0.5	nd
6	57.9	60.1	5.6	0.6	0.48 ± 0.07
7	59.4	60.6	7.1	1.4	0.69 ± 0.07

^a Schöniger oxygen flask method (data repeated from reference 1).^b ¹³C NMR (from reference 1).^c Pyrolyzate yield (based on weight of polymer). Standard deviations are given from multiple runs.^d Trace of chlorobenzene observed by Py-GC-MS.^e Not determined.

APPLICATION OF THERMAL DESORPTION/GAS CHROMATOGRAPHY/
MASS SPECTROMETRY (TD/GC/MS) TO POLYMER CHARACTERIZATION

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Thermal desorption/gas chromatography/mass spectrometry(TD/GC/MS) is a very useful technique for polymer characterization. Some applications of the technique are the detection of pneumatogens in polystyrene foams, the studies of melamine-formaldehyde reaction products and the thermal stability studies of polymers.

Figure 1 shows the instrumentation for the TD/GC/MS technique. It consists of a Chemical Data System Model 120 concentrator/trapper interfaced to a Finnegan 4000 GC/MS/COM system. The concentrator/trapper has a sample desorber connected to a Tenax GC trap. After loading the sample into the desorber, the sample is purged with helium for a given period of time. The sample is heated to a preset temperature for a given time period with helium passing through the sample. The volatiles that come off are trapped and concentrated. The trapped volatiles are desorbed from the trap onto the GC injection port and analyzed.

Different manufacturers use different pneumatogens for the manufacture of polystyrene foams. Fig. 2 shows the RIC (EI ionization) of the volatiles of a polystyrene foam (Desorbing temperature: 150°C for 5 min.; Trap temperature: 255°C for 5 min.; Desorber helium flow rate: 30 ml/min.) and the pneumatogen is found to be trichloromethane (freon 12). For the same brand of polystyrene foam in storage for over a year, the RIC indicates a significant decrease in the amount of detected pneumatogen. Thus, fresh samples are desired for better results.

Instead of using the concentrator/trapper, volatiles of the heated (200°C) melamine-formaldehyde resin were condensed onto a piece of glass at room temperature. The volatiles were then dissolved in methylene chloride and analyzed by the GC/MS system using the EI ion source (Fig. 3). A possible structure of the most abundant species in the RIC of Fig. 3 is shown in Fig. 4.

One of the important applications of the TD/GC/MS technique is the thermal stability studies of polymers. In the thermal stability studies, information concerning the relative stability of polymers, qualitative and quantitative information on the polymer degradation products can be obtained. Fig. 5 shows two polymer chains. The difference between the two polymers is that polymer 1 has C groups while polymer 2 has D groups instead. The relative stability of the two polymers can be determined by the desorbing temperatures at which the C and the D group appear in their RIC's. For example, the C group appears at 120°C while the D group appears at 140°C. Thus, polymer 2 is relatively more thermally stable than polymer 1. In addition, the RIC can also provide qualitative and quantitative information on the poly-

mer degradation products.¹

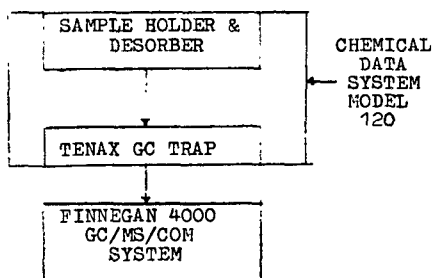


Fig. 1: Instrumentation for thermal desorption/gas chromatography mass spectrometry

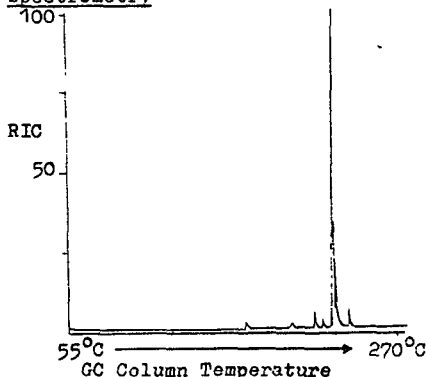


Fig. 3: RIC of the volatiles of melamine-formaldehyde resin (GC Column: 10 meter SP2100 capillary; GC Column Temperature Program: 55°C for 5 min. and 55°C to 270°C at 5°C/min.)

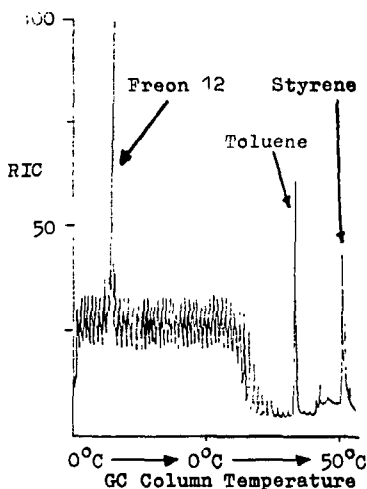


Fig. 2: RIC of the volatiles of polystyrene foam (GC Column: 60 meter SP2100 capillary; GC Column Temperature Program: 0°C for 10 min. and 0°C to 150°C at 5°C/min.)

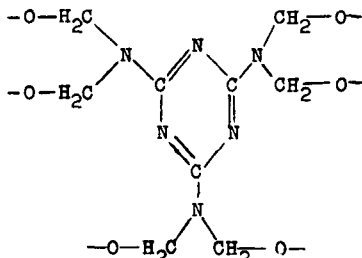


Fig. 4: A possible structure of the most abundant species in the RIC of fig. 3.

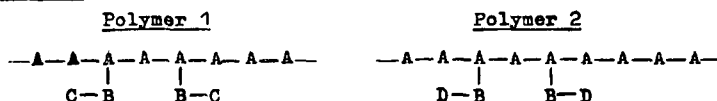


Fig. 5: Thermal stability studies of polymer 1 and polymer 2

Coal Structure and Liquefaction Reactivity Correlations Using
Pyrolysis/Mass Spectrometry/Pattern Recognition

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Pyrolysis/mass spectrometry combined with computerized pattern recognition is a powerful probe into the underlying structure of complex mixtures of organic compounds. In our laboratory, a number of flotation-separated sporinites (spore and pollen fossils from coal) were individually pyrolyzed at 510°C in the inlet to an EI ionization quadrupole mass spectrometer. A separate suite of whole coals which had been well characterized in terms of liquefaction reactivity and yields of liquefaction products by other workers was also pyrolyzed. Because the spectra obtained were exceedingly complex, computerized pattern recognition techniques were used to extract the meaningful information from the spectra.

Non-linear mapping demonstrated that there were differences in the pyrolysis mass spectra which related to rank of the sporinites. Factor analysis revealed seven abstract factors which contained 89% of the variance present in the original spectra. Multiple linear regression was then used to obtain from the seven abstract factors a single new factor which correlated with rank. Figure one is the "factor spectrum" obtained for rank: a plot of the coefficients or loadings on the original mass spectral peaks relative to rank. Peaks in the positive (upward) direction correlate positively with rank and their magnitude relates to the degree of their correlation with rank. Similarly, peaks in the downward direction are negatively correlated.

An analogous treatment was given to the whole coals. Figure 2 is the factor spectrum for percent carbon (which is highly correlated with rank) for the whole coals. Figures 1 and 2 are remarkably similar considering the very different origins of the two data sets. They are also very consistent with currently held beliefs concerning the diagenesis of coal; they show the loss of oxygen and increasing condensation and aromatization characteristic of increasing geochemical maturity.

Figure 3 shows the factor spectrum for asphaltene yield in the Gulf continuous flow reactor for the whole coals. Evidently, the presence of aryl oxygen, particularly of the lower molecular weight alkyl phenols, has a beneficial effect on the yield of asphaltenes in this liquefaction process. Perhaps this improvement is related to involvement of hydroxyl as a homogeneous hydrogen transfer catalyst.

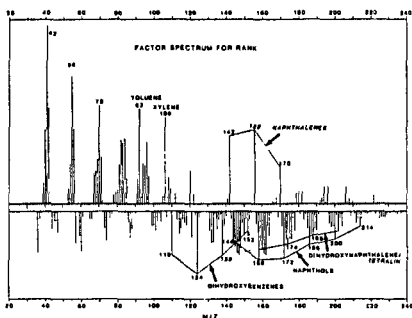


Figure 1. Factor Spectrum for Rank of Sporinities.

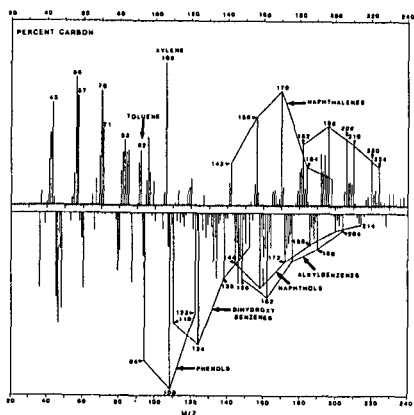


Figure 2. Factor Spectrum for Percent Carbon in Selected Coals.

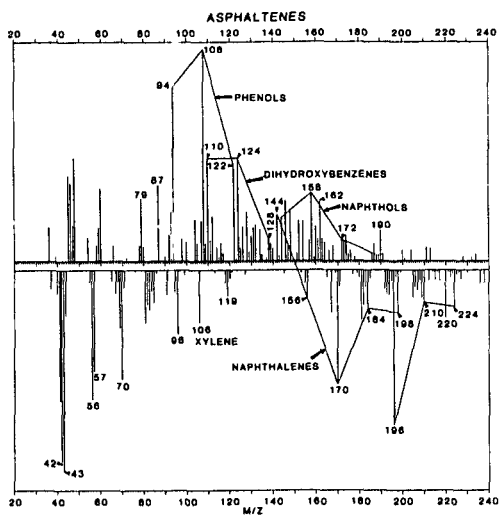


Figure 3. Factor Spectrum for Yield of Asphaltenes.

EXTRACTION AND GC/MS ANALYSIS OF ORGANIC CONTAMINANTS IN SEDIMENTS AND BOTTOM MATERIALS

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The characterization of organic contaminants in sediments and bottom materials is a difficult problem because of the highly complex nature of the matrix and the difficulties associated with the extraction of the organic compounds from the samples. GC/MS analysis is usually the method of choice for characterization of the broad range of compound types present in the matrix. Prior to GC/MS analysis, the organic contaminants must be preferentially extracted from the environmental matrix and rendered to a form suitable for this mode of analysis. There are a number of problems associated with converting the sample to an appropriate analyte. These would include the mode of extraction, the sample pH for extraction, the type of sediment, the time involved, the amount of sample handling required, the procedures for quality control and the sample clean-up prior to GC/MS analysis. Several of these areas will be addressed.

Soxhlet extraction and mechanical dispersion extraction with an ultrasonic probe are two methods included in the EPA interim methods for the analysis of priority pollutants in sediments (1,2). Figure 1 shows the reconstructed chromatograms of one sediment sample which was subjected to both extraction procedures. Employment of the mechanical dispersion extraction technique on the clay-like sediment resulted in the irreversible absorption of the organic contaminants, the surrogate compounds spiked into the sediment sample for quality control and a portion of the dichloromethane-acetone extraction solvent mixture. In contrast, Soxhlet extraction for 16 hours using a dichloromethane-methanol solvent mixture resulted in the successful isolation of the quality control surrogate compounds as well as several semi-volatile priority pollutants and other organic compounds. Soxhlet extraction efficiencies for the six surrogate compounds spiked into this sample ranged from 26% to 82%. In comparison, the mechanical dispersion extraction technique yielded no recovery of the spiked surrogate compounds from this particular sample. Soxhlet extraction yielded superior recoveries in all sediment types thus far analyzed. The most pronounced affect was noted in clay-type sediments.

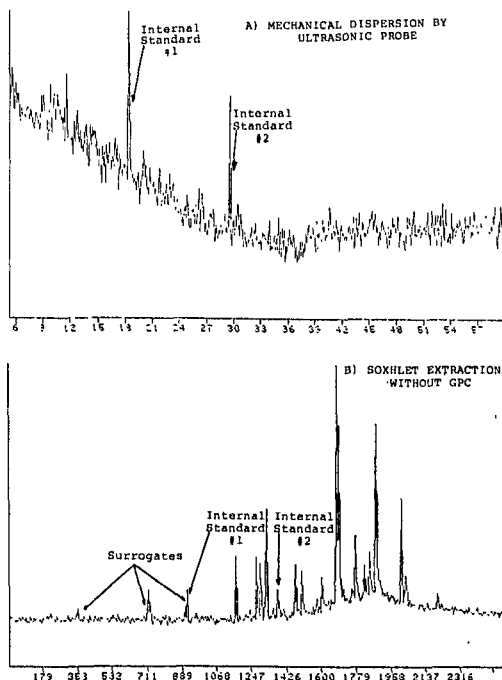
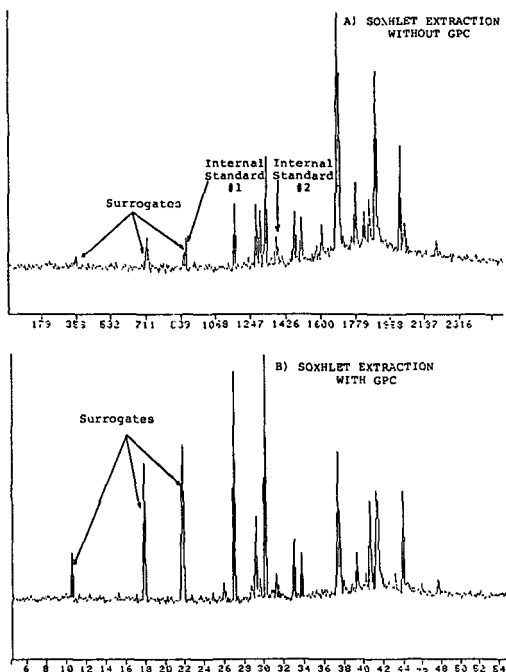


Figure 1: Reconstructed chromatograms of a sediment sample subjected to both a) mechanical dispersion extraction by ultrasonic probe and b) Soxhlet extraction.

Sample clean-up prior to GC/MS analysis is desirable to protect the capillary gas chromatography columns and the mass spectrometer from excessive contamination and to remove matrix interferences which could mask the identity and presence of the desired organic compounds isolated from the sediment sample. Our laboratory has employed open-column gel permeation chromatography (GPC) to remove many of the heavier molecular weight contaminants. The resultant reconstructed chromatograms of a sediment extract with and without GPC are shown in Figure 2. Easier identification of the organic compounds isolated from the sediment sample is possible after removal of the heavier molecular weight substances. This is dramatically displayed by the quality control surrogate compounds. In the reconstructed chromatogram of the sample without GPC clean-up, the surrogate peaks are not as readily evident as in the reconstructed chromatogram of the sample which underwent GPC clean-up.

The GC/MS analysis of organic compounds in sediments and bottom materials requires efficient isolation of the desired compounds from the sediments. Considerations for an efficient isolation procedure includes the extraction method and the sample clean-up. Soxhlet extraction results in greater amounts of organic compounds being isolated from the sediment and bottom material samples, and also has the advantage of reduced sample handling. This is especially important during the analysis of hazardous waste samples. Mechanical dispersion extraction with an ultrasonic probe requires less time since the extraction procedure can be completed in less than one hour per sample. Open column GPC is an efficient method for sample clean-up for GC/MS analysis. The removal of medium to high molecular weight interfering substances is essential for protecting the GC/MS system and for yielding more reliable data.



Figures 2: Reconstructed chromatograms of a sediment sample a) without and b) with GPC. GPC chromatography carried out on 10cm SX-3 Biobead open-column using dichloromethane as the elution solvent.

1. "Interim Methods for the Sampling and Analysis of Priority Pollutants in Sediments and Fish Tissue," U.S. Environmental Protection Agency, Physical and Chemical Methods Branch, Environmental Monitoring Support Laboratory, Cincinnati, Ohio 45268, Publication No. EPA 600/4-81-055, August 1977, Revised October 1980.
2. "Extraction and Analysis of Priority Pollutants in Sediment and Soil," (PPS-11/81) Environmental Protection Agency, Laboratory Services Branch, Sand A Division, Athens, Georgia 30613, November 1981.

IDENTIFICATION OF AMINE TYPES IN COAL LIQUEFACTION PRODUCTS

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The determination of amine types in synfuels is currently of high interest from both environmental and processing viewpoints. Recent reports have indicated that a considerable portion of the mutagenicity associated with coal-liquids resides in the amino polycyclic aromatic hydrocarbons (APAH) fraction. Partially hydrogenated basic nitrogen heterocycles are known to be highly effective as hydrogen donor and shuttling agents in direct coal liquefaction solvents and also as intermediates in nitrogen removal mechanisms. This report deals with developing the methodology and applying the existing instrumental techniques for differentiating between APAH and partially hydrogenated polynuclear aromatic hydrocarbons bearing a secondary nitrogen atom.

Initial efforts have concentrated on a heavy coal-liquid produced at 4550C under 4,000 psi of hydrogen from West Virginia hvAb coal without an added catalyst in the 400 lb/day fixed-bed liquefaction unit at the Pittsburgh Energy Technology Center (PETC). Nitrogen bases were separated from a particulate-free, methylene chloride soluble dialyze of the coal product by a column chromatographic procedure using a cation exchange resin. The APAH and partially hydrogenated nitrogen heterocyclics were separated from the pyridinic bases by derivatization with trifluoroacetyl chloride followed by chromatography employing the cation exchange column. Low voltage/high resolution mass spectrometry was then used to verify the separation of the derivatized APAH from the pyridinic moieties. With few exceptions, each precise mass formula cannot be interpreted unambiguously in terms of primary amino (APAH) or secondary heterocyclic amine structures.

In an effort to resolve these difficulties, MS/MS techniques were used to analyze the derivatized APAH fraction. Shown in Figure 1 is the chemical ionization mass spectrum of the nitrogen base fraction treated with trifluoroacetyl chloride. The derivatized sample consists primarily of three homologous series starting with m/z 204, m/z 230, and m/z 256, respectively. After subtracting the mass of the trifluoroacetyl group and the additional proton, the parent bases belong to homologous series beginning with m/z 107, m/z 133, and m/z 159, respectively. While the series originating at m/z 107 is probably substituted anilines, identification of the m/z 133 group is complicated by the possible presence of tetrahydroquinolines (THQ) in addition to aminoindan. Figure 2 displays the MS/MS daughter spectra for both the derivatized nitrogen bases and the derivatized tetrahydroquinoline standard. The reference compound gave a cleaner daughter spectrum, which indicates the probability of different isomers or minor components being present. However, the major component in the derivatized APAH fraction corresponding to m/z 133 is THQ.

Alternate MS/MS techniques, such as neutral loss scanning, allow additional information to be obtained from a given complex mixture. An example is shown in Figure 3, where neutral loss scanning permits differentiation of partially hydrogenated nitrogen heterocycles from APAH in the derivatized basic extract. In the case of the MS/MS daughter spectrum for derivatized THQ, loss of m/z 28 (ethylene) produced an intense fragment ion at m/z 202. By comparison, loss of m/z 28 was negligible in the MS/MS daughter spectra of APAH reference compounds. One of the major series of ions present, m/z 230, 244, 258, etc., corresponds to the previously identified THQ series. Further identifications of components present in the derivatized nitrogen-base extract, based on neutral loss m/z 28 scanning, are given in Table 1.

This study has provided evidence for the presence of a number of partially hydrogenated nitrogen heterocycles in a coal-liquid consistent with published accounts of nitrogen removal mechanisms. These results were obtained by direct analysis of the derivatized APAH fraction utilizing all three conventional MS/MS scanning modes -- daughter, parent, and neutral loss.

Table 1. Hydrogenated Nitrogen Compound Identification by MS/MS Neutral Loss Scanning.

Derivatized Bases Neutral Loss 28 amu Series	Molecular Weight of Corresponding Base	Possible Structure
230, 244, 258, 272, 286	133, 147, 161, 175	Tetrahydroquinolines
256, 270, 284, 298	159, 173, 187, 201	Tetrahydroazaacenaphthenes
280, 294, 308, 322	183, 197, 211, 225	Tetrahydroazaphenanthrenes

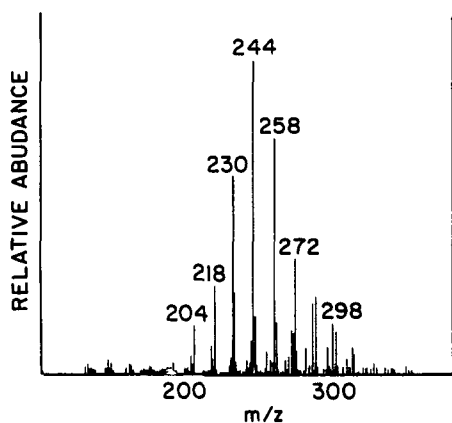


Figure 1-Chemical Ionization Nitrogen Bases Derivatized

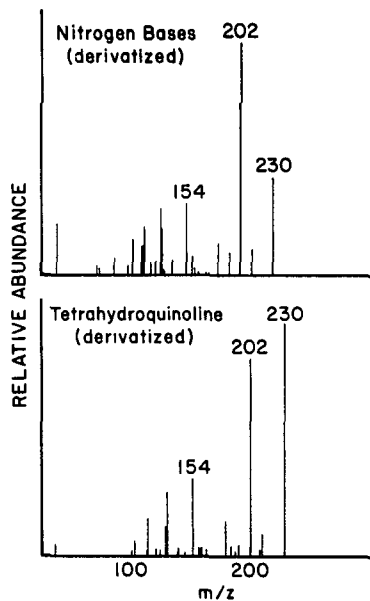


Figure 2-Identification of tetrahydroquinoline.

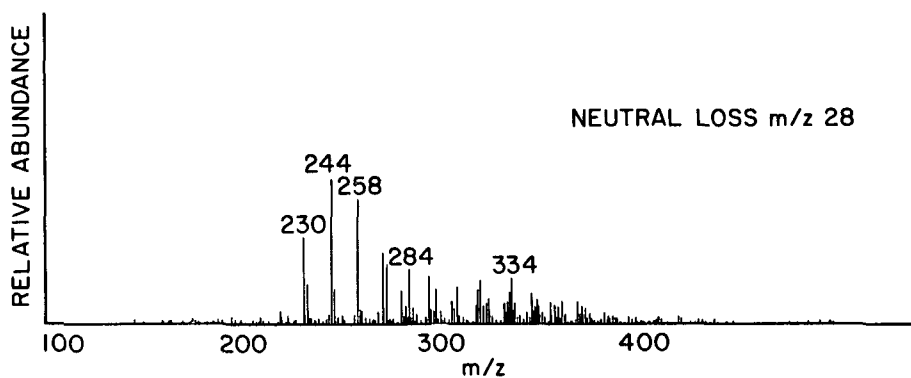


Figure 3-Nitrogen Bases derivatized

QUANTITATIVE ANALYSIS OF TRACE SULFUR GAS SPECIES
FROM OIL SHALE PYROLYSIS BY MS/MS*

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Livermore, CA 94550

We have previously described feasibility studies for using MS/MS to identify trace sulfur species formed in or released from the oil shale in the processing procedure.(1) Additionally, we have shown that the sensitivity, selectivity and specificity of the MS/MS technique makes it the method of choice for on-line studies of these environmentally important trace compounds.(2)

In this paper, we will describe the all glass pyrolysis apparatus we have designed and interfaced with our MS/MS inlet to do analysis of trace sulfur species from pyrolysis of shale.

Various oil shale samples were prepared for analysis. These included raw shales, acid leached shales, shales spiked with pyrite and organic sulfur model compounds, etc., so that differences between trace organic sulfur species and mineral sulfur species might be determined. Retorting or pyrolysis conditions of gas formation in these experiments were designed to duplicate actual Fisher Assay conditions.

The data show that the ratio of the various sulfur gases to the sum of the total sulfur gas is exceptionally constant. This holds true both for shales within a particular geological formation as well as for shales from several geographical areas. The standard deviation within a geological formation is fairly low for most species and the averages are nearly identical between areas. The percent of the total sulfur that ends up as a gaseous sulfur species is not constant but ranges from 2.6 to 25.6%. The results of spiked samples confirm the data seen from the raw shales: the sulfur gases are probably the result of an equilibrium from a series of reactions rather than simple decompositions. This is especially noteable in the case of pyrite and $MgSO_4$. This suggests that in the high temperature, carbon rich reducing atmosphere of shale pyrolysis, almost any form of sulfur will be at least partially converted to a range of organic gaseous species.

- (1) Symposium: Characterization of Shale Oils (Co-Sponsored by ASTM), 30th Annual Conference on Mass Spectrometry, Honolulu, Hawaii, June 1982, Paper No. F089.
- (2) 4th Briefing on Oil Shale Research, Lawrence Livermore National Laboratory, Livermore, CA, Sept. 22 and 23, 1982, P. 525.

*Worked performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.

MOD 1 | ENERGETICS OF STRONG HYDROGEN BONDING IN GAS PHASE IONS FROM ION CYCLOTRON RESONANCE EXCHANGE EQUILIBRIA. J. W. Larson and T. B. McMahon. Department of Chemistry, University of New Brunswick, Fredericton, NB, Canada E3B 6E2.

Bimolecular ion molecule reaction sequences have been used to generate cluster ions in the gas phase in which strong hydrogen bonding occurs. Hydrogen bond energies of halide ions to Bronsted acids will also be shown and the effects of gas phase acidity on hydrogen bond energy discussed. Binding energies of F^- and Cl^- to Lewis acids will be presented.

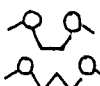
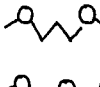
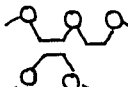
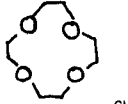
MOD 3 | ION-MOLECULE CHEMISTRY OF TRANSITION METAL CLUSTERS; D. J. Anderson, D. L. Bricker, and D. H. Russell; Department of Chemistry, Texas A&M University, College Station, Texas 77843.

Transition metal clusters play an important role in many areas of chemistry, in particular as synthetic models for biological and industrial catalysis as well as energy storage/conversion materials. We are presently studying the gas phase chemistry of transition metal clusters via Fourier transform mass spectrometry in an effort to model catalytic type reactions. The transition metal clusters utilized in these studies are synthesized by ion molecule reactions (e.g., $M^+ + N(CO)_x \rightarrow MN(CO)_x^+$) and include small homonuclear (2-4 metal atoms) and heteronuclear (2-6 metal atoms) clusters. It is not surprising to find that rather large ligand effects are observed for both cluster formation and reactivity with small hydrocarbons. These effects will be discussed as well as the applicability of FIMS methods for performing studies on transition metal clusters and related systems.

THE IONIC HYDROGEN BOND: PROTONATION AND COMPLEXES OF POLYFUNCTIONAL ETHERS AND AMINO ACID DERIVATIVES. INTRAMOLECULAR, MULTIPLE AND $\text{CH}_3^+ \cdots \text{O}^-$ BONDS.
Michael Meot-Ner (Mautner), National Bureau of Standards, Washington, D.C. 20234

The thermochemistry of protonation of polyfunctional ethers and of the model amino acid derivative $\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{COOCH}_3$ (1) (i.e., N-acetyl methyl ester) indicate intramolecular hydrogen bonding in the protonated species. Table I shows the proton affinities, the entropy changes (upon protonation by structurally simple reference compounds), as well as the increase in the proton affinities of polyfunctional species due to ion stabilization by intramolecular hydrogen bonding.

TABLE I

	PA ^a	$-\Delta S_{\text{prot}}^{\text{b}}$	$\Delta H_{\text{IHB}}^{\text{a}}$
	205	4	10
	214	6	19
	219	14	25
	220	---	22
$\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{COOCH}_3$ 224	15		6

^a kcal mol⁻¹, reference to $\text{PA}(\text{NH}_3) = 204 \text{ kcal mol}^{-1}$; (b) cal mol⁻¹K⁻¹.

When polyfunctional molecules complex with protonated species such as ammonium ions RNH_3^+ , polydentate bonding can occur, i.e., the enthalpies (ΔH_D^0) and entropies (ΔS_D^0) of dissociation of the polydentate complexes are higher than those of analogous complexes of monodentate ligands. Comparison between monodentate and polydentate ligands can identify the stabilization resulting from multiple hydrogen bonding (ΔH_{MB}^0). The thermochemistry (Table II) suggests structures such as 2 - 4.

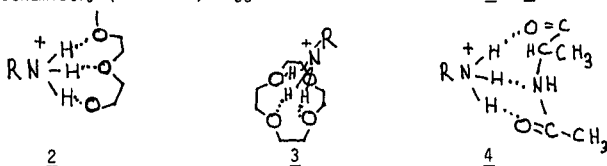


TABLE II

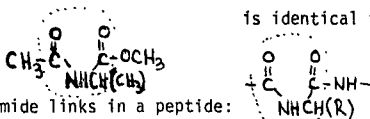
ION	LIGAND	ΔH_D^0	ΔS_D^0	$-\Delta H_{\text{MB}}^0$
$\text{c-C}_6\text{H}_{11}\text{NH}_3^+$		29.4	35.5	8
		39.7	44.6	15

		43.3	43.8	18
		37.2	34.8	12
CH_3NH_3^+	HCONH_2	30.0	30.0	6
	$\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{COOCH}_3$	40.1	35.1	14

Unexpectedly, even when multiple $-\text{NH}^+\cdots\text{O}-$ bonding is blocked, as in complexes of $(\text{CH}_3)_3\text{NH}^+$ with polyfunctional ligands, multiple interactions still seem to occur, as indicated by the thermochemistry of these complexes (Table III) compared with complexes of monofunctional ligands. The multiple interactions in these complexes may involve $-\text{CH}^{\delta+}\cdots\text{O}-$ bonds or multiple ion-polar group interactions.

TABLE III				
ION	LIGAND	ΔH_D°	ΔS_D°	$-\Delta H_{\text{MB}}^\circ$
$(\text{CH}_3)_3\text{NH}^+$		26.7	34.8	7
		32.8	33.1	10
		34.6	40.0	12
		35.8	41.5	13
	$\text{CH}_3\text{CONHCH}(\text{CH}_3)\text{COOCH}_3$	29.7	27.6	2

It should be noted that the geometry of the two carbonyl oxygens in the alanine derivative is identical to the geometry of two



adjacent amide links in a peptide: Therefore, the thermochemistry of protonation of 1 suggests that intramolecular hydrogen bonding between adjacent amide links may stabilize protonated peptides by $\sim 6 \text{ kcal mol}^{-1}$, and analogy with the larger polyethers suggests that intramolecular ionic hydrogen bonding between nonadjacent amide links folded for optimal bonding may stabilize protonated peptides by up to 30 kcal mol^{-1} . Further, in analogy with the complexes in tables II and III, multiple interactions between protonated amines or similar intermediates and amide groups in a protein may stabilize such ionic intermediates in enzymatic reactions by up to 45 kcal mol^{-1} .

THE ELECTRONIC STRUCTURE OF GAS PHASE ORGANOMETALLIC IONS: AN AB INITIO STUDY OF IONS OF THE TYPE CrCH_2^+ ; S.K. Huang, A. Alvarado, J. Harrison, John Allison, Department of Chemistry, Michigan State University, East Lansing, MI 48824.

Recently, experimental information on the bondstrengths of small transition metal-containing ions such as CoCH_3^+ has appeared in the literature (1,2). These have prompted theoretical investigations on such species to determine bondlengths, bondstrengths as well as the nature of the ground electronic states. This paper focuses on calculations at the SCF and MCSCF level on CrCH_2^+ . CrCH_2^+ is of interest since it can be formed by both ground state and excited state Cr^+ with a variety of organic molecules (3). Halle, Armentrout and Beauchamp have experimentally determined the metal-carbon bondstrength, $D^0(\text{Cr}^+-\text{CH}_2) = 65 \pm 7$ kcal/mole (3). They suggest that the bond may possess substantial π -character. Recently, Vincent, Yoshloka and Schaefer (4) have calculated the bondstrength of the ${}^6\text{B}_1$ state of CrCH_2^+ to be 22.3 kcal/mole. They conclude that "it seems likely that the experiments of Beauchamp do not refer to the bond energies" of this high spin state of CrCH_2^+ .

In the calculations reported here, molecular states derived from two states of Cr^+ and two states of CH_2 are considered. The first excited state of Cr^+ (${}^6\text{D}(\text{sd}^4)$) lies 1.6 eV above the ground state (${}^6\text{S}(\text{d}^5)$). The first excited state of CH_2 (${}^1\text{A}_1(\sigma^2)$) lies 10 kcal. above the triplet ground state (${}^3\text{B}_1(\sigma\text{p}_\pi)$). The CrCH_2^+ molecule contains 24 core and 7 valence electrons. Only states in C_{2v} symmetry were considered.

The combination of $\text{Cr}^+({}^6\text{S})$ and $\text{CH}_2({}^3\text{B}_1)$ yields 3 molecular states, ${}^8\text{B}_1$, ${}^6\text{B}_1$ and ${}^4\text{B}_1$. (CrCH_2^+ derived from the ${}^6\text{D}$ state of Cr^+ also may be in a ${}^6\text{B}_1$ state). The ${}^8\text{B}_1$ state contains 7 unpaired electrons and is unlikely to be bound. The ${}^6\text{B}_1$ state contains one sight coupled pair, (i.e. a possible chemical bond between a 3d_g metal orbital and a σ methylene orbital.) The ${}^4\text{B}_1$ state contains two singlet-coupled pairs, i.e. a possible double bond.

At the SCF level, the ${}^6\text{B}_1$ state ($\text{Cr}^+({}^6\text{S}) + \text{CH}_2({}^3\text{B}_1)$) is bound by only ~ 2 kcal. If the bonding electrons are correlated (to insure separation to the proper species) the state becomes bound by 12.5 kcal. By comparison, the ${}^6\text{A}_2$ state ($\text{Cr}^+({}^6\text{D}) + \text{CH}_2({}^3\text{B}_1)$) is much more bound at the SCF level (16.0 kcal) and bound by 22.0 kcal when the bonding electrons are correlated. These results indicate that Cr^+ in the sd^4 excited state forms CrCH_2^+ which is bound much more strongly than the state formed by Cr^+ in the d^5 state. This ${}^6\text{A}_2$ state is not, however, bound relative to

the ground state species.

The 4B_1 state was examined to allow the formation of a double bond. At the single determinant SCF level, this is a very high energy (unbound) state. When the σ and π electron pairs are correlated, the energy is lowered significantly, but the state is only very weakly bound. Analysis of this wavefunction suggests that there is very little double bond character.

Calculations on the strongly bound 6A_2 state (correlating to $Cr^+(^6D)$ and $CH_2(^3B_1)$) were performed at the MCSCF level. The state remains bound relative to the separated species by 21.2 kcal, but is not the molecular ground state.

To summarize, the lowest energy state of C_{2v} symmetry appears to be a 6B_1 state, containing a single bond. Calculated bondstrengths do not presently correlate with experimental results.

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Rare Gas Ion - Hydrogen Interactions.

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About 25 years ago Stevenson and Schissler (1) first studied the reaction between argon ions and hydrogen molecules and found it to behave almost ideally, i.e. according to the simple Collision Theory of Giumousis and Stevenson (2). Since then this system and its related isotopic variations and the analogous reactions with krypton ions have been the subject of numerous investigations, using various methods under a wide range of experimental conditions. It has become evident that these reactions are not so simple and especially the Kr^+ reactions show a number of peculiarities. We have added new experimental evidence by measuring the reactive cross sections of all these systems as a function of relative energy up to about 10 eV c.m.

Experimental: The cross sections were measured in an ion beam apparatus (3). A magnetic sector and electrostatic lens system separate and accelerate an ion beam through a neutral reactant in a reaction cell into a quadrupole and electron-multiplier detector, where both reactant and product ions are counted.

Results: $Ar^+ + H_2/HD/D_2$: The difference in cross section between the various isotopic species is very small, almost within the experimental error, fig. 1. The D_2 data tend to rise above the other cross sections at low energy, in agreement with other low energy measurements. The general trend is a rapid decrease of cross section with increasing energy, E , of the type: $\sigma \approx \exp(-nE)$, where n is about 2/3 and is slightly energy dependent.

$Kr^+ + H_2/HD/D_2$: The high energy Kr^+ cross sections fit the order: $HD > H_2 > D_2$, fig. 2. They show a general similarity to the Ar^+ data with the exponential constant n of about 2/3. At the high energy part of the curve, above 2 eV c.m., H_2 and D_2 show a second but lower maximum, which will be discussed later, while the HD curve is more or less monotonic, like the Ar^+ curves. Inspection of the two channels of $Kr^+ + HD$: $Kr^+ + HD \rightarrow KrH^+ + D$ and $Kr^+ + DH \rightarrow KrD^+ + H$, shows a maximum at a different energy for each, fig. 3, but the excitation curve of the sum of both cross sections is consequently almost smooth.

The intramolecular kinetic isotope effect, I.E., of both systems starts out normal, i.e. $XH^+ > XD^+$, fig. 4. The Ar^+ I.E. remains near unity up to about 2 eV c.m. after which it rises and goes through a sharp maximum. The Kr^+ isotope effect rises continually up to a maximum at about 1 eV c.m., then drops through an inverse I.E. minimum and rises again. This can be explained as follows: The excitation curves of ArH^+ and ArD^+ are almost parallel, fig. 3, and the I.E., therefore is almost constant up to about 2 eV, above that energy the ArD^+ curve falls off more rapidly and the I.E. consequently increases going through a maximum till both excitation curves approach each other again at low cross sections and high energy. The KrH^+ curve rises more steeply than that for KrD^+ , fig. 3, the I.E. therefore increases. Since the second maxima in both curves appear at different energies, the curves cross each other and the I.E. consequently dips through an inverse I.E. minimum and at the next crossing of the excitation curves the I.E. becomes normal again.

To understand the second maxima in the Kr^+ excitation curves, we assume that the low energy part of the excitation curve is similar to that of Ar^+ , we deconvolute (with a cubic fit) and obtain a second apparently endoergic curve with a threshold energy of about 0.6 eV and a maximum slightly shifted to a higher energy, fig. 5. This second curve clearly indicates the opening of a new reaction channel which requires about half an eV of activation energy. One may suggest that this channel is the result of a sequential impulsive collision (4) where the ion collides with one atom of the hydrogen molecule. This atom then collides with the other hydrogen atom, ejecting it with enough kinetic energy to reduce the total energy of the system to permit bonding between the ion and the remaining hydrogen. Preliminary calculations agree with this assumption.

A number of attempts to develop a theory which explains all observations published have been made since Friedman and Klein (5) suggested in 1964 the effect of the displacement of the centers of mass and polarisation. Of all the theories put forward that of P. Hierl (6) "Orientation Isotope Effect" appears to be the most promising. We are at present trying to refine it and to incorporate it in a phase space approach to rationalize our observations.

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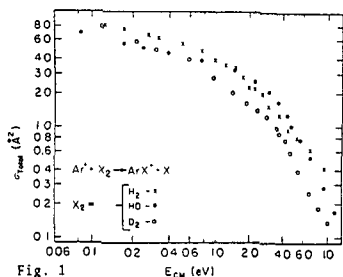


Fig. 1

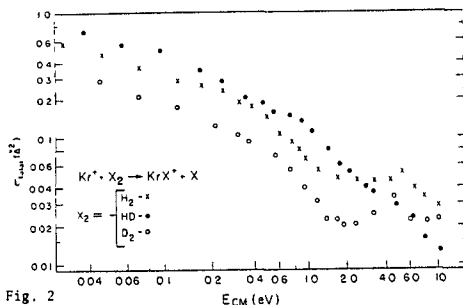


Fig. 2

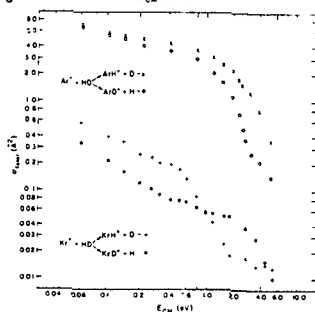


Fig. 3

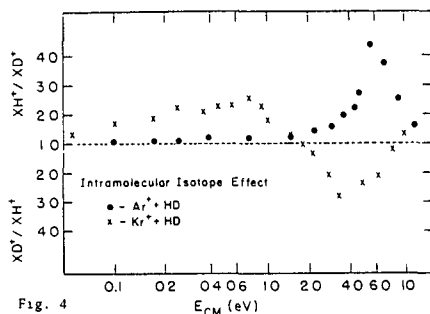


Fig. 4

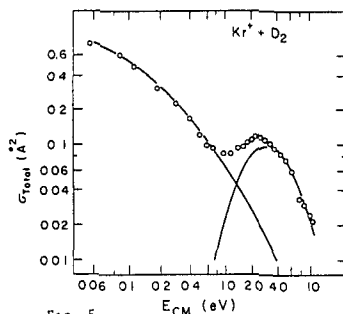
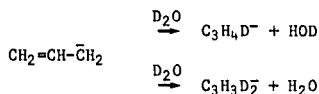


Fig. 5

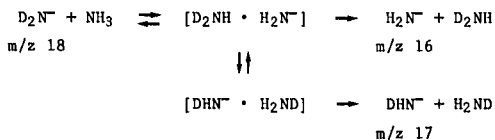
MULTIPLE PROTON TRANSFERS IN THE REACTION OF $\text{HO}^-/\text{H}_2\text{N}^-$ WITH NH_3 AND H_2O

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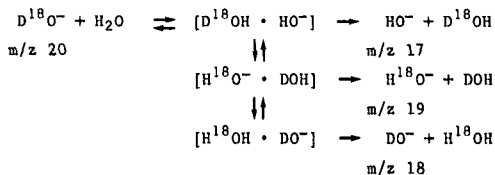
Since gas phase ion-molecule reactions proceed through the intermediacy of a complex, there is often time for more than a single reaction to occur before separation of the reaction products. One way to gain information about the lifetime of the complex and the extent of reaction is to examine the amount of deuterium exchange which occurs within a single ion-neutral encounter. In previous work we have investigated the extent of multiple exchange between a carbanion and D_2O ; we showed, for example, that a relatively large amount of doubly labeled allyl anion results from a single encounter with D_2O .



In order to gain further insight into reactions of this type, we have examined multiple exchange in reactions of amide and hydroxide ions with ammonia and water in our selected ion flow tube. To study exchange with ammonia we injected $\text{D}_2\text{N}^-/\text{H}_2\text{N}^-$ into a flowing afterglow and followed its exchange with NH_3/ND_3 .

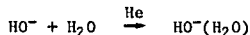


To study exchange with water, D^{18}O^- was injected and its reactions with H_2O measured.



We also studied the extent of multiple exchange in the exothermic reaction between amide ion and water and the endothermic reaction between hydroxide ion and ammonia.

The extent of exchange obviously depends not only on the rates of proton transfer but also on the lifetime of the complex. In order to gain insight into this latter factor we have measured the termolecular association rate coefficient for hydroxide ion reacting with water



In all of the systems studied to date extensive but far from completely random multiple exchange is observed. The maximum exchange is seen in slightly endothermic exchanges where the complex might be expected to be the most long-lived; however, even in the highly exothermic reaction between D_2N^- and H_2O some DO^- is produced, a reaction which must involve at least three proton exchanges.

STUDIES ON THE FORMATION AND REACTIONS OF H_3O^- AT 293K

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The ion H_3O^- has been observed as the product of an endothermic reaction between $\text{OH}^- \cdot \text{H}_2\text{O}$ and H_2 in an ion beam experiment at barycentric energies around 1 eV(1). A bond dissociation energy $D(\text{H}^- - \text{H}_2\text{O}) = 17 \pm 3$ kcal per mol was obtained by comparison of the energy threshold for reaction (1) with the known threshold measured in the same experiment for reactions (2) and (3).



The ion H_3O^- has also been observed by Kleingeld and Nibbering (2) as the product of a reaction between OH^- and H_2CO in an ICR experiment (reaction (4)).



We have been unable to observe H_3O^- from reaction (4) in a selected ion flow tube (SIFT) at 293K. However, we do observe the ions H_3CO^- and H_3CO_2^- , probably produced from reactions (5) and (6), as found by Kleingeld and Nibbering (2)



The decay of the OH^- reactant ion signal in the SIFT experiment has some curvature at low H_2CO flow rates. These results can be explained by a mechanism which includes reactions (4)-(7), together



with the assumption that the H_3O^- ions produced in reaction (4) are excited. The excited H_3O^- ions are apparently not quenched efficiently in collisions with the He carrier gas and live long enough to react with the added H_2CO . Assuming a steady state in the (excited) H_3O^- concentration, this mechanism leads to a rate equation which can be used to obtain a value for k_4 and a lower limit for the lifetime of the excited H_3O^- .

Use of OD^- as the reactant ion led to the observations summarized in reactions (8)-(12)- where again the H_2DO^- product was not observed directly.



When $\text{OD}^- \cdot \text{D}_2\text{O}$ reacted with H_2CO , solvent switching occurred (reaction 13)).



However, no H_3CO^- was observed, so we conclude that H_2DO^- could not have been formed.

The occurrence of H_3CO^- formed from reactions (8) and (9) when OD^- was the reactant, and the failure to observe H_3CO^- when $\text{OD}^- \cdot \text{D}_2\text{O}$ was the reactant leads to lower and upper limits for the bond dissociation energy of H_3O^- . These limits include the value $D(\text{H}^- - \text{H}_2\text{O}) = 17 \pm 3$ kcal derived from the ion beam experiment.

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COLLISIONAL BREAKUP AND ISOTOPIC EXCHANGE REACTIONS OF $(\text{CH}_3\text{O}_2)^+$.

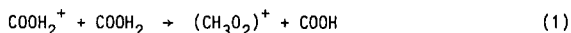
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CH_3^+ forms long lived complexes in collisions with many neutrals and thus undergoes fast ternary association reactions. $(\text{CH}_3\text{O}_2)^+$ formed via association of CH_3^+ with O_2 has the cluster form $\text{CH}_3^+\cdot\text{O}_2$, which can be deduced from observed switching reactions in collisions with CO and N_2 ^{1,2}. These switching reactions show the dissociation energy $D(\text{CH}_3^+ - \text{O}_2)$ to be less than the ones of $\text{CH}_3^+\cdot\text{CO}$ and $\text{CH}_3^+\cdot\text{N}_2$, which in turn are known to be smaller than $D(\text{CH}_3^+ - \text{NH}_3) \sim 4$ eV, from which the heat of formation $\Delta H_f(\text{CH}_3^+\cdot\text{O}_2)$ is estimated to lie between 170 and 262 kcal/mole. The lower one of these two values is still considerably higher than the heat of formation for $(\text{CH}_3\text{O}_2)^+$ of 109 kcal/mole, obtained from appearance potential measurements by Munson and Franklin³, who assumed this ion to be protonated formic acid. These findings imply the existence of different isomeric forms of $(\text{CH}_3\text{O}_2)^+$ depending on the processes of formation of this ion. In order to get more insight into the structure and other properties of $(\text{CH}_3\text{O}_2)^+$ produced in binary reactions, we performed drift tube investigations both on collisional breakup and on isotopic exchange reactions of $(\text{CH}_3\text{O}_2)^+$. The following identical results were obtained both with $(\text{CH}_3\text{O}_2)^+$ being produced in the fast proton transfer reaction

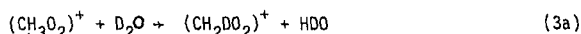


and in the extremely slow reaction

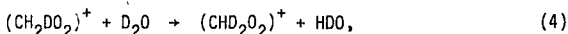


The rate coefficient for the collisional breakup of $(\text{CH}_3\text{O}_2)^+$ in collisions with Ar increase strongly from a few $\times 10^{-13} \text{ cm}^3 \text{ sec}^{-1}$ at relative kinetic energies, KE_{cm} , between $(\text{CH}_3\text{O}_2)^+$ and Ar, of ~ 0.2 eV to several $\times 10^{-11} \text{ cm}^3 \text{ sec}^{-1}$ at $\text{KE}_{\text{cm}} \sim 1$ eV. An Arrhenius plot of the breakup rate coefficients shows a dissociation barrier of at least 22 kcal/mole and a breakup pattern of $\text{HCO}^+:\text{H}_3\text{O}^+ = 50\% : 50\%$, independent on the collision energies. These results imply that $(\text{CH}_3\text{O}_2)^+$ ions produced in (1) and (2) are identical and that ΔH_f of these ions is considerably less than the above mentioned lower limit of $\Delta H_f(\text{CH}_3^+\cdot\text{O}_2)$.

Additional support for this identity was obtained from isotopic exchange reactions of $(\text{CH}_3\text{O}_2)^+$ produced in (1) and (2) with D_2O ^{4,5}. The total reaction rate coefficient for the reaction of $(\text{CH}_3\text{O}_2)^+$ with D_2O is fast at thermal energies ($7 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$), decreases with increasing KE_{cm} to $3 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$ at $\text{KE}_{\text{cm}} \sim 0.7$ eV, turning up sharply at higher energies to $7 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$ at $\text{KE}_{\text{cm}} \sim 1$ eV. The examination of the product distribution of the reaction shows the isotopic exchange (3a)



to be dominant in the energy regime below 0.7 eV, while at higher energies the endothermic proton transfer channel (3b) takes over. At low energies a consecutive second H-D exchange (4) is observed for the ion $(\text{CH}_2\text{DO}_2)^+$ produced via (3a) reacting with D_2O ,



but the product ion $(\text{CHDO}_2)^+$ shows no further exchange of the only H atom left in additional collisions with D_2O . These observations are consistent with the assumption that $(\text{CH}_3\text{O}_2)^+$ produced in the binary reactions (1) and (2) have the structure $\text{HC}(\text{OH})_2^+$. This structure also explains why reaction (2) proceeds at an extremely slow rate ($k \approx 7 \times 10^{-12} \text{ cm}^3 \text{ sec}^{-1}$) at near thermal energies^{6,7}, because considerable rearrangement is required to reach this structure starting from the reactants O_2^+ and CH_4 .

When $\text{HC}(\text{OD})_2^+$, produced in the source system via (3a) and (4) is introduced into the reaction chamber, its total rate coefficient for the reaction with H_2O shows the same energy dependence within experimental errors as the one for the reaction of $\text{HC}(\text{OH})_2^+$ with D_2O , and again, while single isotope exchange is dominant at low energies, above 0.7 eV the endoergic proton transfer is the main channel.

We have also investigated the proton transfer from $\text{HC}(\text{OH})_2^+$ to H_2O and from $\text{HC}(\text{OD})_2^+$ to D_2O . Both are immeasurably slow at room temperature but their identical rate coefficients rise sharply to $k \approx 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$ at $\text{KE}_{\text{cm}} = 0.7 \text{ eV}$ and $k \approx 7 \times 10^{-10} \text{ cm}^3 \text{ sec}^{-1}$ at 1 eV matching the proton transfer in reaction (3). This increase is also in quantitative agreement with the differences of the proton affinities of water and formic acid. Thus the present investigations show clearly the existence of two stable isomeric forms of $(\text{CH}_3\text{O}_2)^+$ depending on whether the ion is produced in a binary reaction or by three body association.

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LABORATORY STUDIES OF H/D EXCHANGE IN THE REACTIONS OF H_3^+ WITH H_2 .

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INTRODUCTION

During the last three years we have carried out detailed studies of isotope exchange in ion-molecule reactions, including $^{13}C/^{12}C$ exchange in the $C^+ + CO$ and the $HC_3O^+ + CO$ reactions (1), $^{15}N/^{14}N$ exchange in the $N_2H^+ + N_2$ reaction (2) and D/H exchange in the $H^+ + H_2$ (3), $CH_5^+ + CH_4$ (4) and $CH_3^+ + H_2$ (5) reactions. We have also considered the relevance of such reactions to isotope fractionation in interstellar molecules (1,2,6). In the $H_3^+ + H_2$ reactions, we first studied the reaction $H_3^+ + HD \rightleftharpoons H_2D^+ + H_2$ because of its interstellar importance (6,7). Now we have studied the other reactions in this series and the results of this study are briefly reported here.

The common approach taken in all these studies of isotope exchange reactions is to measure at one or more temperatures the overall forward (exoergic) rate coefficients, k_f , and the overall reverse (endoergic) rate coefficients, k_r , and also the product ion distributions when there are two or more products. From these data, the equilibrium constants, $K (=k_f/k_r)$, and hence ΔH° and ΔS° for the individual reaction channels can be determined. The ergicities of these reactions correspond to the differences in vibrational zero-point-energies (z.p.e.) and rotational energy contents of the reactants and products.

EXPERIMENTAL

The measurements were made using a variable-temperature selected ion flow tube (SIFT) apparatus, the features of which have been discussed previously (8). The reactant ions H_3^+ , H_2D^+ , HD_2^+ and D_3^+ , are formed in a high pressure ion source containing H_2 , D_2 or H_2/D_2 mixtures. The high source pressure relaxes any vibrational excitation in the ions. The ions are injected at low energy into pure helium carrier gas and the reactant gases, H_2 , HD or D_2 as appropriate, are introduced into the carrier gas in the usual way. Thus, k_f and k_r and the product ion distributions for all reactant ion/reactant gas combinations were determined at two temperatures, i.e. 80 and 300 K.

RESULTS

In four out of the ten reactions in the $H_3^+ + H_2$ series only one binary product channel is possible, whilst in the remaining six reactions two binary product channels are possible. This total study represents a great deal of data which will be presented in detail in a fuller paper (9). Here we choose to discuss just four representative reactions which illustrate the important features of the total data set. The reaction :

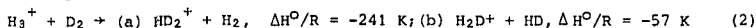


is one involving only one product channel. We have studied this reaction in detail previously (6,7). Both k_f and k_r have been obtained at 80, 200 and 295 K and $\Delta H^\circ/R$ was determined and compared with the values calculated from the z.p.e. and rotational energy contents reactants and products. The quoted value of $\Delta H^\circ/R$ for reaction (1), and indeed for the other reactions discussed below, have been calculated from the differences in vibrational z.p.e. only and do not include contributions due to any differences in rotational energy contents of the species involved. Differences in the rotational energy contents are sometimes very significant at low temperatures (<100 K). This is particularly so for reaction (1) for which $\Delta H^\circ/R$ is very dependent on temperature below 100 K (6), principally due to the so-called "rotational z.p.e." of H_3^+ (10). Note that the exoergic direction for the reaction is that resulting in an increase in the degree of deuteration of the ion (this is a "golden rule" for these $H_3^+ + H_2$ reactions, but not for all D/H exchange reactions (11)).

An important feature of these isotope exchange reactions (and indeed of all the other isotope exchange reactions which we have studied; these are referred to in the Introduction) is that the k_f increase towards the corresponding k_c (the collisional limiting value) as the temperature is reduced. Thus for reactions (1), $k_f(1)$ is $1.3(-9) \text{ cm}^3 \text{ s}^{-1}$ at 80 K ($k_c = 1.7(-9) \text{ cm}^3 \text{ s}^{-1}$). As expected k_r reduces with reducing temperature, T.e.g. $k_r(1)$ at 80 K is $2.9(-10) \text{ cm}^3 \text{ s}^{-1}$. Also when $T \sim \frac{1}{2} \{\Delta H^\circ/R\}$, then $k_f + k_r \rightarrow k_c$ for all the reactions studied, an observation which is particularly significant in predicting k_f and k_r values at the low temperatures of interstellar gas clouds (12).

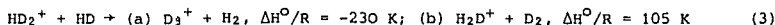
From the six reactions for which two product channels are possible, we choose for discussion a reaction for which both channels are exoergic, one which has an exoergic and an endoergic channel and one for which both product channels are endoergic. The usual accuracy of product ion distributions determined using SIFT apparatuses was not always achievable for these reactions because of the sometimes rapid secondary reactions which occur between the product ions and the reactant gas. This was particularly problematic when HD was the reactant gas.

Both product channels and exoergic in the reaction :



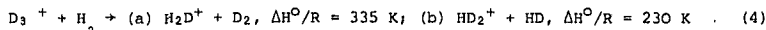
Statistics (entropy) favour H_2D^+ production (2b) which can be viewed as an atom exchange or isotopic scrambling process, whereas HD_2^+ production (2a) can be viewed as the more direct process of proton transfer. In a merged beam experiment (13) only (2a) was observed, presumably because the time scale of the interaction at beam energies does not allow for (2b) which presumably requires the formation of a $(\text{H}_3\text{D}_2^+)^*$ complex. The present SIFT data indicate that (2a) is dominant even at 80 K, but that (2b) is also significant (25% at 80 K). This indicates that, in the thermal SIFT experiment, the $(\text{H}_3\text{D}_2^+)^*$ complex has a sufficiently long lifetime to allow some H/D exchange (partial scrambling) to occur. However, there is more phase space available to (2a) because of the greater exoergicity of this channel; the simpler mechanism of (2a) is also favoured on the grounds of interaction time. The overall rate coefficient for the reaction is $1.4 (-9) \text{ cm}^3 \text{ s}^{-1}$ at 80 K and therefore is within error equal to k_c .

One product channel is exoergic and one endoergic in the reaction :



Clearly D_3^+ production (3a) requires complex formation and predictably is not significant in the beam experiment (13) but it is the major product (~80%) in the present SIFT experiments at 80 K. Although (3b) represents the simpler mechanism and is also favoured entropically, it is a small component of the reaction at 80 K because this channel is appreciably endoergic (i.e. $\Delta H^\circ/R \sim T$). However at 300 K, when $\Delta H^\circ/R < T$, then (3b) becomes the major channel (~70%) indicating that entropic and mechanistic effects rather than enthalpic effects determine the course of the reaction. The net rate coefficient for the reaction is relatively small (~0.5 k_c), even at 80 K, this being a reflection of the high probability for the regeneration of the reactant ion HD_2^+ out of the intermediate $(\text{H}_2\text{D}_3^+)^*$ complex.

Both product channels are significantly endoergic in the reaction :



This reaction has previously been studied in a tandem ICR (14) and both H_2D^+ (70%) and HD_2^+ (30%) were observed products, a result also obtained at 300 K in the present SIFT studies. However, the total rate coefficient in the ICR experiment is only 40% of that indicated by the SIFT study ($1(-9) \text{ cm}^3 \text{ s}^{-1}$). Thus at the higher temperatures (and energies) the direct deuterium transfer process (4a) is favoured; however, at 80 K channel (4b) is favoured (~60%), presumably because it is the least endoergic channel and because it is favoured entropically. That both channels are appreciably endoergic is manifest by the relatively small total rate coefficient for the reaction at 80 K which is $4(-10) \text{ cm}^3 \text{ s}^{-1}$. (i.e. $k \sim 0.2 k_c$).

CONCLUSIONS

Studies of D/H exchange in the $\text{H}_3^+ + \text{H}_2$ system clearly demonstrate the controlling influence of enthalpy and entropy differences on isotopic exchange reactions. They also demonstrate that isotopic scrambling occurs in relatively long lived intermediate complexes at low temperatures, whereas at higher temperatures the simpler mechanism of proton (or deuterium) transfer is favoured. The data on these reactions also add to the body of data on ion-molecule isotope exchange reactions (1, 15) which invariably show that the rate coefficients for the exoergic reactions, k_E , approach the collisional limiting values, k_c , at low temperatures (i.e. when $T < |\Delta H^\circ/R|$).

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THE ANALYSIS OF MUSTARD GAS USING
ION MOBILITY SPECTROMETRY/MASS SPECTROMETRY (IMS/MS)

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MD 21204

Mustard gas, bis-(2-chloroethyl)-thioether, was studied using
IMS/MS techniques (1). For an IMS temperature of 50°C, mustard gas
was found to be ionized via proton transfer reactions to produce
 MH^+ ions (reduced mobility of $1.63 \text{ cm}^2 \text{ volt}^{-1} \text{ sec}^{-1}$) and via elec-
trophilic attachment reactions to produce $(M+32)^-$ ions (reduced
mobility of $1.54 \text{ cm}^2 \text{ volt}^{-1} \text{ sec}^{-1}$). The negative ion response cor-
responds to an attachment of the oxygen ion to the parent molecule
as suggested by Blyth (2). Under conditions of a saturated chloride
reactant ion, $(M+35)^-$ ions (same mobility) were observed.

At 140°C, the MH^+ ion was still observed but the $(M+32)^-$ ion
disappeared in favor of a chloride ion mobility peak. The decrease
in the $(M+32)^-$ ion is due to an increase in the reverse rate con-
stant for



while the increase chloride ion yield is due to the thermal sensi-
tivity of the dissociative capture process.

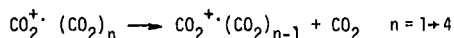
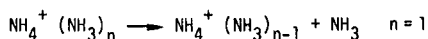
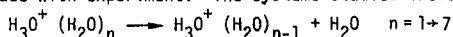
The ionization chemistry for mustard is similar to that for
dimethylsulfoxide (3,4).

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MOD 10 | KINETIC ENERGY DISTRIBUTIONS OF UNIMOLECULAR REACTIONS OF NASCENT COLLISION COMPLEXES: EXPERIMENT AND THEORY; Andreas J. Illies, Martin F. Jarrold and Michael T. Bowers, Department of Chemistry, University of California, Santa Barbara, CA 93106.

Nascent ion-molecule collision complexes are formed in a temperature and pressure variable ion source, extracted, mass selected and unimolecular reactions back to reagents observed in the second field-free region of a reverse geometry mass spectrometer. The metastable peaks are obtained under high energy resolving power ($> 10,000$) and deconvoluted numerically to obtain the kinetic energy distributions. The metastable/main beam intensity ratio is measured as a function of source pressure. A statistical phase space theory model is developed to calculate both the energy distributions and intensity ratios for the dimer to monomer metastable reactions; comparisons are made with experiment. The systems studied include:



In all cases the average kinetic energy release increases with n .

MOF 11 | IONS FORMED BY METHYL TRANSFER DURING FIELD DESORPTION MASS SPECTROMETRY (FDMS); Gordon W. Wood, R. J. Collacott, and D. V. Ramana; University of Windsor, Windsor, Ontario, N9B 3P4 Canada.

A methyl transfer reaction, analogous to the well-known thermal nucleophilic substitution ($\text{S}_\text{N}2$), was identified as a concomitant of FDMS of suitable substrates as early as 1975 (1). Extensions of this observation have made the methyl transfer process one of the most widely acknowledged sources of artifact ions in FDMS (2). Recent work will be presented on betaines and related zwitterions, including labelled analogs, in which attention is focussed on the migration process. Distinction will be made between thermal and field-induced methyl transfer by comparison with EIMS results.

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RECENT ADVANCES IN THERMOSPRAY LC-MS

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Our original approach to LC-MS employed laser heating to rapidly vaporize both the solvent and sample, and molecular beam techniques to transport the sample to the ion source of the mass spectrometer.¹ Later we developed a greatly simplified version of this system by using oxy-hydrogen flames to vaporize the LC effluent and a substantially less elaborate vacuum system.² More recently an electrically heated vaporizer system was installed on a commercial quadrupole instrument.³ This interface required only minor modification of the commercial instrument with a single vacuum pump added to the pumping system normally used for CI operation. LC effluents are thermosprayed directly into the ion source and the excess vapor pumped away by the mechanical pump which is directly coupled to the ion source opposite the vaporizer. In earlier work it was found that, under certain conditions, ions were produced even though the hot filament normally used to produce the primary ionizing beam was turned off.⁴

The thermospray process is defined as the production of a jet of fine liquid particles by heating a liquid stream as it is forced through a small diameter capillary tube. Our recent work has focused on obtaining a better understanding of the processes involved in thermospray LC-MS system. High-speed flash photographic studies of the thermospray jet show clearly that thermospray is quite distinct from the direct liquid injection method.⁵ In direct liquid injection, the inlet line is cooled to prevent vaporization upstream of the spraying aperture, while in thermospray controlled partial or complete vaporization of the liquid is accomplished by strongly heating the capillary tube. This leads to production of a supersonic jet of vapor with liquid droplets and solid particles entrained in the jet. Primary ions are produced by field-assisted evaporation of ions from charged liquid droplets or solid particles. No external electrical fields are required in the ionization process; the local field generated by the charge on the small droplets produced by thermospray vaporization of aqueous electrolytic solutions is sufficient. Only thermal energy is supplied.

A schematic diagram of a recent version of the thermospray system is shown in Figure 1. This system employs a temperature sensor in the vapor jet to allow proper control of the vaporizer and jet heaters. For example, the jet temperature just downstream of the Mach disk can be monitored by sensor #2 and the temperature of the vapor just downstream of the ion sampling point can be monitored by sensor #4. These sensors can be used to control the power inputs to the vaporizer and jet heaters to maintain proper operating conditions even though the solvent flow rate or composition may change. A system for automatically maintaining proper operation over a wide range of conditions is presently under development.

Performance characteristics of the present system are summarized in Table 1. The indicated detection limits have been achieved for a wide variety of relatively nonvolatile

or thermally labile compounds with aqueous solvent flow rates in the 1 to 2 mL/min range. For very difficult samples which are both nonvolatile and thermally labile, the detection limits are often somewhat poorer. It appears that pseudomolecular ions can be obtained on any compound which is ionized in solution, at least up to the molecular weight limitation of our present quadrupole (ca. 2000 amu) but, for very difficult cases, sample quantities in the microgram range are sometimes required.

Several problems remain to be solved before development of the thermospray technique can be considered reasonably complete. Improved automatic control of the vaporizer and jet heaters is required to properly compensate for large variations in solvent flow and composition. Smaller diameter capillaries in the vaporizer would allow operating at lower flow rates and/or higher vapor temperatures. Better control of downstream heating of the jet may lead to improved performance for difficult cases involving samples which are both thermally labile and nonvolatile. Mass range can probably be extended by adaption of the thermospray system to a high performance magnetic mass analyzer. Improved fundamental understanding of the ionization and vaporization mechanisms may lead to further improvements in the apparatus and techniques. Work is continuing in all of these areas.

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TABLE I. SUMMARY OF PERFORMANCE CHARACTERISTICS OF THERMOSPRAY LC-MS

DETECTION LIMITS	1-10 picograms/sec
LINEAR DYNAMIC RANGE	10 ⁴
LIQUID FLOW	0.5 to 2 mL/min
TIME CONSTANT	1 sec
SOLVENT	ANY
BUFFERS	ANY (VOLATILE BUFFERS GIVE BETTER PERFORMANCE)
SAMPLE	ANY (VOLATILITY HELPS SENSITIVITY)
GRADIENT COMPATIBLE?	YES, WITH PROPER CONTROL OF VAPORIZER

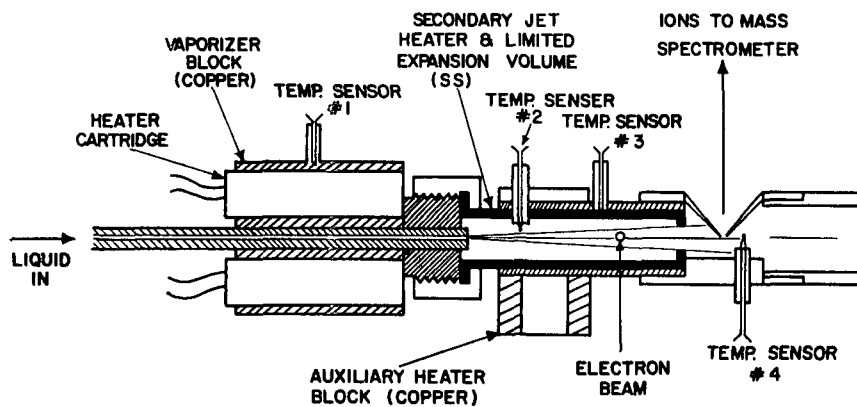


Figure 1. Schematic Diagram of Thermospray Apparatus

APPLICATIONS OF SUPERCRITICAL FLUID CHROMATOGRAPHY- MASS SPECTROMETRY^(a)

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The analysis of complex mixtures is hindered by the molecular weight range and selectivity of present analytical instrumentation. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) are ultimately limited by the volatility of the particular chemical species to the study of compounds having molecular weight less than approximately 300 to 400. Liquid chromatography can be used to separate higher molecular weight and less volatile compounds. However, the chromatographic resolution of HPLC is much less than with modern capillary column GC, the solvating capability of the mobile phase is often insufficient for large compounds containing several functional groups, and a sensitive and selective detector is required. Modern HPLC is rapidly approaching the practical limits on the number of effective theoretical plates and only minor improvements in chromatographic efficiency can be expected without new breakthroughs in column and pump technology. While some advances seem likely using packed capillary columns, open tubular columns for HPLC suffer from the extremely small diameter required ($<10\text{ }\mu\text{m}$) for improvements in efficiency and associated difficulties related to sample size limitations, stationary phase preparation, and detection limits.

The ideal detector for HPLC is the mass spectrometer due to its inherent sensitivity and selectivity. However, existing HPLC-MS combinations to date have all proved to be lacking due to one or more difficulties which include: extreme complexity, inadequate sensitivity, large variations in relative sensitivity, mass spectra complicated by pyrolysis or heterogeneous processes, inadequate (or degradation of) chromatographic resolution or poor interface operation.

The combination of capillary column supercritical fluid chromatography (SFC) with mass spectrometry offers unique advantages. The range of compounds which may be separated with SFC is quite similar to HPLC. Both techniques allow the separation of materials which are thermally labile and of much higher molecular weight than possible using GC. Supercritical fluid chromatography has the added capability of pressure programming, providing precise control of the solvating power of the mobile phase. The much higher volatility of most SFC mobile phases provides a significant advantage in the design of the SFC-MS interface⁽¹⁾. The use of wall coated open tubular capillary columns provides mobile phase flow rates compatible with chemical ionization mass spectrometry. Other advantages accrue from the high chromatographic efficiency possible with capillary columns due to the low pressure drop across the column and the more uniform density of the mobile phase.

The SFC-MS instrumentation development in our laboratory⁽¹⁻³⁾ incorporates a high pressure programmable syringe pump, a constant temperature oven, $0.2\text{ }\mu\text{L}$ injector, capillary column SFC, direct fluid injection (DFI) interface, and a tandem quadrupole mass spectrometer equipped with a dual electron impact-chemical ionization ion source. Fused silica capillary columns, with 50 to $200\text{-}\mu\text{m}$ I.D. and lengths of 10 to 50 m, have been used. Our work to date has utilized isobutane, n-pentane, nitrous oxide and carbon dioxide as well as fluid mixtures. The principal design considerations for the DFI probe involved obtaining constant temperature conditions, proper column restriction to obtain optimum flow rates, and efficient transfer of nonvolatile compounds to the ionization region. The fused silica capillary column is connected to the DFI probe tip by a zero dead volume connection, and platinum-iridium tubing extends through the last 3 cm of the interface to the flow restrictor which determines the mobile phase flow rate. The DFI probes have been designed to couple with a modified direct probe inlet of a chemical ionization (CI) source. A second CI source inlet provides an additional flow of chemical ionization reagent gas (usually the SFC mobile phase). A servo-valve and pressure controller are used to regulate gas flow. Thus, a constant CI source pressure is maintained during SFC pressure programming.

The capillary SFC-MS interface avoids problems related to solvent evaporation by maintaining supercritical conditions up to the DFI restrictor. Additional advantages result from

the much higher volatility of typical mobile phases compared to HPLC, where typical conditions result in extensive cluster formation and persistence of a jet of frozen liquid droplets extending 2 to 5 cm into the vacuum region, and the resulting need for a "desolvation region". The DFI interface produces only clusters of $<100^{\circ}\text{A}$ in diameter which have a very short lifetime under the chemical ionization source conditions and which probably do not persist past the Mach Disk (shock wave)⁽⁴⁾. The result is that the mass spectra show no evidence of ion clusters other than typically observed for chemical ionization. Our experience to date indicates that any material soluble in the supercritical fluid phase is efficiently transferred to the gas phase. Thus, it appears that CI spectra can be obtained for any compound soluble in a supercritical fluid.

The SFC-MS instrumentation has been applied to the separation and analysis of a variety of complex samples including synfuel fractions, various environmental materials, and polymeric samples.

Work to date has demonstrated that SFC-MS is an effective method for the separation and analysis of thermally labile compounds. Recent work with new stationary phases and smaller diameter columns suggests that the optimum column diameter is approximately 50-75 μm . In this range over 3000 plates/meter have been demonstrated, approaching the chromatographic efficiency obtained by capillary gas chromatography. (The application of smaller column diameters suffers from both the decreased sample loading required and the greater pressure drop across the column, which restricts the application of the pressure programming technique.) Thus, the greatly enhanced chromatographic resolution possible compared to HPLC and the efficient and sensitive detection offered by the DFI-MS method, presents the promise of wide range of improved analytical methods based upon SFC-MS.

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DIRECT LIQUID INTRODUCTION LC/MS, Jack Henion, New York State College of Veterinary Medicine, Cornell University, 925 Warren Drive, Ithaca, NY 14850.

Combined LC/MS has not experienced the rate of growth or acceptance that GC/MS was enjoying ten years ago. This results from a number of factors which include the added difficulty of introducing aqueous organic solvents into the vacuum system of mass spectrometers. This is to say that enriching the chromatographic effluent by removing helium is far easier than trying to remove most of the liquid from the solute eluting from a HPLC.

The two most successful approaches to on-line LC/MS are the transport type interface best represented by the moving belt and the direct liquid introduction (DLI) interface first practically demonstrated by McLafferty and co-workers. The former attempts to remove all or most of the LC eluent while the latter actually introduces the LC eluent directly into the ion source of a chemical ionization mass spectrometer and uses the vaporized solvent gases as the CI reagent gas. The transport interface thus affords the possibility of both EI and CI mass spectra while the DLI interface is confined to only CI mass spectra.

We have explored the experimental utility of the DLI LC/MS interface because of its inherent simplicity and the knowledge that problems can be encountered when removing trace levels of labile substances from a heated surface as is required in the moving belt interface. Since the DLI interface transports the solute in solution directly into the chemical ionization source, the sample is "protected" from thermal decomposition or contact from heated surfaces. This has been demonstrated by several workers and most recently in a comparison study of the labile metabolites of ranitidine on each of the commercially LC/MS interfaces (1). Since our work involves drug metabolism studies, we have pursued development studies of the DLI LC/MS interface focussing our efforts on practical implementation and improved detection limits:

Our earliest DLI LC/MS work utilized a glass capillary tube patterned after that reported by McLafferty and Arpino. Melera of Hewlett-Packard added a significant improvement to this approach by incorporating a water cooled probe with a removeable diaphragm containing a laser drilled five micron pinhole. This device eliminated the viscous flow characteristics of the capillary tube and provided an easy means of unplugging the pinhole if it became occluded. This DLI LC/MS interface became commercially available from HP in 1979 and made conventional DLI LC/MS possible but split the LC effluent such that only about 1% of the total effluent entered the mass spectrometer. Thus on-column sensitivity was limited to only 0.5-1 microgram levels. This is suitable for many applications where plenty of sample is available, but is not sufficient sensitivity for most metabolism work.

We initiated DLI micro LC/MS using a JASCO FAMILIC 100 micro LC several years ago to evaluate the feasibility of the technique. The sensitivity achieved was significantly improved over the split effluent interface, but the column efficiency for the micro LC columns at that time was not high enough to be practically useful. Since then commercial availability of high performance micro LC columns (1mm id) has taken place, and with minor modifications of conventional HPLC equipment it is possible to perform respectable micro LC. A DLI micro LC/MS diaphragm interface was therefore developed which allowed the use of this new capability (2). This new approach to micro LC/MS combined the desirable features of the removeable diaphragm and the improved efficiency of the new generation stainless steel 1mm id micro LC columns which were readily adaptable to conventional HPLC fittings. Routine detection limits of drugs and their metabolites in the 5 ng range injected on column were achieved with full scan mass spectra in either the PCI or NCI ioni-

zation mode. These experiments introduced the total micro LC effluent into the mass spectrometer. Improved performance was obtained by changing the HP ion source desolvation chamber to a heated, extended desolvation chamber with internal solvent stripping (3). A five-fold increase in sensitivity was achieved by this rather simple modification which is applicable to either conventional or micro DLI LC/MS.

Although micro LC/MS provided detection limits comparable to GC/MS, many researchers were not anxious to implement this technology into their laboratories. This appeared to be due in part to the inertia already established by conventional HPLC, and partly due to the admittedly persnickety nature of conducting micro HPLC on equipment not specifically designed for such purposes. In any event we have developed methods that attempt to utilize the benefits to LC/MS provided by reduced flow rates and micro LC columns. A current trend in HPLC is to use 2 mm id packed microbore columns which operate at flow rates in the neighborhood of 100-200 microliters/minute. A recent entry into this field are the 2mm id x 10cm and 2mm x 22cm columns provided by Brownlee Labs, Inc. which are equipped with removable guard columns and housed in a convenient cartridge holder. These 2 mm id microbore columns provide good chromatographic efficiency at 100 L/min flow rates and may be utilized with conventional HPLC and DLI LC/MS equipment at a much more favorable split ratio. Thus one can achieve routine 50 ng detection limits of solute injected on column without the necessity of modifying any equipment.

Multidimensional HPLC (heart cutting) has received renewed interest recently and this technology offers some additional analytical capability to LC/MS. Multidimensional LC/MS, or LC/LC/MS, allows the analyst to utilize the combined chromatographic capabilities of normal phase and reversed phase HPLC. This can be particularly useful when one is analyzing crude biological extracts which contain the component(s) of interest in the presence of large amounts of interfering endogenous material. Thus one can load a normal phase silica column with the crude extract and initiate the separation with the effluent passing through the sample loop of a second loop injector connected to a reversed phase column. When the component of interest passes through the detector and into the loop of the second injector, that injector is used to inject that cut (heart cut) of the normal phase effluent onto the reversed phase column. If this second column is connected to a LC/MS interface one can conduct on-line LC/LC/MS experiments to identify the components of interest isolated by LC/LC from rather impure samples.

We have implemented multidimensional micro LC/MS (micro LC/LC/MS) in the manner described above. Figure 1 shows a schematic representation of the apparatus which included a JASCO FAMILIC 100 connected directly to a Rheodyne injector which was fed by a modified Waters HPLC pumping system connected to a Whatman ODS 3 microbore reversed phase column. The exit of this microbore column was connected directly to our DLI micro LC/MS probe which was placed in the solid probe inlet of a HP 5985B GC/MS. Resolution of a crude equine urine extract from a horse which had received an administration of prednisolone could not be achieved by either micro LC column alone. Thus the feasibility of this approach has been demonstrated for those instances where this combination of analytical equipment is required.

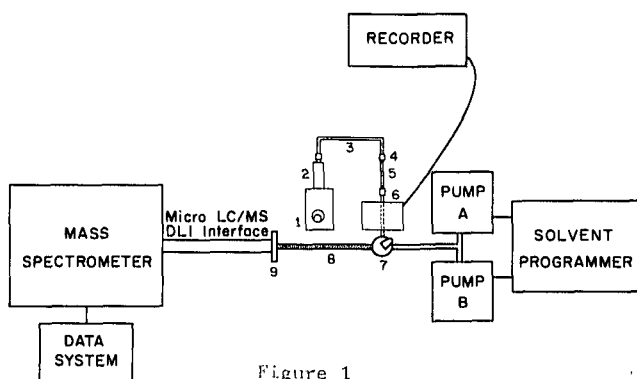


Figure 1

One of the most significant recent advances in LC/MS is the report of thermospray by Vestal and co-workers (4). This approach to LC/MS appears to offer the sensitivity of micro LC/MS without the need to utilize micro LC columns. Thus chromatographers may rely on their experience with conventional HPLC utilizing one or two mL/min eluent flow rates which are introduced unsplit into the ion source of a partially modified CI mass spectrometer. The most amazing aspect of thermospray is that ionization occurs as a result of the heated vaporizer LC/MS probe without any contribution from a conventional filament as a source of ionizing electrons. The end result of this is that ionization of involatile, thermally labile compounds occurs with abundant molecular weight information.

We were intrigued by the enormous potential that thermospray LC/MS appears to offer, so we have implemented Vestal's approach on a commercially available HP 5985B GC/MS. This included adding an auxiliary rough pump to evacuate the ion source from the GC side and construction of a removeable thermospray vaporizer probe interface which would be accommodated by the standard solid probe inlet of this instrument. Figure 2 shows a schematic diagram of this system.

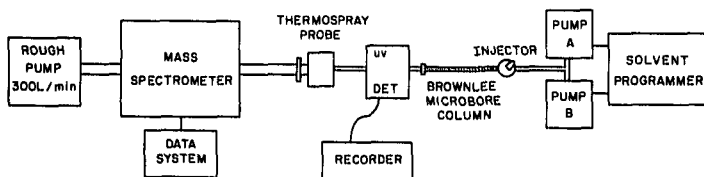


Figure 2

We have achieved the thermospray process, eg. ionization without any external source of ionizing electrons, using an eluent of 50/50 CH₃OH/0.05M NH₄OAc containing 10 ppm adenosine through a Brownlee Labs RP 18 2mm id x 25cm microbore column operated at 150 μ L/min and a vaporizer temperature of 225 degrees C. The ion current resulting from these experiments was very stable and the (M+1) ion at m/z 268 for adenosine was the base peak in the full scan mass spectrum of this compound. An actual thermospray LC/MS experiment involving the separation of theophylline and caffeine provided the customary ion current profiles showing the separation with their respective (M+1) ions as the base peak in each of their mass spectra. Thus the thermospray process is possible from a conventional mass spectrometer utilizing homemade equipment. This system provides the added flexibility of a removeable thermospray interface and eventually the ability to keep the GC interfaced to the MS.

The above discussion attempts to highlight some of our recent experience with DLI LC/MS. In all cases we endeavor to keep things simple and practical. Some of the approaches described could just as well be implemented on the moving belt transport interface and would similarly improve its capability. For trace level work, however, where very low levels of fragile compounds must be thermally volatilized from the belt surface, the DLI method of sample introduction appears to offer some advantages.

LC/MS is a fertile area for development which offers tremendous analytical opportunities when a truly viable LC/MS interface is available. Each analytical technique has its limitations and LC/MS is no exception. However, as we strive to develop better methods, perhaps a near-universal interface will be developed. In the meantime there is a lot of good analytical work that can be accomplished with what we already have.

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PROBLEM SOLVING WITH LC/MS

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Introduction

Just over six years ago we commenced studies with combined liquid chromatography/mass spectrometry (LC/MS). Our investigations have mainly utilized commercially available interfaces of the moving belt type and started with a Finnigan system¹ on our Finnigan 4000 quadrupole mass spectrometer. This interface does not directly enter the mass spectrometer ion source and a few years ago we acquired an interface² from VG Analytical for our VG 70/70 magnetic mass spectrometer which enters the ion source of this instrument. Recently we have been conducting studies³ with Professor K. Levsen (Bonn) on his new Finnigan MAT moving belt interface⁴ and this system also enters the mass spectrometer ion source.

Our reasons for adopting the moving belt approach to LC/MS were, that we are primarily interested in the qualitative analysis of complex mixtures of organic molecules which exhibit a wide range of structural types. For effective analysis in this area as much information as possible is required to provide answers to the analytical problems. To this end the provision of electron impact (EI) and chemical ionization (CI) mass spectral data together with chromatographic retention times was attractive. We also felt that flexibility in choice of chromatographic conditions was important and the moving belt system enables either normal or reversed phase LC to be used.

When assessing any LC/MS system it is important to consider both aspects of the combined system. One can classify the types of compound analyzed by LC in mass spectral terms and they fall into five categories:

1. Compounds amenable to study by GC/MS but analysed by LC because it is a preferable analytical technique for the particular problem being addressed.
2. Compounds which are thermally labile and/or are of insufficient volatility for gas chromatographic study but are amenable to direct probe mass spectral study.
3. Compounds which require the use of desorption chemical ionization (DCI) or related techniques for their mass spectral analysis.
4. Compounds which require the use of mass spectral ionization methods such as thermospray ionization, field desorption, fast atom bombardment, laser desorption or ²⁵²Cf plasma desorption etc. for their analysis.
5. Compounds which are not currently amenable to mass spectral analysis because of mass range limitations or the inability of current ionization methods to provide useful mass spectral data.

On the liquid chromatographic side it is important to remember that one of its major strengths is its ability to resolve complex mixtures. Whilst loss of chromatographic performance may not matter in some cases, because the data system can be used to resolve unresolved components. If isomers are present in the mixture, since they often give very similar mass spectra, then it is important that chromatographic performance is maintained. In addition we do not want to have restrictions placed upon us in terms of the type of mobile phase we can use for LC.

Here we discuss the relative merits of current moving belt interfaces for LC/MS in the light of these two criteria using examples from our studies.

Results and Discussion

1. Compounds amenable to study with moving belt systems

Polynuclear aromatic compounds fall into the first of our compound classifications. We have found that belt systems are capable of proving good mass spectral data from these compounds. We have applied LC/MS to the study of pyrolysate samples and the combination of EI and retention time data enables ready analysis of the mixtures.⁵ Compounds of this type together with nitrogen, oxygen and sulphur heterocyclic compounds have also been readily analyzed by LC/MS in partially purified petroleum fractions.⁵

Studies of the second category of compounds are also readily effected by use of LC/MS with belt systems. *Calophyllum inophyllum* is a rich source of natural coumarins and biogenetically related acids.⁶ Some of the coumarins present in extracts from the seeds of this plant are amenable to GC/MS study,⁷ but others are thermally degraded.⁶ We have found EI LC/MS a very effective method for analyzing the constituents of these extracts.⁸ A further example of the utility of LC/MS in this category of compound is provided by our studies of the metabolites present in *Penicillium patulum*. Fig.1 shows the computer reconstructed total ion current trace obtained by EI LC/MS of a phenolic fraction from the extract of this fermentation broth. The compounds present proved to be a mixture of xanthenes, and benzophenones together with griseofulvin, the major metabolite produced by the broth. Some of the compounds proved to be new metabolites and a combination of EI and CI mass spectral data was necessary for characterization, since some components failed to provide molecular ions in their EI spectra.

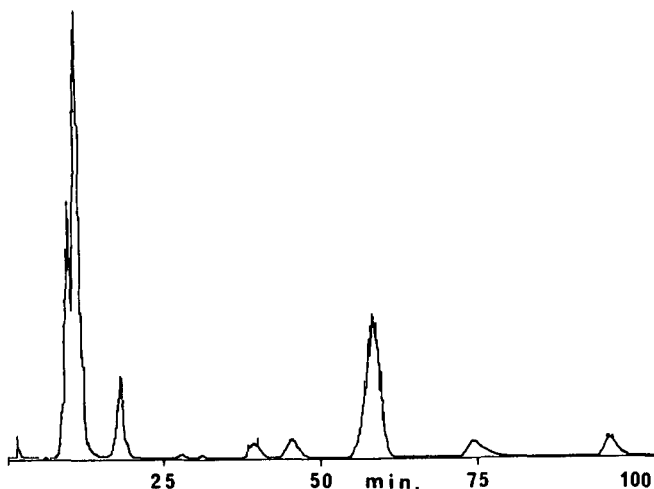


Figure 1. LC/MS trace obtained under EI conditions of base extract from *Penicillium patulum*. Column: Partisil 5, 100 x 4,5 mm, mobile phase, hexane + ethanol (96:4), 0,5 ml min⁻¹

In studies of this second class of compound problems can be encountered with belt interfaces. If the compounds are thermally labile, at relatively large sample amounts injected on-column good spectra are obtained. However as the amount of sample is reduced thermal decomposition occurs resulting in the loss of some mass spectral information. Table 1 illustrates the behaviour of gibberellin A₃ (Mol.wt. 346). Although the effect is also present when CI spectra are obtained, molecular weight information is usually obtainable at lower levels and we have found for gibberellin A₃ negative chemical ionization is particularly useful in this context.

Table 1.

EI Mass Spectra Obtained from Gibberellin A₃ Using a Moving Belt Interface.

Amount	m/z (% rel. int.)							
10 μ g	346(7),	328(17),	310(6),	306(19),	284(36),	238(17),	155(26),	136(100)
1 μ g	346(0.8),	328(5),	310(1),	306(6),	284(100),	238(33),	155(62),	136(27)
300ng				306(1),	284(100),	238(35),	155(80),	136(20)

Ability to provide molecular weight data from sugars is one convenient method for defining limitations of mass spectral ionization techniques. Both our Finnigan and VG Analytical systems provide this data readily from monosaccharides. Such data is also obtainable from disaccharides but not from trisaccharides. The new Finnigan MAT system is superior in this regard being able to handle trisaccharides. In a joint study with Professor Levsen we have found with a wide range of compounds that the new system is consistently superior to the other systems and the spectra obtained are very similar to those obtained by DCI.³

Thus the most recent commercial moving belt system extends the range of compounds which can be handled into the third category. In order to further extend the range of compounds with interfaces of this type some form of surface ionization is necessary and there have already been a number of studies showing the possibilities of using SIMS,⁹ laser desorption,¹⁰ FAB⁴ and ²⁵²Cf desorption¹¹ with transport systems. Although these approaches are attractive ones there will be considerable difficulties in developing true combined LC/MS systems with these techniques particularly if no constraints are going to be placed on the chromatography. It would appear at the present time that thermospray ionization¹² presents the most attractive LC/MS method for handling the fourth group of compounds in our classification.

2. Constraints on Liquid Chromatography

For any liquid chromatographic/mass spectrometric system to be really effective then there should be minimal constraints on the mobile phases used for LC and the system should function as a good detector for the liquid chromatograph. It has been demonstrated^{13,14} that the moving belt system is a good liquid chromatographic detector, providing minimal loss of chromatographic performance as compared with UV detectors. This approach to LC/MS is particularly useful if microbore LC is being used since the columns can be directly coupled to the interface ensuring minimal dead volume connections. With regard to the use of LC/MS as a universal detector we have already noted in the previous section that some classes of compound are not amenable to study with moving belt systems. However we were concerned in our definition with the provision of molecular weight information from the mass spectra obtained. Many of the compounds e.g. larger saccharides will give total ion current traces and hence the system can be used as a detector. It is worthy of note that the belt system because it can provide both EI and CI data has advantages over the direct liquid introduction approach as a universal detection system. One of the strengths of CI is that by selection of reagent gases it can be used for the selective detection of compounds. However in a universal detection system one is looking for the detection of all compounds being eluted from the liquid chromatograph and the use of EI ionization has advantages in this regard.

A problem with interfaces of the moving belt type has been an inability to readily handle aqueous mobile phases with a high proportion of water and the necessity of using a splitter to handle many other solvent systems. A number of methods for overcoming these problems have been developed.¹⁵ Our approach has been to utilize microbore LC, this approach uses much lower flow rates than in conventional LC and we have found that the use of 1 mm i.d. glass lined stainless steel columns (Whatman) to be particularly effective.¹⁶ By running ethanol onto the belt system together with the eluent from the liquid chromatograph we have been able to handle mobile phases containing up to 90% water. Additional advantages of the use of microbore LC with belt systems include the ability to conduct fast analysis by increasing the flow rate of the mobile phase and we have also been able to use solvent flow programming. We have applied this approach in studies of natural products¹⁶ and in examination of environmental samples.¹⁷ In the latter case we developed

a gradient elution system for use with the microbore columns.

Use of microbore LC has problems, a major one being that if chromatographic performance is to be maintained, sample amounts, both content and volume, which can be injected are much lower than with conventional columns. These problems can be overcome to some extent by use of column switching and column preconcentration techniques. We have also found use of 2mm i.d. columns assists with these problems.

In liquid chromatography a wide range of modifiers are used to improve chromatographic performance e.g. acids, bases, buffers and ion-pair systems. Use of volatile modifiers present no problems with moving belt interfaces, however problems are encountered with use of non-volatile additives and ion-pair reagents. A scrubber system has been developed which assists in the handling of these systems, but we feel that the use of a continuous extraction system¹⁸ is preferable. We have recently constructed a system of this type and preliminary experiments show it to be a useful technique for handling ion-pair systems.

Although mass spectrometry is a very powerful technique it cannot resolve racemic mixtures of chiral compounds. Chromatographic methods can resolve racemic mixtures and there is currently considerable interest in the use of liquid chromatography in this context. We have conducted preliminary studies with a Pirkle column and have been able to resolve some standard compounds using this system with LC/MS. Although current commercially available columns are limited, in the types of solvent systems which can be used and in their chromatographic performance, developments in this area should enable a wider range of compounds to be handled and provide a further dimension to our ability to solve problems by LC/MS.

Conclusions

Moving belt interfaces for LC/MS enable a wide range of samples to be studied with few restrictions on the mobile phases used for LC. The new Finnigan MAT interface extends the range of compounds which can be handled, but surface ionization methods will have to be used to enable more mass spectrometrically difficult molecules to be effectively studied. At the present time the use of thermospray ionization appears to be a more effective method for handling mass spectrometrically difficult compounds.

Acknowledgements

We thank the S.E.R.C. and Royal Society for assistance in the purchase of mass spectral and chromatographic equipment.

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The moving belt interface provides a versatile approach for on-line HPLC-MS coupling as it allows operation of the mass spectrometer in the EI, CI, SIMS and other related modes. A drawback of the commercially available system using normal bore columns has been its inability to maintain chromatographic fidelity; band broadening and poor peak shape, particularly when using highly polar or aqueous solvents. In this paper, parameters which influence chromatographic fidelity and mass spectrometric sensitivity have been evaluated. In determining the chromatographic performance of the interface several variables have to be considered including solvent transfer onto the belt, belt speed, solvent vaporization and sample desorption conditions. For the quantitative evaluation of these effects on extra column band broadening it was necessary to calculate variances by the method of statistical moments rather than by peak width measurements. An aerosol system was designed for sample deposition. It was found that placement of the nebulizer at $\sim 30^\circ$ angle to the belt provided optimum conditions for sample transfer onto the moving belt. Under optimized conditions a direct correspondence between HPLC-MS and HPLC-UV chromatograms was obtained for a variety of column types including normal bore, microbore and columns using 3 micron packings. Figure 1 shows a chromatogram of polynuclear aromatic hydrocarbons (PAH) using high water reversed phase conditions at a flow rate of 1ml/min. It was also found that linear calibration curves can be obtained for PAH's from 40pg-60pg injected on column with a reproducibility of 4-7% relative standard deviation. Additional details of this work may be obtained from references 1 and 2.

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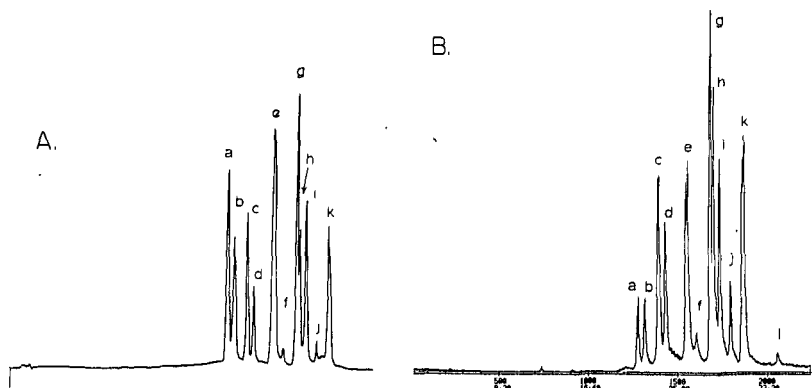


Figure 9 Comparison of LC/UV and LC/MS profiles for reverse phase gradient chromatography, belt speed 45 pm, spray pressure 8 psi, gas temperature 85°C , 15 cm x 4.6mm, 7.5 μm Zorbax BP-C8 column 65:35 (v/v) water/acetonitrile to 100% acetonitrile in 20 min, flow 1 ml/min, a) phenanthrene, b) anthracene, c) fluoranthene, d) pyrene, e) chrysene, f) unknown, g) benzo(b)fluoranthene, h) benzo(k)fluoranthene, i) benzo(a)pyrene, j) benzo(a)anthracene, k) indeno(1,2,3-c,d)pyrene, l) unknown.

MATRIX EFFECTS OBSERVED IN ORGANIC SIMS WITH A LIQUID METAL ION SOURCE

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Recently it was demonstrated that liquid metal ion (LMI) sources can be used effectively in practical analytical applications of matrix assisted SIMS (1-3). Two effects were observed in the initial experiments with LMI sources which we tentatively attributed to the matrix (3). We observed at incident current densities on the order of $10^{-5}\text{A}/\text{cm}^2$ that a stable ion emission ($\sim 10^{-13}\text{A}$) from the glycerol matrix is established after approximately 10-30 sec of irradiation. In the course of bombardment of the sample a sharp increase in the intensity of the molecular ions of certain compounds occurs, when the glycerol is almost depleted.

While it is not possible at present to fully explain these phenomena, they suggest directions for future investigations of the role of the liquid matrix in the sputter process.

A more complete description of the matrix effects is being published elsewhere (4).

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MOLECULAR SIMS WITH A LIQUID METAL FIELD ION POINT SOURCE

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Since its introduction by Benninghoven et al. in 1976, SIMS of organic solids has become increasingly more important for the mass spectrometry of organic solids. A breakthrough in application was achieved by Barber and Bordoli by using a liquid matrix for the sample. In addition to the sample preparation, much attention has been given to the properties of the primary beam. Using Xe instead of Ar as the primary beam results in an increase in secondary ion abundance (1) which could be further increased using a primary Mercury beam (2). In all these experiments gas discharge ion sources were used as the primary beam. An alternative means of primary ion production is to draw an alkali beam out of a thermal ionization source (3).

Recently the use of a Liquid Metal Field Ion Point Source as a primary ion source for organic SIMS was reported (4). In the present paper we report the preliminary results of this unique ion emitter with a ZAB 2F mass spectrometer equipped with a modified FD source. In a LMI source metal ions are formed by a field emission process from a needle tip, resulting in an ion source of high brightness.

The LMI sources employed in the following experiments utilized tungsten tips covered with gallium or a silicon gold eutectic alloy. The tips were placed close to the aperture of a counter electrode and the LMI source was operated by applying a voltage difference of 4 to 6 kV between the tip and resistive heating of the emitter. This simple emitter/cathode assembly was mounted about 15 mm above the target perpendicular to the optical axis of the mass spectrometer. The sample target was a 2 mm diameter stainless steel rod cut approximately 60 degrees to the axis of the mass spectrometer.

Polypeptides such as litorin and bombesin were used as test compounds dissolved in a glycerol matrix.

The LMI source operated stably in the presence of relatively high organic vapor pressures (10^{-6} torr) for several hours with an anode current of 5 to 15 microamperes. The sources could be rejuvenated by rewetting them with fresh metal. The LMI source produces a negligible load on the vacuum system compared to gas discharge ion sources and does not heat the secondary ion source like a thermionic alkali ion source. Protonated and deprotonated molecules were produced from all the test compounds in relatively high abundances with little fragmentation.

CONCLUSION

LMI sources are very well suited as primary ion sources for SIMS because they produce abundant secondary ion currents, have good vacuum compatibility, are small, and are simple to operate.

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SECONDARY ION MASS SPECTROMETRY USING A LIQUID METAL SUBSTRATE

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Secondary ion mass spectrometry (SIMS) has been used to study organic compounds from a variety of matrices: silver, NH_4Cl (1), carbon (2), and liquid gallium/indium alloy (3). The objectives of this work were: 1) to determine if organic adsorbates could be analyzed directly from carbon particles with SIMS, and 2) to determine how the various matrices affect the secondary ion emission of the organic molecules.

The polycyclic aromatic compounds (PAC) phenanthrene, anthracene, and several substituted derivatives (e.g., $-\text{NH}_2$, $-\text{OH}$, $-\text{CHO}$, $-\text{CO}_2\text{H}$, $=\text{O}$) were chosen because of their environmental significance and their known affiliation with carbon particulate matter, or soot. SIMS analyses were done on two instruments. Dynamic SIMS experiments were performed with a CAMECA IMS 300 ion microscope using the liquid metal substrate. Static SIMS experiments were performed with a double-focusing instrument using all four matrices. Approximately 10-50 μg of the PAC were dispersed on the liquid metal. Also, an amount of the PAC was either deposited from solution on silver or mixed in a 1:100 dilution ratio with NH_4Cl or carbon and then burnished on silver so that the final quantity of the PAC on silver was 1 μg . The ion microscope was operated using a 5.5 keV Ar^+ or O_2^+ ion beam at $>1 \times 10^{-6} \text{ A/cm}^2$. Static SIMS experiments were performed using a 5 keV Ar^+ ion or Ar^0 neutral beam at $\geq 1 \times 10^{-9} \text{ A/cm}^2$.

Initial experiments using the ion microscope demonstrated that the liquid metal provided a substrate from which long-lived M^+ ion emission was observed using a dynamic primary ion beam. This liquid metal substrate offers several advantages over conventional liquid or solid matrices. For example, the gallium/indium alloy has a smooth surface onto which solids can be dispersed. The liquid metal is also conducting and has a low vapor pressure.

Static and dynamic SIMS experiments were conducted on the double-focusing instrument using the Ag, NH_4Cl , carbon, and liquid metal matrices. Relative intensities of M^+ , (M -substituent group) $^+$, and $(\text{M}+\text{C})^+$, where C is Ag or Ga and In, were measured. A comparison of the SIMS spectra of phenanthrene from the four matrices is shown in Figure 1. In most cases, the signal-to-background ratio for the carbon matrix was greater than that for the Ag and NH_4Cl matrices.

The molecular ion signal for phenanthrene and 1,10-phenanthroline was also measured as a function of time from the Ag, NH_4Cl , and carbon matrices. The relative ion yields of the PACs and the ionization efficiency of each matrix were calculated and are presented in Table 1. The ion yields and ionization efficiencies for phenanthrene were similar for each matrix. The NH_4Cl , on the other hand, significantly enhanced the secondary ion yield of phenanthroline (as well as most other substituted PAC) relative to the Ag or carbon matrix. These observations suggest that chemical as well as physical factors influence the effect of NH_4Cl on the secondary ion emission.

Further comparison of the matrices showed that the silver matrix was the easiest to prepare and required a static beam to ensure low background and good S/N. The NH_4Cl matrix enhanced the ion emission for most substituted compounds and also worked best (i.e., high S/N) with a static ion beam. The unsubstituted PAC were easily analyzed from carbon and a greater primary ion beam current density was possible without increasing the background ion intensity. The liquid metal required a dynamic ion beam for optimum performance.

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SIMS SPECTRA OF PHENANTHRENE

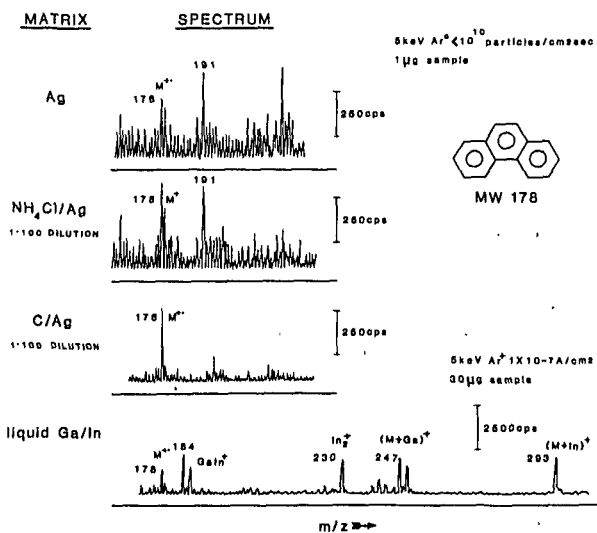


Figure 1

Table 1

Compound	Matrix	Ionization ¹ Efficiency	Efficiency Ratio	Ion Yield ²
	NH ₄ Cl	4x10 ⁻⁴	10	1x10 ⁻²
Phenanthroline	Carbon	1x10 ⁻⁴	2.5	5x10 ⁻³
	Ag	4x10 ⁻⁵	1	4x10 ⁻³
	NH ₄ Cl	7x10 ⁻⁸	1.75	5x10 ⁻⁴
Phenanthrene	Ag	5x10 ⁻⁸	1.25	4x10 ⁻⁴
	Carbon	4x10 ⁻⁸	1	3x10 ⁻⁴

1: #secondary ions desorbed/#sample molecules

2: #secondary ions desorbed/#primary ions

ENERGY DISTRIBUTION OF ORGANIC IONS EMITTED IN SIMS AND FAB EXPERIMENTS

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An important aspect of SIMS and FAB experiments is the energy distribution of secondary organic ions (molecular, fragments, clusters, etc.) sputtered from the surface of the target. The knowledge of this parameter provides important information on the mechanism of the secondary emission of ions, as well as for designing SIMS and FAB experiments for successful analysis of the compounds of interest. The mechanism of emission of large intact organic molecules from the sample target involves, as in inorganic SIMS, momentum transfer from the bombarding particle to the target atoms or molecules. For the process of momentum transfer it is unimportant if the primary particles are ions or neutrals (1). This is especially true for the first phase of sputtering, when the momentum of the particle is transferred to the surface atoms. The charge on the primary particle may play a significant role in the secondary process of emission when the sample molecules, in form of intact ions or neutrals, are transferred into the gas phase near the surface. A number of processes can then occur including direct ionization or neutralization of secondary particles by the incident beam. Consequently, the charge on the primary particles may influence the type of secondary ions formed (molecular or fragment). Emission of secondary particles is governed by two principal mechanisms: (i) direct-knock-on sputtering and (ii) linear cascade sputtering. Both mechanisms have been analyzed in detail regarding sputtering of organics in (1,2). Direct-knock-on sputtering produces energetic secondary particles (10-100eV), whereas linear cascade sputtering produces secondary particles with low kinetic energy. Thus, the relative contribution of these two mechanisms would determine in large part the character of the energy distribution of secondary ions.

We have initiated a systematic study of the energetics of various organic ions in relation to the different experimental parameters which occur in the SIMS and FAB analysis of organics:

1. Primary beam - energy and nature of the primary particles, charge of primary beam, current density, static versus pulsed beam.
2. Target conditions - type of material, target bias, orientation and conductivity.
3. Nature of secondary ions - chemical structure, molecular weight, molecular ions, fragments, clusters, cationized ions, etc.
4. Charge of secondary particles - positive, negative or neutrals.

We report here preliminary data on some of these experiments, which were performed using a secondary emission mass spectrometer system described previously (3,4). Our data indicate that quadrupole mass spectrometers should be preceded by an energy analyzer, which improves significantly the instrument performance (resolution, signal/noise ratio), and offers interesting applications in specific cases of analysis. We observed similarities in the energy distribution of secondary organic ions with inorganic SIMS experiments. We also observed a tendency for organic cluster ions to have lower kinetic energy compared to molecular ions, even though this effect is not as pronounced as for inorganics (5,6,7). For example, the energy of G_3H^+ glycerol cluster is smaller than the energy of glycerol molecular ion (GH^+) by only a few tenths of an eV. Water (8) and silicon clusters (9) exhibit much larger differences, specifically in the high energy tail, suggesting a direct-knock-on mechanism playing a more important role for inorganics than for organic materials. Fragments and molecular ions had similar energy distributions but this fact should be confirmed on a wide variety of compounds of different chemical structure. In contrast to cluster ions, molecular ions of similar structure (such as polyethylene glycols) exhibit an increase in kinetic energy for larger molecules. Molecular ions of some organic compounds such as the alkaloid crinine (MH^+272) exhibit lower kinetic energy (about 1.5 eV) than that observed for comparably sized glycerol clusters (G_3H^+ , 277). This allows one to energy select ions of interest and suppress contributions from the matrix. This technique has been described for the analysis of As in Si (10).

Continuation of these experiments will permit us to:

- 1) Understand fragmentation and ionization processes of secondary ions under various

conditions in SIMS instruments;

- 2) Analyze complex mixtures using particle induced ionization;
- 3) Obtain information on molecular weight and structure of various labile compounds of biological interest
- 4) Design better conditions for efficient liquid SIMS experiments.

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Relative FAB Ionization Efficiencies of Some Symmetrical Quaternary Ammonium Salts, T. Keough and A. J. DeStefano, The Procter & Gamble Co., Miami Valley Laboratories, PO Box 39175, Cincinnati, OH 45247.

We have examined the behavior of a series of tetra-n-alkyl quaternary ammonium iodides under fast atom bombardment (FAB) conditions. The purpose of these experiments was to determine the influence of cation size, anion affinity, matrix, solvent, and concentration on FAB ionization efficiencies. We also wanted to evaluate FAB for the routine screening of unknown quaternary ammonium salt mixtures and to see if the technique offers any promise for quantitative analysis.

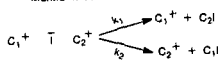
We initially investigated the gas-phase fragmentation behavior of some unsymmetrical cluster ions comprised of two quaternary ammonium cations, C_1^+ and C_2^+ , bound by an iodide anion (Figure 1). We note that the rate of formation of the larger cation is greater than the rate of formation of the smaller cation for each cluster ion studied. Assuming normal bond lengths and angles, we can calculate a radius for each symmetrical quaternary ammonium cation and observe (in the gas phase) that the relative rate of cation formation varies linearly with r^2 .

Relative ionization efficiencies (IE) were determined in FAB and SIMS by a two-step procedure. First, the pure salts were analyzed to determine the extent of fragmentation as a function of cation size ($A=I_0^+/\sum I_i^+$). FAB and SIMS results are compared in Figure 2. Not surprisingly, the extent of fragmentation increases with size for both techniques. Second, we analyzed a series of equimolar two component mixtures of salts and measured the relative abundance of each molecular cation ($B=I_0^+$). These latter measurements required integration of sample related signal until both components totally desorbed since the various salts exhibit different desorption profiles under FAB conditions. Typical desorption behavior is illustrated in Figure 3. The ionization efficiency of each component (Figure 4) is then given as the total ion current produced by identical quantities of each material ($\sum I_i^+=B/A$). A significant solvent effect on relative ionization efficiency is clearly evident in FAB.

Knowledge of the relative ionization efficiency and the extent of fragmentation allows us to assess the utility of FAB and SIMS for routine screening of unknown salt mixtures. In equimolar salt mixtures, the relative intensity of each molecular cation (I_0^+) is calculated as $I_0^+=(A) \cdot (IE)$. Figure 5 compares the calculated molecular cation intensities which would be obtained from FAB and SIMS of equimolar salt mixtures. With SIMS, the increase in fragmentation with size offsets the increase in ionization efficiency so a steady decrease in I_0^+ with size is observed. In FAB, the smaller salts are not detected because of their very low ionization efficiency. The molecular cation intensity then rises through a maximum and falls off once the extent of fragmentation begins to dominate the ionization efficiency term. Obviously, there exists an optimum range of quaternary ammonium salts that can be detected by FAB. Confirmation of this argument is given in Figure 6 which compares the SIMS and FAB spectra of an equimolar mixture of tetraethyl and tetraheptyl ammonium iodide. As expected, the tetraethyl ammonium cation is clearly evident in SIMS but absent from the FAB spectrum.

This study has clarified some of the chemical factors influencing ionization of quaternary ammonium salts under FAB and SIMS conditions and provides a basis for beginning an investigation into the quantitative capabilities of FAB. Results of our quantitative studies are given in the next paper.

MS/MS of Gas-Phase Cluster Ions



C_1^+	C_2^+	k_1/k_2
$(C_7H_{13})_4N^+$	$(C_8H_{13})_4N^+$	1.4
$(C_8H_{13})_4N^+$	$(C_9H_{13})_4N^+$	2.4
$(C_9H_{13})_4N^+$	$(C_{10}H_{13})_4N^+$	2.2
$(C_{10}H_{13})_4N^+$	$(C_{11}H_{13})_4N^+$	2.7
$(C_{11}H_{13})_4N^+$	$(C_{12}H_{13})_4N^+$	92.5

Figure 1: MS/MS of unsymmetrical cluster ions.

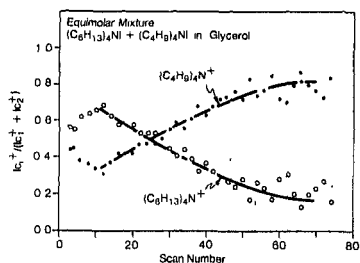


Figure 3: FAB desorption profiles of quaternary ammonium salts in glycerol.

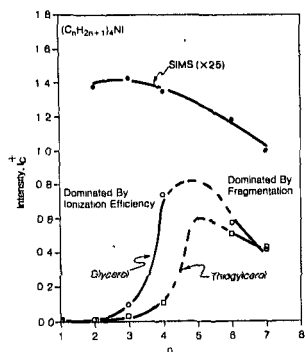


Figure 5: Calculated molecular cation intensities as a function of size.

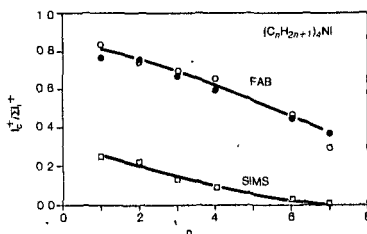


Figure 2: Normalized cation intensity as a function of size.

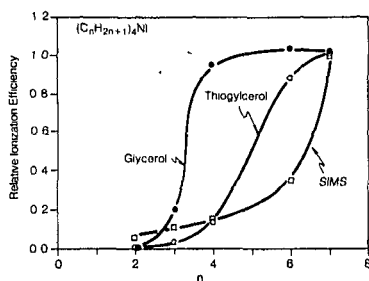


Figure 4: Relative ionization efficiencies of some quaternary ammonium salts.

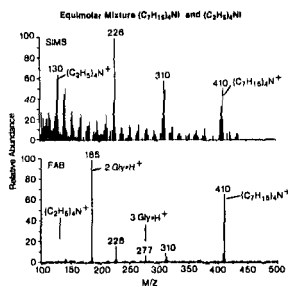


Figure 6: Comparison of SIMS and FAB spectra of an equimolar salt mixture.

SURFACTANT ANALYSIS USING FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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Quantitation of quaternary ammonium salts by fast atom bombardment mass spectrometry (FAB MS) can be difficult since the intensities of the ions of interest are complex functions of effects such as solubilities, surface activities, counter-ion affinities, ionization efficiencies and extents of fragmentation. In this study we explored several types of internal standards intended to minimize these effects. The three types of internal standards investigated were (1) a quaternary ammonium salt quite different in mass from the analyte, (2) a homolog or other quaternary ammonium salt close in mass to the analyte, and (3) a heavy-isotope labelled form of the analyte. As anticipated, the accuracy of the results improved as we went from 1 to 3.

Attempts at using quaternary ammonium salts far in mass from the analyte were abandoned quickly. Figure 1 shows scan 3, scan 11 and the average of 30 scans from the FAB analysis of an equimolar mixture of hexadecyltrimethylammonium chloride and dioctadecyldimethylammonium bromide (thioglycerol solvent). The spectra changed rapidly with time and the peak heights from the averaged spectra did not reflect the correct relative concentrations after corrections for the ionization efficiencies and degrees of fragmentation of the two components. Figure 2 shows the desorption profiles generated from this analysis and illustrates potential problems that can be caused by selective desorption from the FAB probe.

The use of a homolog as an internal standard was studied through a standard-addition experiment using dodecyltrimethylammonium chloride (C_{12} TMAC) as the analyte and tetradecyltrimethylammonium chloride (C_{14} TMAC) as the standard. Initially, C_{14} TMAC was added to the C_{12} TMAC solution until sufficient ion currents were observed for both species. Each point on the calibration curve (Figure 3) was obtained by running the analysis until all sample-related ion current was gone and only glycerol remained in the spectrum (~30 minutes), and then integrating the ion currents from the molecular cations. The resulting intensity ratio was plotted vs. the amount of C_{12} TMAC added to the solution and the line extrapolated to C_{12} TMAC added=0 to obtain the original amount of C_{12} TMAC in solution. The results were within 10% of those determined colorimetrically. A similar experiment using ditallowdimethylammonium chloride (DTDMAC) as the analyte and tetrahexylammonium iodide as the internal standard gave results an order of magnitude lower than those determined colorimetrically and cast doubt on the generality of this method.

The need to integrate homolog signals until all sample signal is gone is demonstrated in Figure 4 where $I_{C_{12}TMAC}/I_{C_{14}TMAC}$ is plotted vs. MS scan number for an equimolar mixture of the two components. The ratio varies by a factor of 25 over the course of the analysis, so integrated intensities are needed to produce reproducible results. A similar experiment using d_3 - C_{12} TMAC ($C_{12}H_{25}NCD_3(CH_3)_2Cl$) as the internal standard showed a linear response with zero slope and a standard deviation of ~3% about the mean value. This implies the calibration curve can be constructed from any scan or an average of several scans, cutting the analysis time down by a factor of 10 from the homolog method. Calibration curves generated for C_{12} TMAC and DTDMAC using d_3 -labelled internal standards are shown in Figures 5 and 6, respectively. The DTDMAC curve deviated from linearity rapidly above $I_{DTDMAC}/I_{d_3-DTDMAC}=10$. Deviation of the slopes from unity was in part due to residual solvents. Drying the reagents brought the slopes of both curves to 1.0.

In summary, heavy-isotope-labelled internal standards appear to offer the best chance for quantitation by FAB. The aforementioned problems were too numerous and too serious to be circumvented by the other standards we investigated. Limited dynamic range appears to be the major drawback to this otherwise promising technique.

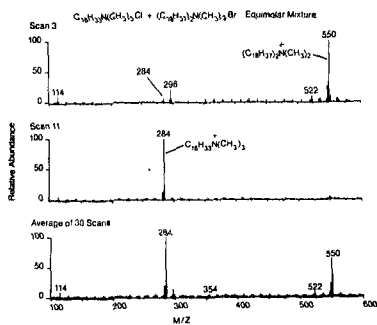


Figure 1. FAB analysis of 1:1 mixture of $C_{16}TMAC:(C_{18}H_{37})_2N(CH_2)_3Br$ in thioglycerol.

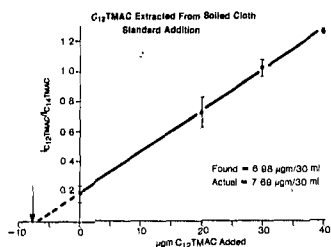


Figure 3. Quantitation of $C_{12}TMAC$ by standard addition.

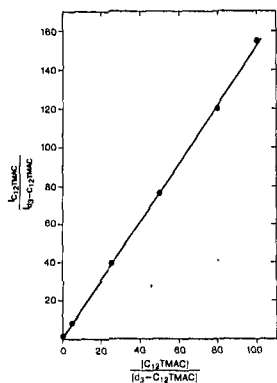


Figure 5. Quantitation of $C_{12}TMAC$ using $d_3-C_{12}TMAC$.

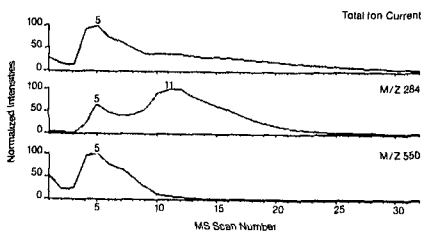


Figure 2. Reconstructed ion chromatograms from analysis shown in Figure 1.

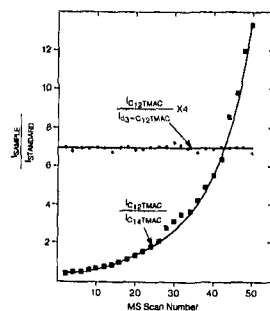


Figure 4. Sample/standard intensity ratio vs. MS scan for 1:1 $C_{12}TMAC:C_{14}TMAC$ and $C_{12}TMAC/d_3-C_{12}TMAC$ mixtures.

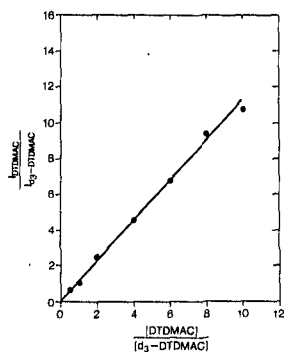


Figure 6. Quantitation of DTDMAC using $d_3-DTDMAC$.

DISSOCIATION OF LARGE ALKALI IODIDE CLUSTER IONS

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Secondary Ion Mass Spectrometry (SIMS) and Fast Atom Bombardment Mass Spectrometry (FABMS) of cesium iodide (CsI) and sodium iodide (NaI) have allowed the production, mass analysis and detection of massive cluster ions ($> m/z$ 25,000) with a conventional double-focusing mass spectrometer [1]. The secondary cluster ion spectra of the alkali iodides (AI) show clusters of the type $[A(AI)_n]^+$ and $[I(AI)_n]^-$, whose distributions exponentially decrease. A bond-breaking model has been developed and excellent agreement has been observed between the theoretical distributions and experiment [2]. Ion abundance enhancements in the distributions occur at certain n -values, and they correspond to close-packed cubic structures. For example, enhancements occur at $n=13, 22$, etc. corresponding to $3 \times 3 \times 3$, $3 \times 3 \times 5$ etc. cubic-like structures. The two n -values immediately following the enhancements are decreased in abundance, relative to the envelope of the distribution. These, $n=13, 14, 15$; $n=22, 23, 24$; etc., regions, we refer to as anomalous [3,4]. The intensity enhancements were also found to be a direct function of the ionic radii of the alkali metal salt studied [5].

When we extended our investigation by using the unique high-mass, metastable diagnostic, and collision spectroscopy features of a ZAB-HF reverse-geometry instrument, we observed discrepancies in the anomalous regions compared to our earlier work on a different mass spectrometer. Variation of the accelerating potential revealed that the magnitude of the deviation in the anomalous regions from the general distributions was related to the ion flight time. This indicated that the origin of the anomalies was due to unimolecular dissociation of certain n -valued cluster ions to certain highly-stable structures, for example, $n=14^* \rightarrow n=13$.

The single-focusing data obtained with the ZAB-2H revealed two major unimolecular dissociation pathways for almost every massive cluster ion:



and



where A and B are the alkali metal and iodine for positive ion clusters and iodine and metal for negative ions. The CsI clusters preferentially decayed by loss of one neutral molecule (Figure 1) while the NaI clusters preferentially decayed by loss of two neutral molecules.

The dissociations were further studied with mass-analyzed ion kinetic energy spectrometry (MIKES) on the ZAB-HF mass spectrometer. Variation of the collision cell gas pressure indicated the observed dissociations were indeed unimolecular. However, collision-induced dissociations (CID) were observed at relatively low collision-gas pressures, especially for those inherently unstable species (e.g., $n=14, 15$). These studies suggest that the larger cluster ions are produced in highly-excited states.

When a voltage was applied to the collision cell to differentiate unimolecular from bimolecular processes, several artifact peaks appeared in the CID-MIKES spectra. These were determined to be a result of unimolecular dissociation occurring in the second field-free region (FFR) prior to the collision cell, followed by a consecutive bimolecular dissociation within the collision cell (Figure 2).

This observation suggests a novel method for studying consecutive reactions in the second FFR of a reverse-geometry mass spectrometer. If the region incorporates two collision cells to which voltages V_a and V_b can be applied, respectively, then the reaction $m_1 \rightarrow m_2 \rightarrow m_3$ can be studied. For example, if m_1 is accelerated to an energy eV_a and $m_1 \rightarrow m_2$ in the first cell at voltage V_a and $m_2 \rightarrow m_3$ is the second cell at V_b , then the kinetic energy of m_3 in the consecutive reaction $m_1 \rightarrow m_2 \rightarrow m_3$ is given as

$$T_{m_3} = -\frac{m_3}{m_1} e(V_a - V_b) + \frac{m_3}{m_2} e(V_b - V_c) + eV_c.$$

Computer simulation of consecutive reactions from various precursors such as 2-pentanone with the tandem collision cell arrangement shows this type of experiment to be promising. Further work on implementing the system is in progress.

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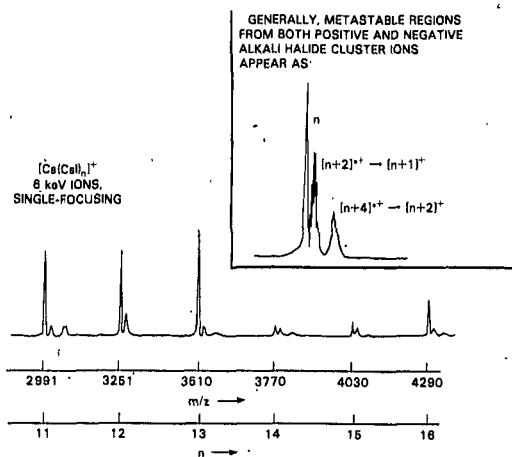


Figure 1

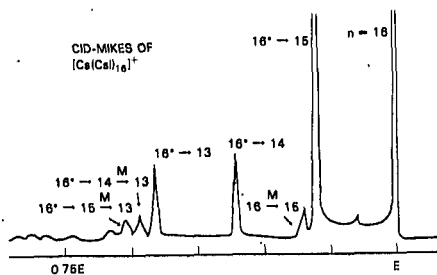


Figure 2

MS/MS OF DESORBED IONS

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Novel ions can be created directly from the condensed phase by the impact of an argon ion or atom beam of several thousand volts kinetic energy. These include intact cations from precharged organic salts, cationized species incorporating both metal and an organic molecule, and cluster ions between sample molecules themselves, or between sample and matrix. We report SIMS and MS/MS spectra for organic samples dissolved in glycerol. An EBE triple sector mass spectrometer was used with the source at 10 keV; argon ions were created in a source held above this potential. Beam currents at the sample were estimated at about 1-10 $\mu\text{A}/\text{cm}^2$. Under such bombardment conditions, secondary ions from neat samples were transient, lasting about ten to thirty seconds. With dissolution in glycerol, beams persisted for five to ten minutes. Sample sizes of 1 mg dissolved in 5-10 μl of glycerol were supported on aluminum. A needle collision cell was fitted in the region between the magnet and the second electric sector, and helium was used as the collision gas at a pressure sufficient to attenuate the parent ion intensity by 50%.

Figure 1 gives the MS/MS spectrum of the 265^+ ion from thiamine hydrochloride, which correlates well with the SIMS spectrum itself, lending credence to a unimolecular origin of fragment ions. Of considerable interest are the ions in the positive ion SIMS spectrum above the mass of the intact cation. Abundant ions at 267^+ , 297^+ , 327^+ , and 357^+ are observed. The latter three can be identified as the adduct of 265^+ with glycerol, and fragments formed by H_2CO loss, while the 267^+ corresponds to hydrogenation of the thiamine. However, while glycerol adducts normally contain an abundant ion 93^+ in their MS/MS spectrum (corresponding to protonated glycerol), none of these ions so fragment. The MS/MS spectrum of 357^+ is given in Figure 2; losses to give 265^+ , 238^+ , 144^+ , 123^+ , and 81^+ occur, but 93^+ is not observed. Formation of 238^+ probably occurs via loss of glycerol and HCN. This ion does not appear at all in the spectrum of the intact cation 265^+ , but is noted in the MS/MS spectra of each of the adduct ions, decreasing in relative abundance in the order $357^+ > 327^+ > 297^+ > 267^+$. We conclude that the formation of 238^+ reflects a significant change in the structure or reactivity of the thiamine, suggesting a strong sample solvent interaction.

Isomer differentiation by MS/MS is evident in comparison of the MS/MS spectra of two isomeric alkaloids, O-methylcandicine (Figure 3) and coryphanthine (Figure 4). In each case, the SIMS spectrum contains an abundant intact cation 194^+ , and the pattern of fragment ions can be used to distinguish the structures. The MS/MS spectra confirm the distinction, especially in the presence of interfering peaks at the masses of the fragments. The relative abundance of 135^+ (loss of trimethylamine) is characteristic, as well as the ratio of 103^+ to 105^+ , and unique fragment ions 117^+ and 120^+ . This distinction is preserved when the fragment ion 135^+ itself is selected for MS/MS analysis.

The ability of MS/MS to make an *in situ* isotopic selection in parent ions proves valuable in the interpretation of spectra. Mass shifts of the daughter ions are apparent even with the relatively low resolution provided by an electric sector analysis. This technique has been discussed for several types of MS/MS spectra, such as daughter, parent, and neutral loss scans, and while most often applied in analysis of halogenated compounds, the distinctive isotopic clusters of cationized species such as those formed in SIMS facilitate its use in the structural elucidation of metal-containing species. Persistence of ion emission makes it possible to signal average over an extended time, and isotope-resolved MS/MS data can be garnered from peaks containing such minor isotopes as ^{13}C , ^{34}S , and the like.

SIMS spectra often contain cluster ions composed of both the sample and the matrix in which it is found. Formation of clusters with glycerol is well-known. Often $C_{x+1}A_y^+$ clusters are formed from ionic solids. Carnitine hydrochloride forms cluster ions in a series $C_y-H_{y-1}^+$. The intact cation 162^+ is the base peak in the SIMS spectrum, but ions at 232^+ , 484^+ , and 645^+ are also observed, representing this series for $y = 2$ through 4. The MS/MS spectra of each shows dissociation to the intact cation 162^+ . This data emphasizes the multiple origins of stable species observed in the SIMS spectra. For a general cation C^+ observed in the spectrum, sources include desorption directly from the surface as C^+ , dissociation of clusters such as $C_{x+1}A_y^+$, dissociation of clusters such as $C_y-H_{y-1}^+$, and dissociation of clusters such as $(C+S)_y^+$, where S represents a generic solvent. This complex chemistry can affect quantitation using internal standards if the standard is not carried identically through all of the processes which form C^+ .

FIGURE 1

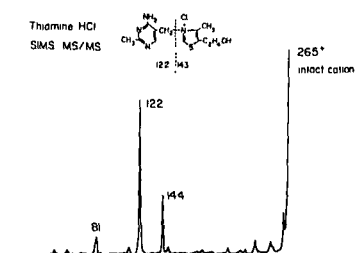


FIGURE 2

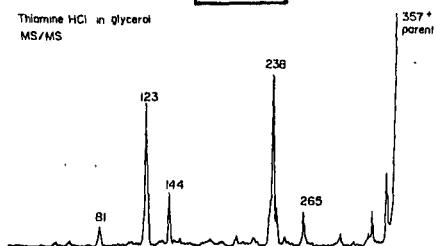


FIGURE 3

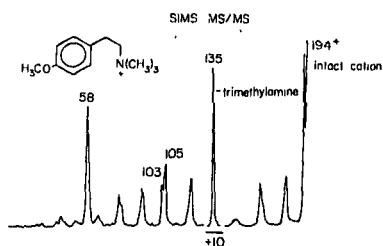
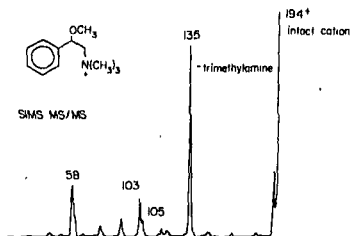


FIGURE 4



CATION EFFECTS ON SIMS MS/MS SPECTRA

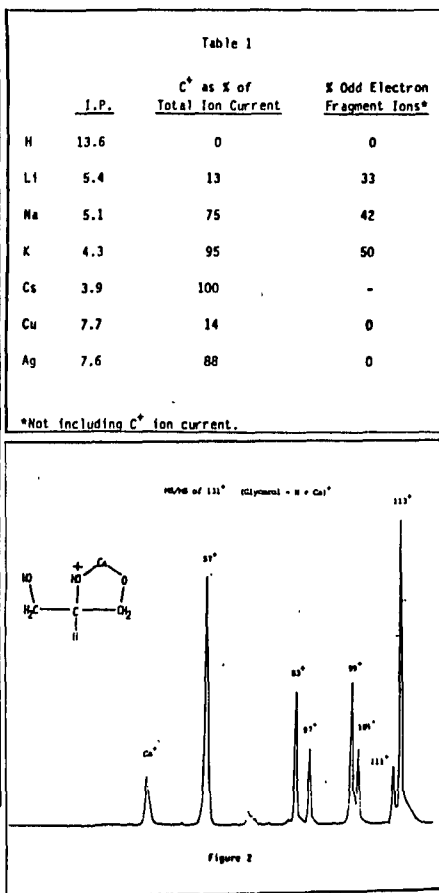
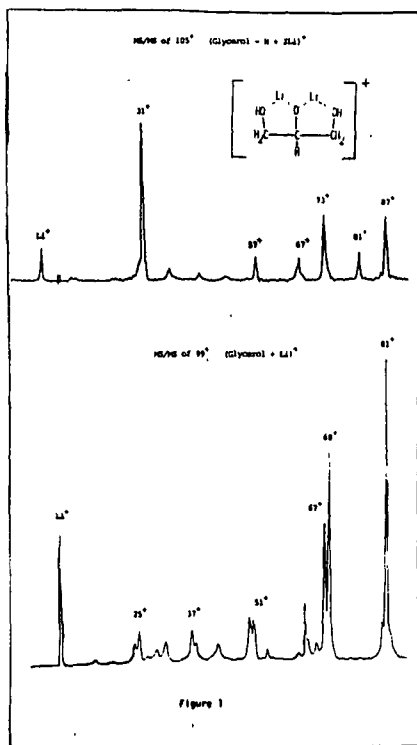
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The nature of the cation in ions formed by cationization via desorption ionization methods is relatively unimportant when obtaining a mass spectrum. However, this is not true when doing mass spectrometry/mass spectrometry (MS/MS). The species used to cationize a molecule will influence the amount of structurally significant fragmentation and the fragmentation pathways.

The fact that any structurally significant fragmentation is observed is itself somewhat surprising given the low cation affinities of metal ions. The amount of structurally significant fragmentation depends upon the CAD cross section and the amount of fragment current carried away by the cationizing species. Table 1 shows the latter of these for the cationization of glycerol by some group I elements. The CAD cross-sections were not measured quantitatively, but the qualitative trends show a definite increase from Li thru Cs with H, Cu, and Ag being on the high end. For the alkali's a definite correlation exists between cation fragment current and IP, as one might expect; however, the group I transition metals do not fit into this correlation. The overall trend can be correlated with the order of cation affinities (i.e., $H > Li < Cu > Na > Ag > K > Cs$). This suggests it may be possible to get relative ordering of cation affinities by measuring the amount of fragment ion current carried by the cationizing species in an MS/MS experiment. This would be somewhat analogous to the proton affinity measurements of Cooks et al;¹ however, more experiments need to be done to fully demonstrate this and to determine possible complications such as bridging of the cation as discussed below.

Besides the amount of structurally significant fragmentation, the fragmentation pathway is influenced by the cation. The last column in Table 1 shows the amount of odd electron fragmentation observed in the MS/MS spectra. This correlates very well with the square of the electron transfer parameter of Kroeger and Drago.² This can be rationalized as showing how much charge is transferred from the cation to the molecule. As less charge is transferred, more fragmentation occurs from the cationized species that is similar to fragmentation from an excited neutral. This type of fragmentation can make the MS/MS spectra more difficult to interpret.

This postulate of the degree of charge on the molecule vs. cation is further supported by the MS/MS spectra of the $(glycerol+2C-H)^+$ species where C is an alkali. These spectra show predominately the even electron species as shown in Fig. 1 (along with the MS/MS spectrum of $(M+C)^+$ for comparison. This is compatible with the charge delocalization hypothesis given the structure shown in Fig. 1, which is consistent with the fragmentation observed. Additional evidence is provided by the mass spectra and MS/MS spectra of glycerol cationized by the group II elements Mg and Ca. Instead of $(M+C)^+$, $(M+C-H)^+$ ions are observed in the mass spectrum with only even electron ions observed in the MS/MS spectra (Fig. 2). The fragmentation can be best rationalized via the structure shown in Fig. 2. Comparing Fig. 2 with Fig. 1 shows the drastic change possible in MS/MS spectra with cationizing species. The mass spectra of glycerol cationized by Mg or Ca indicate that two ligands are needed as the intensity of $(2M+C-H)^+ > (M+C-H)^+$ with the latter species being of very low relative intensity where C = Mg, presumably due to its small ionic radius and thus inferior bridging ability vs. Ca. The fragmentation can be best rationalized via the structure shown in Fig. 2.



Acknowledgment

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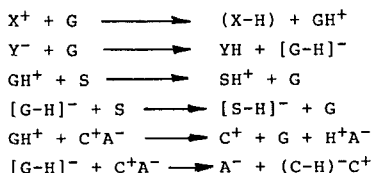
A CHARGE-CASCADE MODEL OF IONIZATION IN FAB AND SECONDARY ION MASS SPECTROMETRY

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Although the concept of the "desorption of performed ions" is a popular one as an explanation of the mass spectra observed in FAB and organic SIMS, a variety of experimental observations can be better explained using a model based on sequential ion-molecule reactions in a dense gas. That ion-molecule reactions explain their observations has been noted by a number of authors, including some working in the related field of laser desorption. We suggest that an ion-molecule charge-cascade is the primary means by which observed ions are formed, although other mechanisms may contribute also.

In terms of the FAB experiment, the model consists of three stages: (1) the conversion of the matrix and sample from a liquid solution into the equivalent of a high density gas (but translational and internal energies are not in equilibrium), with the simultaneous formation of primary ions by molecular destruction; (2) the reaction of the primary ions with matrix molecules to form the major reactant ions; and (3) the reaction of the matrix reactant ions with sample molecules to form sample ions.

Calculations suggest that the collision cascade induced by the impact of the energetic particle into a liquid results in a disturbed, energetic region 250Å or more in diameter. Molecules are separated and set into motion with a wide range of translational energies, and undergo a number of collisions before the energy of the system is dissipated and the liquid state is regained. Molecules undergoing the strongest interactions with the bombarding particle energy may be ionized by heterolytic bond cleavage, electronic excitation, shock wave ionization, etc., to form the initial species X^+ and Y^- . Since the matrix material is of the highest concentration, the charge is transferred into this "sink" until a collision with a sample molecule transfers the charge to the sample (if it is thermodynamically favorable).



This model provides a means of explaining a number of experimental observations, including: suppression of the matrix ions, cross-suppression of samples, the observation of both positive and negative ion species from many compounds, the CI-like nature of the FAB spectra, the response variation with chemical character, the spectral variation with bombarding particle energy and mass, the ability to observe spectra from pure alkanes, and the behavior of the spectra as frozen liquids become fluid.

Such a model may also provide a good basis for understanding SIMS spectra of organics and inorganics.

MASS SPECTROMETRIC DETERMINATION OF LOW LEVEL NEURAMINIDASE
ACTIVITY UTILIZING N-ACETYLNEURAMINIC ACID RELEASED FROM
SODIUM COLOMINATE

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Neuraminidase (N'ase) is an exohydrolase enzyme which cleaves N-acetyl-neuraminic acid (NANA) residues, bound by glycosidic bonds, from the nonreducing ends of oligosaccharides, glycolipids, and glycoproteins. There are ongoing clinical trials on the utilization of N'ase treated leukemic cells in the active immunotherapy of leukemia (1). When N'ase is used to remove surface NANA from leukemic cells, the actual activity of the enzyme preparation must be known for the establishment of optimal conditions for the preparation of the immunogen because the efficacy of the immunotherapy depends upon the ratio of susceptible NANA to the number of viable cells treated (2). The objectives of this work were: (a) to find a substrate which is chemically pure, readily available and inexpensive so that the N'ase activity of commercial enzyme preparations can be standardized and compared; (b) to determine very low levels of N'ase activity and also activity in very small biological samples.

We selected Na-colominate as the substrate because it is a readily available (high purity, inexpensive) homopolymer of NANA with ketosidic linkages between C2 and C8 of the monomers. Analytical techniques utilized for the quantification of N'ase susceptible NANA were an improved colorimetric technique utilizing the thiobarbituric acid (TBA) reaction with pH-dependent extraction with cyclohexanone to remove deoxyribose and other interferences (3) and chemical ionization (isobutane) GC-MS with selected ion monitoring of the fully silylated (7TMS) NANA ($m/z=814$) and of methoxyneuraminic acid ($m/z=714$), the internal standard (4).

Na-colominate yielded NANA upon hydrolysis by N'ases from 5 natural sources (Clostridium perfringens, influenza virus, Vibrio cholerae, Athrobacter ureafaciens, and Athrobacter sialophilus) as well as by hydrolysis with strong acids. Total ion monitoring of the GC effluents showed no other hydrolysis products. The free NANA content of Na-colominate was in the 1.5 - 3.0 % range in contrast to N-acetylneuraminyl (NAN) - lactose, the most commonly used substrate, which had >5% free NANA.

The Michaelis constants for the N'ases from all natural sources were in the range 1.6×10^{-4} to 3.5×10^{-4} with Na-colominate in contrast to values an order of magnitude higher and also ranging within an order of magnitude with NAN-lactose. The presence of Mn, Zn, Fe, and/or Cu ions in the incubate did not influence analysis, but the presence of Ca^{++} was as beneficial as in the case of NAN-lactose. Good linearity and reproducibility were achieved in the 6-128 mU N'ase range. The TBA and GC-MS methods gave the same results in the 6-70 mU N'ase range. The TBA method is recommended as long as at least 2 ug (≈ 6 mU N'ase) of released NANA is present. The sensitivity of the GC-MS technique is 4 ng of liberated NANA (≈ 0.013 mU N'ase) in a 50 ul sample size.

In conclusion, we recommend that Na-colominate be used as the substrate for the determination of N'ase activity because of lower blank, increased specificity and reproducibility, convenience, and the possibility of interlaboratory standardization. (Supported by T.J. Martell Memorial Foundation for Leukemia Research).

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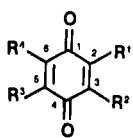
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MASS SPECTRA OF AZIDO UBIQUINONE DERIVATIVES

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The essential role of the coenzyme ubiquinone (Q) in mitochondrial and photosynthetic electron transfer systems has been well established (1-4). The reaction mechanism of ubiquinone-mediated redox reactions is not well understood (2,3). The low solubility of the naturally occurring ubiquinone (Q₆ and higher homologs) in aqueous solution and the low absorption of Q in the visible region have made the study of the reaction mechanism and of the Q:protein interaction difficult. Low molecular weight synthetic Q analogs, especially those with a reporting group (i.e., spin, fluorescent label) offer a promising alternative approach to study of the Q:protein interaction. A more specific photoaffinity-labeled Q-derivative for identification of the Q-binding protein from the mitochondrial electron transfer chain has been successful (5). Recently, we have developed a method to synthesize azido-Q derivatives by directly substituting one hydrogen atom or a bromine atom on the benzoquinone ring with an azido group under weakly acidic conditions (6). Six compounds were synthesized and their biological activity as compared with a standard (7,8), (2,3-dimethoxy-6-methyl-5(10-bromodecyl)-1,4-benzoquinone) are shown below:

	Cpd.No.	R ¹	R ²	R ³	R ⁴	Biological Activity
	I	Methyl	Geranyl	Methoxy	Azido	22
	II	Azido	Geranyl	Methoxy	Methoxy	39
	III	Methoxy	Geranyl	Methyl	Azido	12
	IV	Azido	Geranyl	Methyl	Methyl	—
	V	Azido	Decyl	Methoxy	Methoxy	22
	VI	Methyl	10-Azidodecyl	Methoxy	Methoxy	95
	STD	Methyl	10-Bromo-decyl	Methoxy	Methoxy	100

Mass spectra of these azido-ubiquinones have not previously been reported. The electron impact mass spectra were obtained on LKB-9000 and Varian 731 instruments using a direct probe inlet and recorded on an oscillograph; for the high resolution mass spectra, the CEC-110-B spectrometer was used and the spectra were recorded on a photoplate.

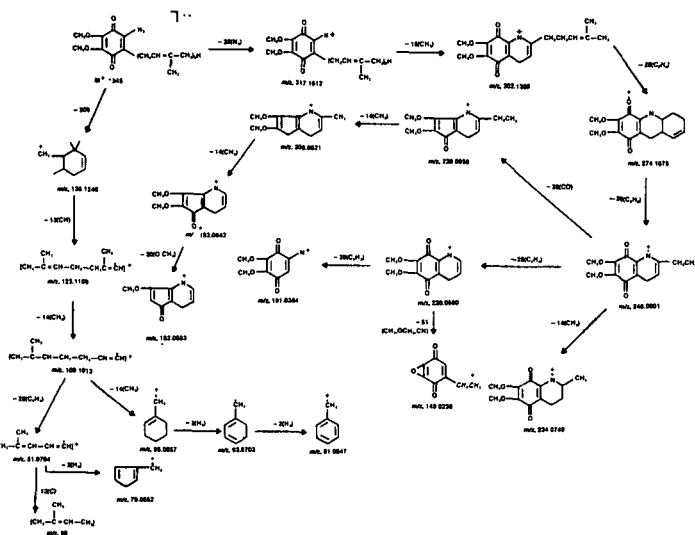
Mass spectra and partial fragmentation patterns are shown for Compound III. (2-azido-3-methyl-6-methoxy-5-geranyl-1,4-benzoquinone (Fig. 1) and Compound II (6-azido-2,3-dimethoxy-5-geranyl-1,4-benzoquinone) (Fig. 2). The fragmentations for compounds I-IV show the peaks at m/z 69 and 123 representing mevalonyl and geranyl sidechains respectively. Fig. 2 (Compound II) does not show the M⁺ -345 but gives the M⁺ -28 ion due to a loss of N₂ whereas Fig. 1 (Compound III), which has the azido group on C-2 instead of C-6, shows the molecular ion. Scheme I shows the complete fragmentation pattern (determined by high resolution mass spectrometry), of III which is typical of Compound I. Compounds II and IV and Compounds V and VI differ primarily in the side chain at carbon -3.

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ACKNOWLEDGMENTS

This work is supported in part by grants from NIH (GM 30721) and USDA (82-CRCR-1-1049).



Scheme 1 Fragmentation pattern of 2-Azido-3-Geranyl-5,6-Dimethoxy-1,4-Benzoquinone

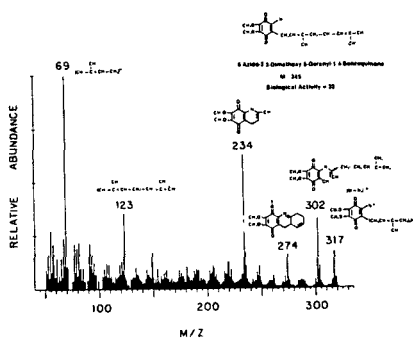


Figure 1 Mass spectrum and partial fragmentation pattern of 2-Azido-3-Geranyl-5,6-Dimethoxy-1,4-Benzoquinone

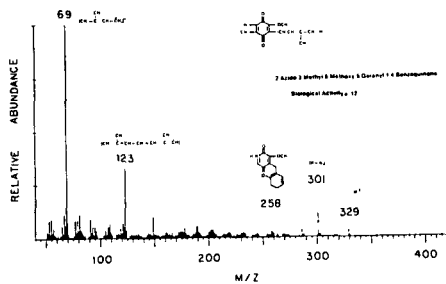


Figure 2 Mass spectrum and partial fragmentation pattern of 2-Methoxy-3-Geranyl-5-Methyl-6-Azido-1,4-Benzoquinone

USE OF MASS SPECTROMETRY FOR IDENTIFICATION OF MINOR CONSTITUENTS OF NUCLEIC ACIDS

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Identification of modified bases or nucleosides in tRNA helps to understand the biochemical function of tRNA in the synthesis of peptides. Although classical identification methods usually rely on chromatographic properties of hydrolysis products, there are cases where a method which is both more sensitive and selective is desired. Furthermore, elemental composition and structural information may also be required when an unidentified nucleoside has been detected. High resolution mass spectrometry is both highly selective and sensitive and may be useful for detection and identification of minor constituents of tRNA (1,2).

Prior to analysis 20 to 100 μ g of the nucleic acid is enzymatically degraded to mononucleosides. The digest mixture is then dried in vacuum over P_2O_5 and derivatized using N,O-bis(trimethylsilyl)acetamide (BSA) and dry pyridine (10:1). This reaction mixture is heated at 100° for 1 hr. and analyzed directly from the derivatizing solution.

Sample introduction via a directly coupled capillary GC column is desirable because it increases the selectivity of the analysis and because it provides chromatographic information which may be useful for identification of unknowns. The commercially available fused silica columns are particularly inert and can often elute subnanogram quantities of derivatized nucleosides without appreciable losses.

The selectivity of the analysis may also be increased by using high resolution. Although the sensitivity of the instrument may be reduced by as much as a factor of 20 when the resolving power is increased to 10,000, the detection limit may still be satisfactory. The result of a typical low level measurement is given in Fig. 1. A 320 pg quantity of persilylated queuine (MW 709) was applied to a 14M fused silica column using a falling needle injector. The mass spectrometer (MAT 731) was tuned for a resolution of 10,000 and continuously swept through a narrow mass range (300 ppm) which was centered about the exact mass (379.1806) of a prominent fragment ion of the derivatized queuine (3). A pulse amplifier/multichannel analyzer signal acquisition system was used to record the mass profile during the time interval in which the queuine eluted from the column. Although a total of only 149 ions was detected, they did have the anticipated retention time and elemental composition. Thus, the selectivity of this method is very high and the probability of having a false positive is very low.

The utility of the method for verifying the presence of modified nucleosides in an enzymatic digest is illustrated in Fig. 2. An equivalent of 3 μ g of *E. coli* tRNA^{Ala} was used for each measurement. The experimental conditions were similar to those described above except for substitution of a direct insertion probe for the capillary GC. Peak profiles and the total number of ions detected are given for five of the six modified nucleosides known to be present in this tRNA. A peak for 4-thiouridine was not observed under the present conditions. The peak at m/z 656.3096 is due to the 7-deazaguanosine moiety and is a diagnostic indicator for queuosine (Q) and related compounds.

Although a resolving power of 10,000 was enough to completely separate the representative ions for ms⁻¹A, ψ , 2'OmG and Q from matrix ions, the molecular ion for thymine riboside required a resolution of 18,000 for isolation. The choice of the method of sample introduction and the mass resolving power should be selected for the particular problem. Maximum selectivity is obtained using both the capillary GC and high resolution while maximum sensitivity is achieved using capillary GC and low resolution. Although most modified nucleosides are eluted from short capillary columns without appreciable losses, hypermodified nucleosides must be run via the direct insertion probe.

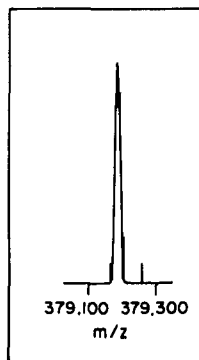


Fig. 1 Time integrated mass profile of a major fragment ion (379.1806) in the mass spectrum of persilylated queuine (MW 709). A falling needle injector and a 14M fused silica column were used to introduce the sample which contained 320 pg of queuine. The resolution was 10,000. A total of 149 ions was detected for 150 scans of a 300 ppm mass interval.

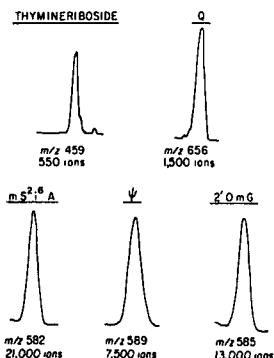


Fig. 2 High resolution mass profiles and integrated ion count for five modified nucleosides known to be present in *E. coli* tRNA^{Yr}. An equivalent of 3 µg of tRNA was used for each measurement.

References

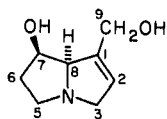
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MASS SPECTROMETRY OF ADDUCTS OF PYRROLIZIDINE ALKALOID METABOLITES AND NUCLEIC ACIDS

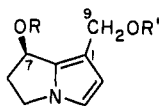
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Retronecine (I) when esterified with various necic acids forms the "pyrrolizidine" nucleus for many of the pyrrolizidine alkaloids. Structural features required for toxicity are the presence of a 1,2 double bond and esterification at C-9. The toxic action of the alkaloids, however, is probably exerted through pyrrole derivatives such as II. One would expect such pyrrole esters to be very reactive alkylating agents and schemes have been proposed for the enzymatic conversion of pyrrolizidine alkaloids to pyrroles in the liver.



(I)



II R = R' = H

The reaction of retronecine pyrrole (II) with nucleic acids at pH 7.4 produces adducts which can be characterized by liquid secondary ion mass spectrometry (LSIMS). Negative ion LSIMS shows an $(M - H)^-$ at m/z 481 for the 5'-deoxyguanosine monophosphate adduct of II (Fig. 1) and intense ions at m/z 346 and 97 for loss of the alkaloid residue and phosphate group, respectively. The sodium, potassium mixed salt of this adduct gives ions with peaks at m/z 503 and 519, as well as the $(M - H)^-$ at m/z 481 (Fig. 2). High field proton NMR spectral analysis shows substitution occurred at C-7 of the alkaloid residue and at N-2 on the 5'-deoxyguanosine monophosphate.

Nucleoside adducts have also been prepared. Guanosine reacts with retronecine pyrrole to form the C-7 - N-2 adduct as previously determined by NMR (1). The negative ion LSIMS spectrum of the adduct shows an $(M - H)^-$ at m/z 471 and a peak at m/z 431 for the methyl ether, which results from alkylation of the HPLC eluant during isolation. Adenosine also reacts with II to form an adduct, presumably with C-7 - N-6 binding which shows a peak at m/z 401 by LSIMS for the $(M - H)^-$. High resolution mass spectrometry of the permethylated adduct shows a peak at m/z 308 with an exact mass corresponding to a protonated N-6 which was both demethylated and dealkylated. Several additional peaks corresponding to exact masses for alkylated adenosine derivatives were present (2).

Positive ion LSIMS was found to be much less useful for characterization of either nucleosides or nucleotides. Quasi-molecular ions were usually weak or absent.

Acknowledgments. This work was supported by NIH Division of Research Resources Grant RR01614 (to A.L.B.) and NIEH Program Project Grant ES-00040 (to M.D.)

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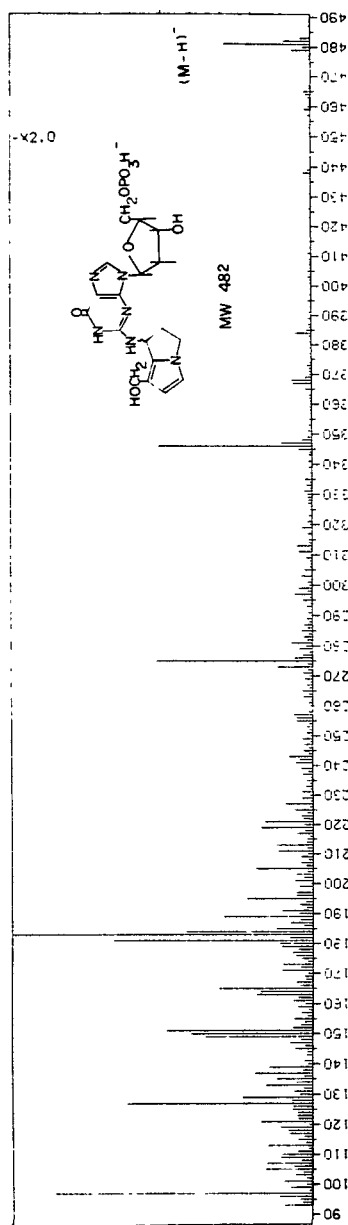


Fig. 1 Negative ion LSIMS of adduct of retronectine pyrrole and 5'-deoxyguanosine monophosphate.

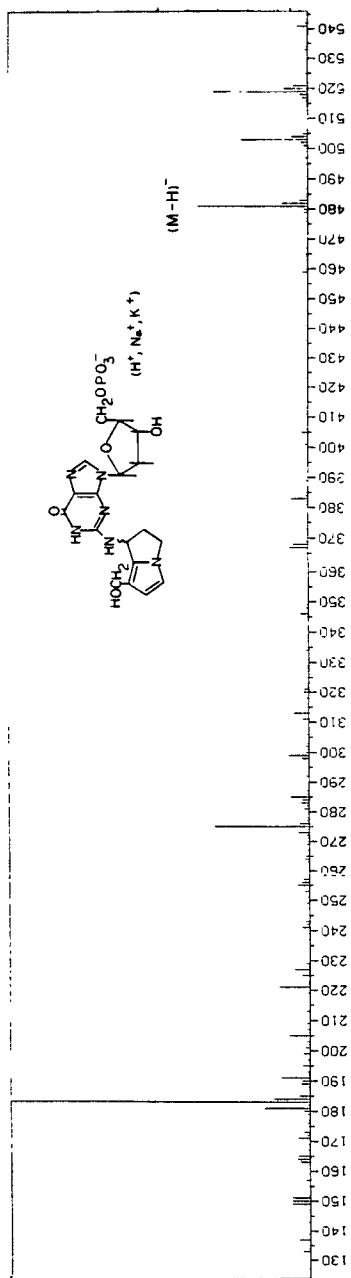


Fig. 2 Negative ion LSIMS of adduct of retronecine pyrrole and 5'-deoxyguanosine monophosphate, sodium potassium mixed salts.

USE OF GC/MS AND FAB FOR PROTEIN SEQUENCE CHECKING
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The development of new techniques for DNA sequencing has made it possible to sequence the genes for many proteins with much greater ease and certainty than was ever possible before. This places an even greater importance on the development of fast, sensitive methods with which to study protein primary structure in order to determine the DNA reading frame, identify the initiating and terminating DNA sequences, confirm the DNA sequence, and determine the modifications made after translation. In this regard, we have used gas chromatography/mass spectrometry (GC/MS) and fast atom bombardment (FAB) mass spectrometry to identify the products of proteolytic digestions in order to verify protein sequences. This is illustrated by applying them to the analyses of bacteriophage P22 tail protein.

The N-terminal and C-terminal of the intact tail protein were investigated by hydrolyzing the protein with dipeptidyl aminopeptidase I and IV (DAP I/IV) or dipeptidyl carboxypeptidase (DCP) and identifying the dipeptide product by GC/MS.² The fourteen dipeptides identified from the DAP I/IV digestion were consistent with the predicted N-terminal sequence and indicated that the first predicted residue, formyl-methionine, had been removed. The fourteen dipeptides identified from the DCP hydrolysis were consistent with the DNA predicted sequence ending 666 amino acids after the N-terminal, and are in the same DNA reading frame as the N-terminal sequence.

Quantitation of the N-terminal amino acid was achieved by dansylation of the bacteriophage P22 tail protein followed by acid hydrolysis, and identification of the dansyl amino acids by FAB mass spectrometry. The identification of threonine as the N-terminal residue is consistent with the results obtained by DAP I/IV hydrolysis, in that threonine is the second predicted residue. Using dansyl aminobutyric acid as an internal standard, the yield of dansyl threonine was determined to be 95% of that expected, indicating that there is one mole of N-terminal threonine per mole of protein.

Mixtures of peptides obtained by CNBr cleavage of P22 tail protein were digested with DAP I/IV or DCP, the products identified by GC/MS and FAB mass spectrometry, and the dipeptides correlated to the predicted sequence in a nearest-neighbor type of analysis. In this manner, five predicted sequences, ranging from 18 to 22 residues, were verified. In addition, two other CNBr fragments were purified by HPLC and their sequences determined by obtaining overlapping sets of dipeptides. Dipeptides containing arginine, histidine and lysine are not identified by GC/MS, hence they were identified by FAB mass spectrometry.

Using these methods, we were able to rapidly verify the predicted sequence of a protein of about 72,000 daltons to a high degree of confidence. It showed that all of the sequences found were in the proper DNA reading frame and ruled out possible errors such as the deletion or insertion of a base, or that large portions of the protein had been removed by proteolysis after the translation process.

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TRIFLUOROACETALDEHYDE ETHYL HEMIACETAL (TFA). FORMATION OF SCHIFF BASES, α -KETOACYL AMINO ACID ESTERS, AND CLEAVAGE PRODUCTS USEFUL FOR GC/MS: PARTIAL HYDRAZINOLYSIS PRODUCTS. DAY, R.A., METRAL, C.J., JAYASIMHULU, K., KORNEL, A., AND VYAS, D., UNIVERSITY OF CINCINNATI, CINCINNATI, OH 45221.

We had found that α -ketoacyl derivatives are useful in MS determination of peptide structures (1-3). TFA is an effective transaminating reagent; it and its by-product amine are volatile, rendering it compatible with MS or GCMS analyses. The α -ketoacyl derivatives frequently possess good GC or GCMS properties. Also, we report here, for the first time partial hydrazinolysis with N_2H_4 or unsym-dimethylhydrazine (DMH) as a method which theoretically should retain identities of all amino acid residues as well as sequence information in di-, tri-, tetra-... peptide hydrazides. The latter are made volatile by TFA treatment or silylation (BSTFA). The final reaction mixtures were themselves suitable for injection on both packed and capillary columns.

EXPERIMENTAL

TFA Treatment. Typically, up to 20 μ moles of peptide, its ester or hydrazide, was heated 10 m at 110° in 200 μ l 1:1 TFA/pyridine. A suitable FAME was used as an internal standard.

Partial Hydrazinolysis. Anhydrous N_2H_4 or DMH were used in ~1000X excess. N_2H_4 reaction times and temperatures were less than required for the Akabori procedure (4). The DMH reaction required 10X longer times than N_2H_4 at a given T.

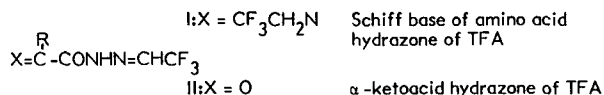
Silylation. The procedure of Krütsch & Pisano was used (5). (BSTFA = bis-silyltrifluoroacetamide.)

GCMS Analysis. The final reaction mixtures were analyzed by injection onto packed columns with 3% OV-1 or OV-17 (1-2 m) and onto fused silica, bonded Supelco SPB-1 (60 m) columns interfaced with either an RMU-7 or MS-80 mass spectrometer. The latter was commonly used to collect exact mass data.

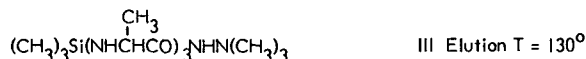
RESULTS

Transamination took place in excellent yields for many peptides (Table I). MS fragmentation occurred as described (3). The major fragments are indicated in the table.

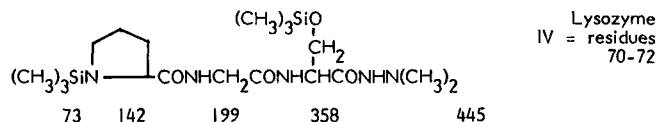
Partial hydrazinolysis gave the expected amino acid and peptide hydrazides. Treatment with TFA gave the volatile Schiff base (e.g. I) or α -ketoacyl (e.g. II) hydrazone.



The synthetic tripeptide, AAA, gave the expected derivatives when treated in various reagent combinations; the best GCMS results were obtained from the silylated dimethylhydrazide III



AAA and AA were identified from the partial dimethylhydrazinolysate of S-carboxymethylated lysozyme. Treatment of S-carboxymethylated lysozyme with DMH followed by BSTFA afforded a mixture of silyl peptide dimethylhydrazides with acceptable GCMS properties, e.g. IV:



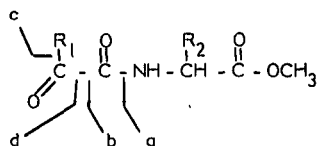
The indicated sequence ions and their identifying ions were seen, e.g. m/z 70 from PRO and m/z 59 characteristic of the dimethylhydrazyl fragment. Many ions were verified by exact mass measurements. Typically, among the most intense ions were M-45, M-60 and m/z 73. M-45 arose from loss of $\text{NH}(\text{CH}_2)_2$ and M-60 from loss of $\text{NH}(\text{CH}_2)_2$ and CH_3 , not $\text{NHNH}(\text{CH}_2)_2$. Treatment of large polypeptides by DMH/BSTFA provides immediately a significant amount of sequence information if the resulting mixture is analyzed by a capillary GCMS system.

Table I. α -Ketoacyl Amino Acid Methyl Ester Formation:^a

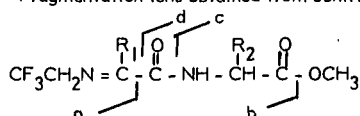
Dipeptide	% Yield*	Fragmentation (Intensity %)			m/z (% base peak) d.	M ⁺
		a.	b.	c.		
Ala-Ala	—	102(98)	130(71)	15(0)	43(100)	—
Leu-Val	63	130(100)	158(22)	57(94)	85(75)	243(24)
Phe-Ala	—	102(87)	130(8)	91(100)	119(6)	—
Ile-Ala	84	102(99)	130(100)	57(92)	85(97)	215(67)
Asp-Phe	38	178(100)	206(1)	59(3)	87(8)	—
Leu-Leu	71	144(100)	172(27)	57(99)	85(99)	257(28)
Leu-Gly	52	88(100)	116(99)	57(95)	85(98)	201(99)
Val-Leu ^b	62	152(100) 110(40) ^c	265(44)	144(41)	172(5)	

* Yield obtained by normalizing the number of C atoms. Derivative compared to FAME.

^a Fragmentation of α -ketoacyl amino acid Me ester.



^b Fragmentation ions obtained from Schiff base:



^c The ion obtained is $(a-R_1)^+$.

We conclude that considerable sequence information may be deduced from peptides treated according to various combinations of the above protocols. N-Terminal ILE and LEU may be readily differentiated through their α -ketoacyl (3) or oxazinone derivatives (2). The partial hydrazinolysis with hydrazine, DMH or trimethylhydrazine- d_2 affords a new approach to peptide sequencing problems.

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COMPUTER AIDED IDENTIFICATION OF DIPEPTIDES IN A GC/MS RUN USING DS-55 FOR-TRAN IV SUB-ROUTINES. SEQUENCING OF PEPTIDES FROM DIPEPTIDES.

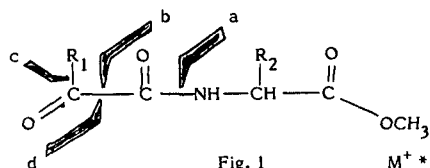
INTRODUCTION

Transamination reactions of amino acids and peptides have been shown to yield affordable quantities of volatile α -keto acyl esters or Schiff bases¹. Degradation of large peptides with diamino peptidases (DAPS) can in principle be used to generate most of the dipeptides from any given peptide. In this presentation we wish to show a computer program for the automatic identification of products of a random mixture of dipeptides which have been transaminated as presented elsewhere¹ : In addition, we wish to show a second computer program which attempts to sequence a peptide from the dipeptide data set.

Discussion

Program 1 GC-Peak Identification

The mass spectra of α -keto acyl esters can be uniquely characterized by four major peaks as shown in fig 1.



*In some cases M^+ may be used.

Identification of any given dipetide can be effected by simply matching these major fragments, and the corresponding intensities, against the GC peaks of a given run. In addition, the search is accelerated by restricting the retention index range at which a given dipeptide is expected to be found. The flow chart of the program is shown in fig 2.

Sequencing Program

The result of the GC-peak identification program shown above is to produce a list(s) of the dipeptides in a set of runs. If such dipeptides correspond to the set arising from degradation of a larger peptide, then sequencing can be carried out. The sequencing program uses the list of dipeptides arising from a given peptide to generate all possible sequences consistent with the data set. Although ambiguities may occur they are not significant for peptide lengths below 15. The logic of the program is best illustrated by the example below.

DIPEPTIDE LIST: OL, SC, HO, OO, CH

Sequence Attempt#	Sequence	Reason for Stop	Match?
1	OL	No <u>L</u> Links	NO
2	SCCHHOOL	No <u>L</u> Links	NO
3	SCCHHOOOOL	No <u>L</u> Links	YES
4	HOOL	No <u>L</u> Links	NO
5	OO-OL	"	NO
6	CHHOOL	"	NO
7	CH-HO-OO-OL	"	NO

Probable Identity S-C-H-O-O-L

The results of program I on the mixture of AA, IA, LV, VI, LL. are shown below:

SCANS: BV1045.310-564
RANGE: 20-400
REPORT DEVICE: \$TT01
ENTER FILENAME
MEST.DA
THRESHOLD(1-7)IE5

```

THRESHOLD: 10
SCAN # 312 SEEMS TO INDICATE PEPTIDE PEPTIDE ID: AA
SCAN # 452 SEEMS TO INDICATE PEPTIDE PEPTIDE ID: IA
SCAN # 521 SEEMS TO INDICATE PEPTIDE PEPTIDE ID: LV
SCAN # 531 SEEMS TO INDICATE PEPTIDE PEPTIDE ID: VI
SCAN # 563 SEEMS TO INDICATE PEPTIDE PEPTIDE ID: LL
NO MORE MATCHES OR SCANS TO BE PROCESSED
STOP

```

```

graph TD
    START([START]) --> Init[SN=-1  
IL=0  
PN=0  
MC=0]
    Init --> EnterName[ENTER  
"NAME"]
    EnterName --> EnterThr[ENTER  
THR]
    EnterThr --> ILinc[IL=IL+1]
    ILinc --> ReadEntry[1  
READ ENTRY(IL)  
RT1, RT4, I2, I1, M2,  
I2, M3, I3, M4, I4,  
NA]
    ReadEntry --> LastEntry{3  
LAST  
ENTRY  
?}
    LastEntry -- N --> IL1[IL=1  
SN=SN+1]
    IL1 --> ReadEntry
    LastEntry -- Y --> Stop([STOP])
    ReadEntry --> SNinc[2  
SN=SN+1]
    SNinc --> TIC{1  
TIC(SN)  
≤  
THR?}
    TIC -- Y --> ReadPeak[READ  
PEAK(PN)]
    ReadPeak --> MassMatch{MASS  
AND INT.  
MATCH?}
    MassMatch -- Y --> MCinc[MC=MC+1]
    MassMatch -- N --> PNinc[PN=PN+1]
    MCinc --> MCge4{MC ≥ 4}
    PNinc --> LastPeak{LAST  
MASS  
PEAK  
?}
    LastPeak -- Y --> MCge4
    LastPeak -- N --> PNinc
    MCge4 -- Y --> Output[OUTPUT  
PEPTIDE  
I.D.]
    Output --> LastScan{LAST  
SCAN  
?}
    LastScan -- Y --> Stop
    LastScan -- N --> MC0[4  
MC=0  
IL=1]
    MC0 --> ReadEntry
    MCge4 -- N --> TIC
  
```

Fig. 2

Closing Notes

Closing Notes

1. Program I can in principle be used as a utility program for a fast search of a compound in GC trace since all that is required is that the data entry be consistent with the program; that is, that it contain 2 retention times 4 masses and 4 in tensities.
2. This program makes use of DS-55 routines and as such can not be used in other systems. The logic, however, can be transferred. For the program to work in other systems the user must provide means to read the scan TIC and the mass spectrum (mass in descending order).

Ret I C.J. Metral, K. Jaysimhulu, A. Kornel, and R.A. DAY - 30th Annual Conference on Mass Spectrometry and allied topics pp. 724-726.

CURIE-POINT PYROLYSIS MASS SPECTROMETRY OF POLYURETHANE ARTIFICIAL HEART IMPLANTS. Joseph Richards, William H. McClennen, Henk L.C. Meuzelaar, Biomaterials Profiling Center; Donald E. Gregonis, Monty Reichert; Bioengineering Department, University of Utah, Salt Lake City, Utah 84112.

The recent implantation of the first permanent total artificial heart in a human subject has led to heightened interest in analytical techniques for stringent quality control and retrieved sample evaluation of Biomer®, the segmented poly(ether-urethane-urea) comprising the main portions of the implant. Dusel *et al.* [1] have demonstrated the enhanced capabilities of pyrolysis mass spectrometry (Py-MS) in discerning the structure of copolyurethanes beyond more conventional spectrometric methods. In this work, Curie-point Py-MS analysis [2] along with computerized multivariate statistical analysis of the spectral data [3] is used to confirm the basic Biomer structure [4], evaluate batch differences in the polymer and study the effects of implantation in the physiological environment.

The Curie-point Py-MS was performed as described elsewhere [2] using 10 µg samples from dimethylacetamide solution with low energy (12 eV) electron impact ionization and quadrupole mass analysis. Comparisons of the mass spectra with literature data [1,4] and model compounds have confirmed the basic Biomer composition to include a soft segment of polytetramethyleneglycol (PTMG) and a hard segment of diphenylmethanediisocyanate (MDI) and various chain extenders including ethylenediamine (EDA). The contributions of each of these building blocks to the spectrum of a model polymer are shown in Figure 1.

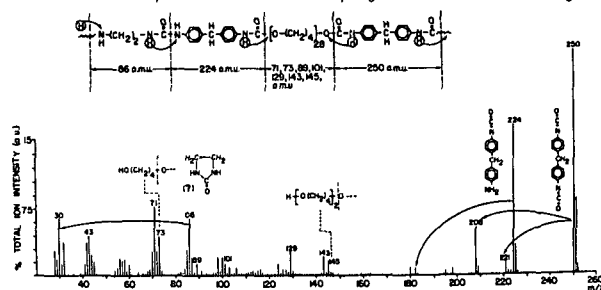


Figure 1. Pyrolysis mass spectrum of a model poly(ether-urethane-urea) consisting of MDI (diphenylmethanediisocyanate), PTMG (polytetramethyleneglycol) and EDA (ethylenediamine) building blocks. The high intensities of the various MDI peaks (relative to Figure 2) are due to the use of a heated inlet.

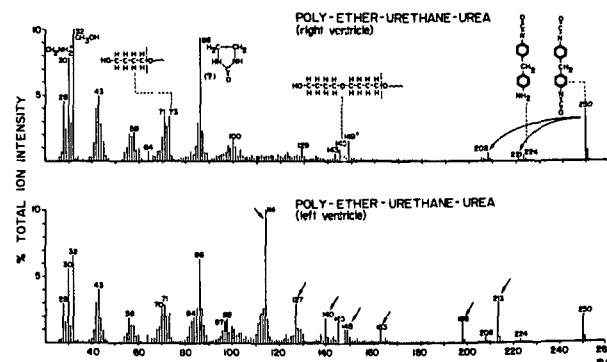


Figure 2. Average pyrolysis mass spectra of two ventricles made from separate batches of Biomer showing the major changes from apparently different polymer formulations. Compare the obvious spectral differences here with the similarities between Figure 2a and the model polymer of Figure 1.

The significance of possible batch differences is demonstrated in the spectra of the left and right ventricles retrieved from one experiment as shown in Figure 2. The right ventricle spectrum (Figure 2a) showed striking similarity to samples from several other hearts as well as the model polymer of Figure 1 (except for the attenuated MDI peaks at m/z 250, 224, 221, 208 due to use of a non-heated inlet). The obviously different spectrum of the right ventricle with additional peaks at m/z 114, 127, 140, 148, 163, 198 and 213 indicates a major change in the basic polymer structure, probably from another manufactured formulation [3].

Although neither of these particular ventricles from separate Biomer batches showed any adverse properties, the fact that their difference was not observed by other quality control techniques, such as proton NMR or transmission IR, emphasizes the utility of the Py-MS analysis.

In another study, samples were obtained from different parts of a Jarvik V and a Jarvik VII model artificial hearts implanted for 71 days in calves. These hearts were made from Biomer with much smaller, and perhaps more typical, batch-to-batch variation. However, comparison of samples from portions of both hearts directly exposed to blood and tissues with samples from relatively unexposed portions showed definite though subtle differences in pyrolysis patterns. Computer-assisted evaluation of these differences by means of factor analysis and discriminant analysis [3] clearly separated the physiologically exposed and nonexposed parts as shown in Figure 3. The difference spectrum in Figure 4 was derived from these two groups and indicated the presence of adsorbed proteins as well as the occurrence of oxidative and/or hydrolytic changes in the polyol moieties of the exposed polyurethane samples.

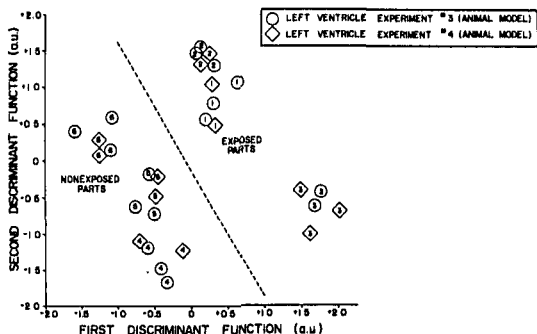
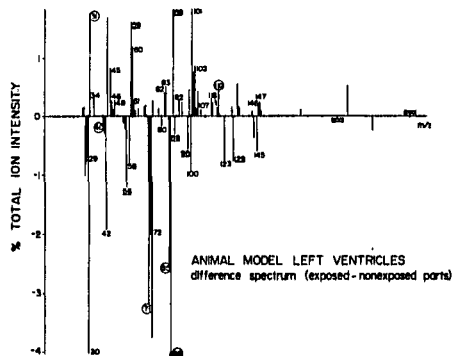


Figure 3. Plot of the discriminant analysis of the pyrolysis mass spectra from retrieved total artificial hearts used in two animal experiments. The computer data analysis of the visually very similar spectra was able to clearly distinguish between the parts directly exposed or not exposed to the physiological environment.

Figure 4. Difference spectrum derived from the data used in Figure 3 with the positive peaks representing ions stronger in the physiologically exposed heart portions (blood diaphragm, inside and outside housing) versus the negative peaks which were stronger in the unexposed parts (air and intermediate diaphragms).



It is concluded that Curie-point pyrolysis MS techniques show considerable potential for structural identification and quality control of polyurethane and other polymer implant materials as well as for the investigation of biodegradation phenomena.

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THE MASS SPECTROMETRY OF SOME 2-METHYLTHIO-OXAZOLINE
DERIVATIVES OF PENTOSE AND HEXOSE

G. D. Byrd, R. M. Davidson, E. White V, B. Coxon, and S. A. Margolis

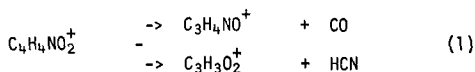
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Oxazoline derivatives of carbohydrates have played an important role as intermediates in the stereospecific synthesis of certain anti-tumor nucleosides such as ara C. In order to extend the range of available intermediates, efforts have been made to synthesize and characterize novel oxazoline derivatives of various carbohydrates. This work describes the mass spectrometry of a series of 2-methylthio-oxazoline derivatives of pentoses and hexoses that were recently prepared as potential intermediates for these syntheses [1].

The compounds were prepared by converting the free aldoses to 2-thiono(1,2-deoxy-D-aldo)[1,2-d]-2-oxazoline derivatives (some of which were previously known) by treatment with an alkali metal thiocyanate and concentrated HCl. Methylation of the 2-thiono derivatives was carried out by methyl iodide in the presence of a base catalyst. These conditions might be expected to give one or more of several products, including those resulting from N-, O-, or S-methylation. Previous ^{13}C NMR studies on these compounds have concluded, in general, that a single S-methylation has occurred.

The 70 eV electron impact (EI) mass spectra of these compounds were taken on a double-focusing high-resolution instrument and yielded molecular ions for most species. Exact mass measurements and ^{13}C and ^{15}N labelling indicate that the major ions in these spectra contain the elements of the oxazole portion of the molecule which presumably remains intact as a ring. The molecular weight data along with the regular occurrence of MeS^+ (m/z 47) in these spectra supports a single S-methylated structure for these derivatives as anticipated. The furanoid and pyranoid derivatives can be easily differentiated as can be seen from the spectra in Figures 1 and 2. However, isomers within a ring system are less readily distinguished.

As illustrated in Figure 1, the furanoid derivatives are characterized by an intense peak in their EI mass spectra at m/z 98 which is assigned the formula $\text{C}_4\text{H}_4\text{NO}_2^+$ through exact mass measurements. Loss of MeS^+ and cleavage of the carbohydrate ring at the C1'-O and C3'-C4' linkages occur in separate steps to produce the C5 formylated oxazole structure suggested in Figure 1 for m/z 98. Linked E/B scans show that this ion decomposes as shown in Reaction 1 by loss of either CO or HCN which is consistent with an oxazole



ring structure. The spectra of the hexose furanoids usually have a weaker molecular ion as illustrated in Figure 3. Observed metastable transitions indicate that the $(\text{M}-\text{H}_2\text{O})^+$ peak at m/z 217 originates at least in part from the molecular ion rather than from thermal dehydration of the sample.

The mass spectra of the pyranoid derivatives are characterized by the dominant peak at m/z 116 while m/z 98 is now negligible as shown by the example in Figure 2. The ion at m/z 116 may have the 2-methylthio-oxazole structure indicated. This species arises by both direct cleavage of the carbohydrate ring from the parent and through sequential cleavage of the C1'-O and C3'-C4' linkages (m/z 146) followed by the loss of H_2CO .

FDMS and LDMS provide molecular weight information for those compounds that have little or no molecular ion in their EI mass spectra. Both desorption methods usually produce $(M+H)^+$ species with LDMS yielding alkali ion adducts as well.

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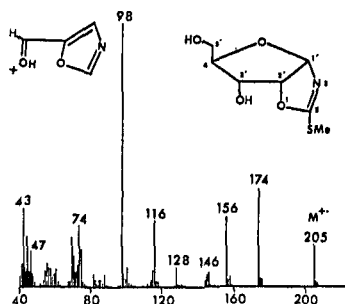


Figure 1

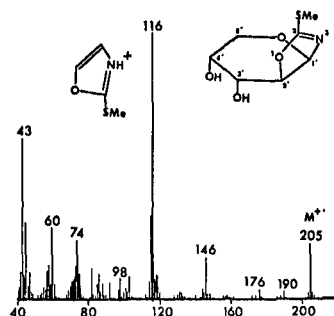


Figure 2

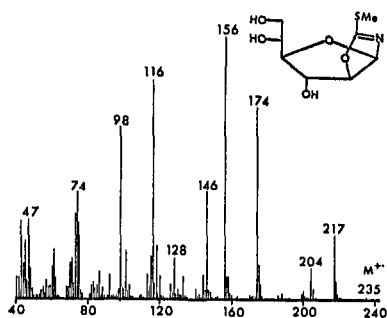


Figure 3

MASS SPECTROMETRY OF A NEW DERIVATIVE OF THE TETRAHYDRISOQUINOLINE, SALSOLINOL; *Sisak, M. and Murphy, R.C.: Univ. Colorado School of Medicine, Department of Pharmacology, Denver, CO 80262.

It has been known for many years that salsolinol (6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline) can be formed by the condensation of acetaldehyde and the neurotransmitter dopamine. The possibility of *in vivo* formation was suggested by Cohen and Collins when they demonstrated the formation of tetrahydroisoquinolines (TIQs) in adrenal glands perfused with dilute solutions of acetaldehyde (1). Noting that acetaldehyde was the primary metabolite of ethanol and that plant derived TIQ's exhibited significant pharmacological effects such as excitation, narcosis, blood pressure changes and convulsions, they further suggested that the TIQ formation might play a role in the behavioral changes during and after intoxication.

Later studies by Meyers and Melchior showed that TIQ's chronically infused into the ventricle of rats increased their voluntary intake of alcohol causing some to drink to intoxication leading to withdrawal symptoms when the alcohol was removed (2).

These findings lead many research groups to develop assays to measure the levels of salsolinol in brain, urine and CSF of ethanol dependent animals and urine of CSF of alcoholic patients. The results of these studies were inconsistent regarding the identification and quantitation of salsolinol. Sjöquist *et al.* has consistently reported endogenous TIQ levels in controls and increased levels in urine, CSF and brain of ethanol dependent animals and alcoholic patients (3). Barker confirms salsolinol as a normal constituent of rat brain but at 10 fold lower concentrations (4). Collins and Bigdeli reported salsolinol present in the tissues of intoxicated animals but only when the acetaldehyde levels were increased by the administration of pyrogallol and pargyline prior to the ethanol (5). O'Neill and Rahwan reported the absence of salsolinol in the brain of ethanol dependent mice (6).

In an effort to resolve some of the controversies, we have begun work on a new assay for salsolinol. Our main objectives have been to utilize gas chromatography/mass spectrometry to allow for positive identification and to find a derivative whose spectrum contains high mass ions that carry a large percentage of the total ion current to allow for high sensitivity with selected ion monitoring and contain all the atoms from salsolinol for possible labelling studies.

Reaction of salsolinol with t-butyldimethylsilyl (tBDMS) chloride in imidazole/DMF (1/2.5) for 30 minutes resulted in two derivatives. The first was the expected O,O-tBDMS-salsolinol and the second was derivative with a molecular weight 28 mass units higher. This second derivative was apparently the result of formylation of the nitrogen by the solvent.

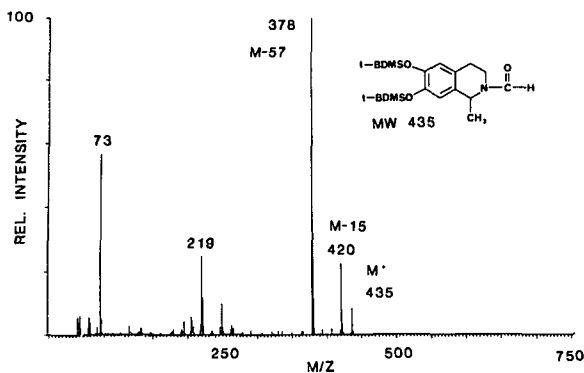


Figure 1: Mass Spectra of O,O-tBDMS,N-formyl salsolinol

The silylation was found to be complete in less than 10 minutes by adding trimethylsilyl chloride at various times during the reaction and following the formation of the O,O-tBDMS and O,O-TMS derivatives. The formylation was studied by removing aliquots and monitoring the non-formylated and formylated derivative. The reaction proceeded according to the first order kinetics and was essentially complete after 90 minutes.

Deuterated internal standard was synthesized by reacting D₃-dopamine with acetaldehyde at pH 7.0. Analysis by GC/MS showed that two products were formed that exhibited different retention times but produced identical mass spectrum. These isomers are apparently due to ring closures at the 5 or 2 positions. The major product (90%) is the favored ring closure at the 2 position to form salsolinol as shown by coelution with authentic salsolinol.

Samples were analyzed on a VG Micromass 16 mass spectrometer fitted with an SE-30 capillary column. Monitoring the base peak under selected ion monitoring conditions, we were able to obtain a calibration curve over the range of 200 pg to 12.8 ng.

Because salsolinol is a catechol, absorption to alumina was employed to isolate these TIQs from the biological matrix. Although radioactivity showed approximately 80% recovery of salsolinol through a alumina column, we were unable to derivatize these samples. We believe that alumina fines that were getting through the column frit were interfering with the derivatization. We were able to overcome this by extracting the salsolinol into a 25% n-butanol/isopropanol solution prior to derivatization.

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Gas Chromatographic/Mass Spectrometric
Analysis of Di-(2-ethylhexyl) Phthalate Metabolites

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Di-(2-ethylhexyl) phthalate (DEHP) is an ubiquitous environmental pollutant with high chemical stability. The widespread use of DEHP as a plasticizer and recent toxicological studies indicating possible weak carcinogenicity have focused attention on this compound, requiring the development of analytical techniques for the determination of DEHP metabolite structures. In an effort to develop GC/MS techniques useful in identifying DEHP metabolites in rat urine, extracts of urine collected from rats fed unlabelled, ^{13}C or ^{14}C labelled DEHP were methylated with diazomethane and HPLC fractions collected for GC/MS experiments (1). In addition, some fractions were derivatized by reacting 10-100 μg quantities of metabolite with 50 μl bis-trimethylsilyl trifluoroacetamide containing 5% trimethylsilyl imidazole, and 50 μl of methylene chloride at room temperature for 20 min. The deuterated TMS derivatives were made by substituting d9-TMS imidazole in the mixture. Butylboronates were prepared by methods developed by Brooks and MacLean (2).

GC/MS analysis was performed using a HP 5710 gas chromatograph having a 30 m DB-5 capillary column directly coupled to the source of a V.G. 7070E mass spectrometer. After on-column injection of the derivatized metabolites the column was heated to 150°C and held for 8 min, followed by heating to 270°C at 8°C/min. Full scan spectra were acquired over the mass range of 30-600 amu at 0.7 sec/dec and 5 KeV source potential for both electron impact and isobutane chemical ionization. Reconstructed ion chromatograms for total ion current and selected masses were examined for peaks characteristic for DEHP metabolites.

Common fragment ions in E.I. spectra for diazomethane treated samples included m/z 163 which was always the base peak, m/z 149 and m/z 181. In addition, appropriate shifts in mass for ions found in spectra of extracts from rats fed ^{13}C labelled DEHP provided evidence for the presence of metabolites. Fragment ions indicative of the oxidized metabolite side chain (R) were always found in diazomethane treated samples, with $(R-1)^+$ typically found in E.I. spectra and R^+ in IBCI spectra. For samples that were TMS derivatized, ions characteristic for TMS adducts to the oxidized side chain were observed corresponding to one and only one member of the series $[\text{TMS-O} = \text{CH} - (\text{CH}_2)_n\text{H}]^+$ where $n = 0$ to 3, locating the positions of the hydroxyl groups relative to the end of the hydrocarbon chain. In the case of a COOCH_3 terminated chain major diagnostic fragments corresponded to the series $[\text{TMS-O} = \text{CH} - (\text{CH}_2)_n - \text{COOCH}_3]^+$. In both series the mass increased by 9 units when the d9-TMS derivatives were used. For butylboronate derivatized samples, one metabolite's spectrum contained an ion at m/z 141, suggesting formation of a butylboronate cyclic fragment indicative of two hydroxy groups with no more than one CH_2 group separating them.

The utilization of appropriate derivatives, combined with isotopic labelling of the DEHP precursor thus provides useful structural information concerning DEHP metabolites. On the basis of these results it is concluded that GC/MS analysis of DEHP metabolites is a useful technique for determining metabolite identity.

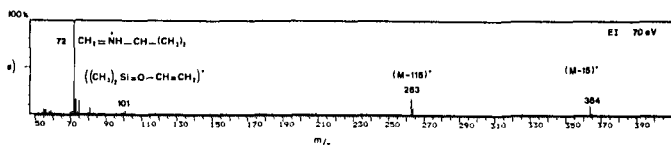
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ELECTRON IMPACT INDUCED REARRANGEMENT OF THE ARYLOXY ISOPROPANOL SIDE CHAIN OF β -BLOCKERS

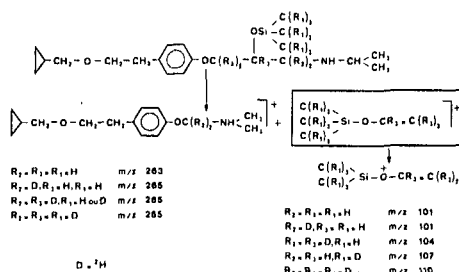
J. Fraisse-André, Ph. Hermann, J. Allen and J.P. Thénor, Dept. of Clinical Research, LERS-Synthelabo, 75013 - PARIS, France.

Most of the ions obtained in the e.i. fragmentation of betaxolol, a new β -blocker recently approved for the treatment of hypertension, can be explained by simple cleavages but the loss of 116 amu fragment results from a rearrangement.



Deuterium labelling in several positions indicates that the lost fragment contains two carbon atoms of the isopropanol and the TMS group.

PROPOSED STRUCTURE

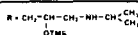


Other β -blockers give the same rearrangement under e.i. conditions with the exception of pronethalol that lacks the oxygen in a position of the phenyl ring.

COMPARISON WITH OTHER β -BLOCKERS

Many other β -blockers have an isopropylamino-propanol side chain attached to an aromatic ring

	Structure	observed M-116 ion m/z	% of base peak
Alprenolol		205	3.8
Betaxolol		283	17.9
Prenalterol		253	4.4
Pronethalol		0	
Propranolol		215	5.5



The rearrangement may result from an attack of the base peak ion m/z 72 on the oxygen in α of the aromatic ring.

ANALYSIS OF PHOSPHOLIPIDS BY POSITIVE AND NEGATIVE
ION CI-MS VIA A MOVING BELT LC/MS INTERFACE.

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Phospholipids are important constituents of all cell-membranes. Various methods have been published for the analysis of these lipids from biological sources. However, very few reports have been published on the analysis of various phospholipids by HPLC (1) and to our knowledge combined use of HPLC-MS for the analysis of phospholipids has not been previously reported. The most convenient detection method for the HPLC analysis of these lipids is by monitoring the elution with a U.V. detector in the 200 nm range. However, this method of detection does not allow direct quantitation of the separated phospholipids since the response varies with the number of functional groups mainly the number of double bonds in the phospholipid molecules. Again, severe limitations are imposed in the choice of chromatographic solvents used since solvents which absorb U.V. light at 200 nm cannot be used. LC/MS of phospholipids can overcome these limitations besides providing extensive information on the molecular structure of each phospholipid and relative abundance of each molecular species of individual class of phospholipids.

Experimental:

HPLC was performed with a Spectra Physics gradient pump and an Accupak 3 μ M silica gel column (4.6 mm i.d. x 10 cm). A gradient was run with CH_2Cl_2 - CH_3OH - H_2O - HOAc A. 91:8:0.5:0.5 and B, 65:30:4:1 starting at 20% B and programming to 100% B in 10 min with a 5 min hold at 100% B at a flow rate of 0.8 ml/min. The HPLC solvent was applied to the Finnigan LC/MS interface moving belt via a drawn amorphous silica capillary to form a steady stream. A polyimide transport belt was used with the evaporator at 330°C (indicated) and the clean-up heater at 210°C (indicated). Ammonia (0.7 torr) or methane (0.6 torr) were used as CI reagent gasses with the ion source at 150°C. Alternate positive ion/negative ion spectra were obtained by the system under the control of a Teknivent model 56K MS data system. MS data was collected from m/z 150-900 with approximately 7 sec. scans.

Results:

The ammonia positive ion spectra of various synthetic phosphatidylcholines contained as major ions M^+-41 (100%), M^+ -phosphocholine (75%) and M^++1 . Prominent ions for fatty acids, $\text{RCO}^+(30\%)$ and $\text{RCO}^+(40\%)$ and several fatty acyl containing glycerol ions were also identified. The negative ion ammonia CI spectra of synthetic phosphatidylcholine differed considerably from the positive ion spectra. They contained M^--1 , M^--60 and M^--184 as the major ions in the high mass range.

The methane positive ion CI spectra of various synthetic phosphatidylcholines were similar to that of ammonia positive ion spectra especially in the high mass-range. They contained, M^++1 , M^+-41 , M^+-182 and M^+-147 ions. The diglyceride ion M^+-182 being 100%. The negative ion methane CI spectra contained M^--1 , M^--24 (100%), M^--130 , M^--148 and M^--184 .

The ammonia positive ion spectra of lung phosphatidylcholine clearly identified the presence of expected 14 different molecular species. Similarly egg phosphatidylcholine molecular species were identified and quantitated by the relative abundance of various m/z in the high mass range.

The ammonia positive ion CI spectra of synthetic and natural phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SP) were also analyzed. In the PE spectra M^++1 , M^+-105 and M^+ -phosphoethanolamine ion M^+-140 were the major ions in the high mass range, M^+-140 being at 100%. In the PS spectra, the major ions were the diglyceride ion M^+-194 , and M^+159 . In the PI spectra also the diglyceride ion, M^+-259 , and the corresponding M^+-224 were the major ions. In the SP spectra the major ions were the ceramide ion, M^+-182 and $\text{M}^+-182-18$. The individual molecular species

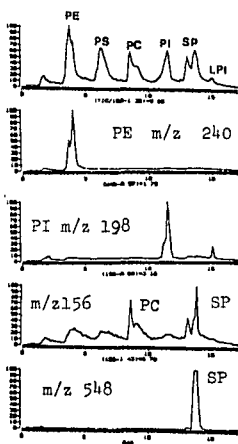


Fig. 1

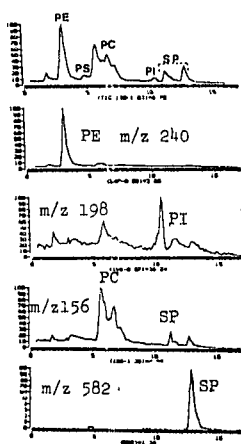


Fig. 2

HPLC-CIMS of phospholipid standards, 5 μ g each (Fig. 1) and of phospholipids from mouse kidney extract (Fig. 2). Reconstructed plots of total ion current and specific ions indicated are given.

of these phospholipids were also characterized from the mass-spectra of naturally occurring phospholipids.

The HPLC/CIMS of a mixture of phospholipids standards (5 μ g each) from natural sources is given in Fig. 1. The reconstructed plot of the total ion current shows the order of elution of each phospholipid. Each phospholipid species showed asymmetric peak and peak with shoulders which were identified by MS as due to different fatty acyl molecular species separations. The reconstructed specific ion plot is given in the fig. for each phospholipid. M/z 240 appears to be characteristic of all naturally occurring PE. M/z 184 and 156 are derived from phosphocholine moiety and are thus characteristic ions in the spectra PC and SP. M/z 180 and M/z 198 (180+18) are due to inositol and are thus specific for PI and lyso PI. The ceramide ions are easily recognized in the MS of SP.

The HPLC/CIMS of phospholipids extracted from mouse kidney is given in Fig. 2. The major phospholipids were well-resolved and identified by their characteristic ions. PC under this condition resolved into four separate peaks, each of which had different fatty acyl molecular composition. Similarly SP resolved into three separate peaks, the faster moving SP contained mostly 22:0 and 24:1 fatty acid, whereas the second major peak contained mostly 16:0 fatty acid. Reconstructed plots for specific ions of phospholipids are given in Fig. 2.

In summary, we have developed a rapid and sensitive HPLC-CIMS method for the analysis of individual phospholipids. The relative percentage composition of individual acyl and alkyl molecular species can be also determined simultaneously by this method.

References:

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DATA/CONTROL SYSTEM FOR TIME-RESOLVED MAGNETIC DISPERSION MASS SPECTROMETRY

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Simultaneous measurement of ion momentum and velocity provides a means for making energy independent mass assignments (1). These mass assignments are valid both for stable ions and for those ions that undergo metastable or collision-induced dissociation after leaving the ion source. Identification of the parent mass for each daughter ion is made from the velocity measurement.

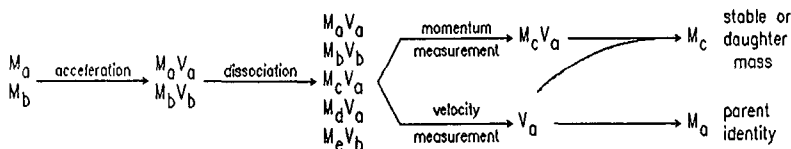


Figure 1. Outline of operation

Acceleration to constant energy gives each ion a velocity related to its mass. Dissociation in the field-free region between the source and the magnet yields daughter ions, each with the velocity of its parent. The magnet selects a particular momentum while a gated window in the detection circuit selects a particular velocity (flight time). The combination of momentum (mv) and velocity (v) measurements allows the assignment of the stable ion or daughter ion mass independent of the ion's energy. The parent mass identity is obtained from the flight time of the daughter.

Data obtained for *n*-decane is presented in Figure 2 as a plot of magnetic field strength versus flight time. All stable ions appear on the diagonal line. Isobaric daughter ions appear on a hyperbolic line (constant field strength-flight time product). All daughters of the same parent appear at the same flight time (vertical line). The lines presented were calculated after calibration. Observed points are plotted as diamonds.

A data/control system has been constructed for the time-resolved magnetic dispersion mass spectrometer and is shown schematically in Figure 3. The computer, built around the Intel 8088 microprocessor, was designed for maximum flexibility in instrument control applications. A trigger from the computer initiates both the ion source pulse and the programmable delay generator (Evans Assoc., Model 4145; 10 ns resolution). The gated integrator (Princeton Applied Research, Model 165), triggered by the delay generator, integrates over a window that is adjustable to 2 ns. Averaging of multiple pulses is achieved in either the gated integrator or the computer. The sampled signal is digitized with a 12-bit A/D converter. The magnet controller is interfaced to the computer with a 16-bit D/A converter.

Software is written in the FORTH programming language. FORTH allows low-level code to be built up to high-level commands that provide easy access to the control and measurement parameters. These commands allow programs for new experiments to be easily written by the user. Utility programs have been written to provide for calibration, mass assignments, and

graphic display of the data. Any type of MS/MS scan can be selected (parent, daughter, neutral loss). The aperture duration and averaging time can be adjusted to provide the best compromise among scan speed, resolution, sensitivity, and signal-to-noise. The hardware and software provide the basis for a flexible control and acquisition system, comparable to other advanced MS/MS systems, for this new type of mass spectrometer.

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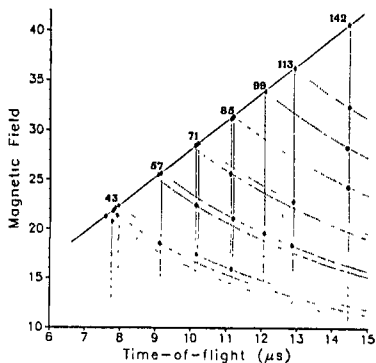


Figure 2

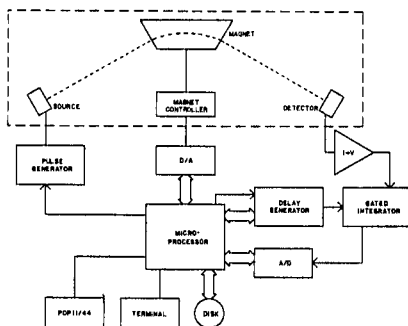


Figure 3

AUTOMATION OF A QUANTITATIVE MASS SPECTROMETER FOR TRACE NOBLE GAS DETERMINATION*

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Noble gases are added as tracers prior to detonation or are generated during underground testing of nuclear devices in Nevada. Gases, consisting principally of air, are pumped from the cavity and sampled. Noble gas concentrations have been routinely determined at the ppm level at Livermore for several years. However, these analyses required numerous manual operations. Automation of the mass spectrometer has eliminated all of the manual steps during data acquisition and calculation of composition.

The gas samples are pre-concentrated by gettering with molten Ca. This provides about a 100-fold increase in the concentration of noble gases and eliminates interfering peaks from the active gases. The mass spectrometer is calibrated with known gas mixtures or air concentrated like the samples. The percent of noble gases or concentration factor is obtained by automated mass spectrometer analysis of the original sample.

The mass spectrometer is a CEC Model 21-103C with modified electronics. The spectrum is obtained by scanning the accelerating voltage with a 16-bit DAC under the control of an LSI-11 computer. The magnet is set to the requested field with a digital field control based on a Bruker digital gaussmeter with a Hall probe. The output signal of the gaussmeter is conditioned and used to control the magnet power supply.

The ion signal is detected with a Keithley Model 642 electrometer having an input resistance of 10^{12} ohms on the most sensitive range. This electrometer has been modified to allow the computer to select current ranges, voltage mode, charge mode, signal damping and zero check. A 5 MHz voltage-to-frequency converter and a 20-bit counter are used to digitize the peak heights. Integration periods are variable. The codes for instrument control and on-line calculations are written in BASIC with assembly language calls.

The automation of the technique has also improved the precision for absolute concentrations and isotope ratios. The data in the table show the precision at the 95% confidence level obtained when a secondary standard was analyzed six times in duplicate. Isotope ratios are typically measured with a precision of 1-2% standard deviation.

Table I. Secondary Standard - 12 Analyses

	<u>Ave. ppm</u>	<u>Std. Dev.</u>	<u>% Std. Dev.</u>
^3He	224.8	1.7	0.8
^4He	588.2	3.0	0.5
^{22}Ne	1873.0	10.0	0.5
^{82}Kr	135.0	0.7	0.5
^{129}Xe	10.7	0.2	1.5
Ar	Balance		

*This work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

"CONTINUUM ACQUISITION":
A COMPUTER TECHNIQUE TO EVALUATE HIGH MASS PEAKS IN THE SPECTRUM

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A computer technique referred to as "continuum acquisition" can be extremely useful in determining the masses of relatively high molecular weight (>1500 amu) compounds and obtaining peak intensity data of sufficient accuracy that a peak cluster is defined well enough for the first meaningful peak in the cluster to be ascertained.

To use the technique, a limited portion of the spectrum, the molecular ion cluster, for example, is scanned repeatedly. If desired, these scans can be summed. Masses are assigned by comparing the positions of the sample peaks with the positions of one or two reference peaks acquired in two successive runs. The capability of summing scans enhances the signal to noise ratio and, consequently, dramatically improves the accuracy of the peak intensity data. With this enhancement the instrument can be operated at higher resolution with less concern over the subsequent loss of sensitivity.

A typical procedure to assign the MH^+ peak (m/z 2067) in the FAB spectrum of ristocetin A, a glycopeptide antibiotic, involves the following:

- Acquire a "continuum acquisition" spectrum of a standard compound (KI) over a mass range that includes both the ristocetin A MH^+ peak and a standard peak (m/z 2029, K_13I_2) that can serve as a reference.
- Acquire another "continuum acquisition" spectrum on ristocetin A using identical mass spectrometer scanning conditions.
- Use the position (time) and the mass of the reference peak in the standard spectrum and the position of the closest peak to it in the ristocetin A spectrum to assign the mass of the closest ristocetin A peak. Then, starting with this peak, simply count the ristocetin A spectrum to obtain the unit mass of the MH^+ peak.

An alternate procedure can be used for more accurate mass assignments. In this case the mass range scanned includes two reference peaks that bracket the sample peak which is to be mass assigned. The mass of the sample peak is then determined by interpolation either manually or with an available computer program. Two different runs using this procedure provided values of 2067.7 and 2067.8 for the MH^+ peak of ristocetin A. The correct value is 2067.7 for $C_{95}H_{111}N_8O_{44}$.

Another compound, a polypeptide--one of the adrenocorticotrophic hormone (ACTH) fragments (residues 1-24)--has been subjected to "continuum acquisition" in order to mass assign the MH^+ peak in its FAB spectrum. $Na_{20}I_9^+$ (m/z 2870.9) and $Na_{21}I_{20}^+$ (m/z 3020.9) cluster ions served as the reference peaks. Two different measurements produced the same value, 2932.9 amu, as the mass of the MH^+ peak. The correct value is 2932.6 for $C_{136}H_{211}N_{40}O_{31}S$. To produce sufficiently intense sample peaks the instrument tuning parameters had to be varied somewhat between the reference compound spectrum and the ACTH spectrum. These slight differences are possibly a factor in causing the error.

An example of the improved peak intensity data possible with "continuum acquisition" is evident in the spectrum of the molecular ion cluster of ACTH (1-24) (Figure 1). The molecular ion cluster (MH^+ , m/z 2932.9) of the compound is of very low intensity and one scan does not define it very well. As the number of scans increases, however, it is obvious that the cluster does become better defined, even to the point of enabling one to pick out the MH^+ peak.

This work was conducted in the FAB mode of operation on a VG ZAB-1F HF mass spectrometer equipped with a standard FAB ion source. The solid sample was mixed on the stainless steel probe in a glycerol/thioglycerol matrix containing a small amount of oxalic acid and examined at R. T. Accelerating voltages were 8 kV for ristocetin A and 7 kV for ACTH (1-24). 8 keV xenon atoms at a discharge current of 1 mA were used to bombard the sample.

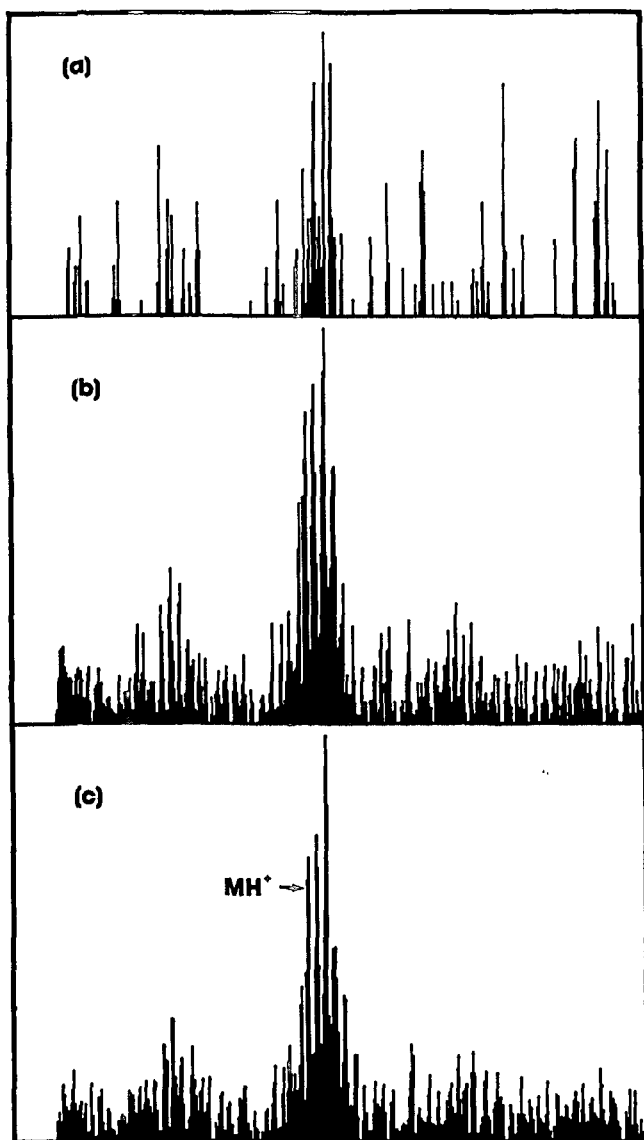


Figure 1. AETH (1-24) "continuum acquisition" FAB spectrum. (a) Single scan. (b) Fifteen scans. (c) Thirty scans.

REAL TIME DATA ACQUISITION SYSTEM FOR A TIME-OF-FLIGHT MASS SPECTROMETER

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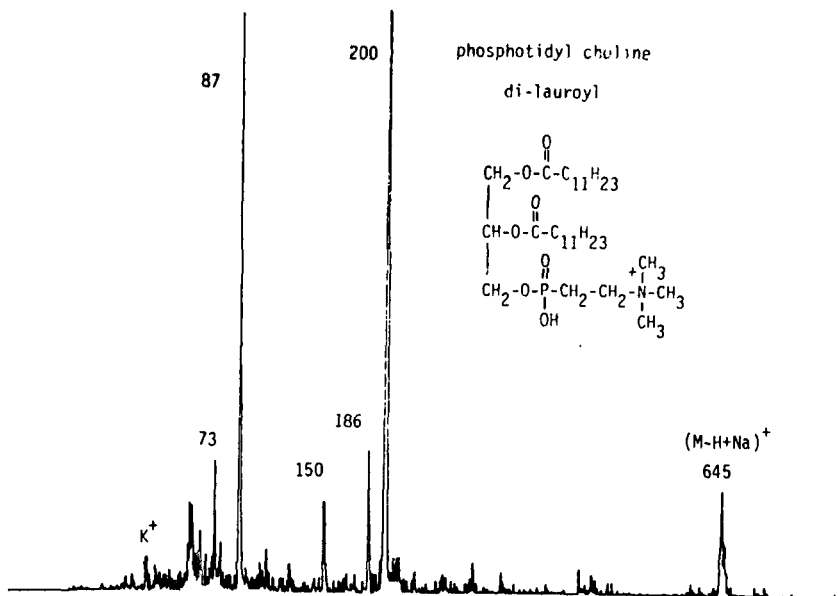
In conventional time-of-flight mass spectrometers, an entire mass spectrum is produced in 20 to 100 microseconds, and can be observed in real time on an oscilloscope display. Permanent recording of the spectrum is accomplished by photographing the single (or repetitive) trace, or by scanning a gated detector for many cycles. In the latter method, the recorded spectrum does not originate from a single ionizing pulse, but relies on repetitive ($\sim 10\text{KHz}$) ionization. Thus, this "analog scanning" approach is not suitable for those ionization techniques, such as laser ionization, in which low repetition rates, or even "single shot" events are used, or when spectra are time dependent, as in the case of pyrolysis or reaction kinetics studies.

The data acquisition system in our laboratory uses a transient waveform recorder, which acts as a fast (100 MHz) digital-to-analog converter/buffer interfaced to an 8-bit microcomputer. (1) Single or multiple real time spectra can be acquired and processed. Successive spectra can be added/averaged to improve peak shapes and increase dynamic range. Peak heights and areas can be computed for quantitative measurements. In addition, mass calibration, centroiding, and calculations of time and energy spreads can be accomplished. While the system is much less expensive than the data systems based upon minicomputers, which are offered commercially, its chief advantage is the ability to acquire time-dependent data.

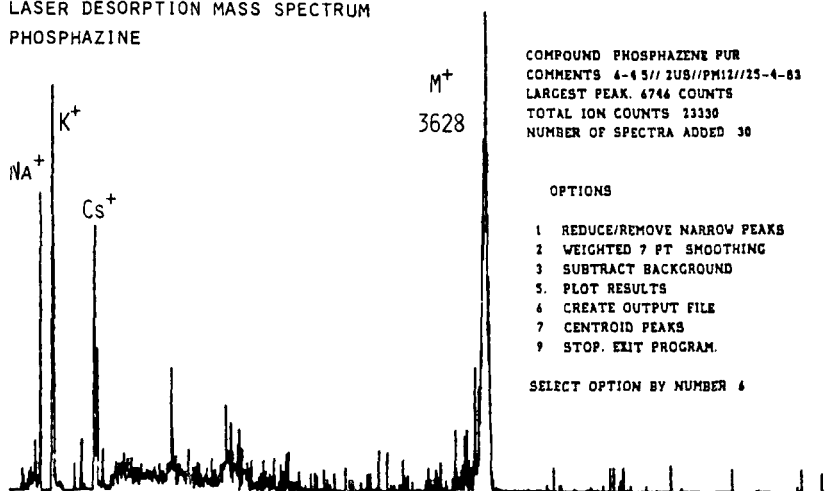
Mass calibrated laser desorption spectra (Figures 1 and 2) demonstrate the system.

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This work was funded by a grant, CHE 80-16440, from the National Science Foundation.



LASER DESORPTION MASS SPECTRUM
PHOSPHAZINE



TRANSIENT RESPONSE EXPERIMENTS FOLLOWED BY SIM MASS SPECTROMETRY

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The mechanism of a heterogeneous catalytic reaction can seldom be elucidated using only kinetic experiments under a steady state regime. The multitude of mathematical models that can be postulated will usually be screened to several that will fit the steady state experimental data equally well. In transient response experiments, the system is perturbed from one steady state to another by changing a variable (composition of the feed, temperature of reaction, pressure of reaction, residence time). Under gradientless conditions in the reactor, the changes with time of concentrations of reactants, products and intermediate species (surface species) can be described by the set of ordinary differential equations derived from the postulated elementary steps of the reaction. Transient responses data are usually more difficult to fit to several different models, therefore they are more informative than their steady state counterparts.

The major requirement of transient experiments is accurate, reproducible and reasonably dense data. Mass spectrometry is the best technique for obtaining the evolution with time of the concentrations of reactants and products in the gas phase. In this paper a system is described that consists of a magnetic sector mass spectrometer controlled by a microcomputer. The NUCLEIDE spectrometer houses 12-inch and 4-inch radius analyzers, contains total-ion monitor, two electron multipliers and Faraday cup collector. Continuous gas inlet to the spectrometer allows the analyst to monitor the variable composition of one of several gas streams, selected by a motor-driven rotary microvalve. The intensity at several mass/charge values can be followed simultaneously by means of stepwise changes of magnetic field and ion accelerating potential in a periodical fashion. These changes are commanded in real-time by a DEC Minc computer, which also reads ion current intensity data at each mass number and stores them appropriately. Optionally, the analyst can perform computer controlled transient experiments by including automatic valve actuation through an IEEE-488 interface.

The software was developed under DEC RT-11 and IBM CMS and MVS operating systems, and written in several languages to compromise efficiency with ease of use and modification by the analyst. Time critical operations, like spectrometer control and data acquisition were programmed in Macro-11 and Fortran, file handling, graphics and data handling were programmed in Basic to allow quick custom modification. All the programs are interactive, menu-oriented type. The graphics capabilities of the DEC VT-105 terminal were exploited in order to provide maximum visual interaction with the experiment performance. A complete experiment requires the following sequence: a) Identification of peaks of interest according to previous qualitative analysis of reaction products. This is performed by a program which also calculates the cracking coefficients of every component in the mixture. This task is usually performed once for each mixture or type of experiment. b) Calibration of the spectrometer with a standard mixture, which is usually done before every experimental run. c) Data collection and experiment control. d) Data communication to a large mainframe for further analysis. In this project a program was developed to transmit raw experimental data to an IBM 3081. This program can run automatically by execution of an optional command file. e) Analysis and plotting of data.

Figure 1 is an overview of the electrical system. The motor actuated valves are responsible for the production of steps or pulses of reactants sent to the catalytic reactor.

Figure 2 is the outcome of a step of methanol 3.5% in He over molybdenum oxide catalyst at 30 degrees C, followed by a step of He and temperature programmed desorption of methanol and products.

The system is very well suited for experiments where the transient response time constant is of the order of one second or more. Optimum performance can be obtained while tracing the concentrations of several components of close mass numbers, in which case peaks can be sampled every 0.25 seconds.

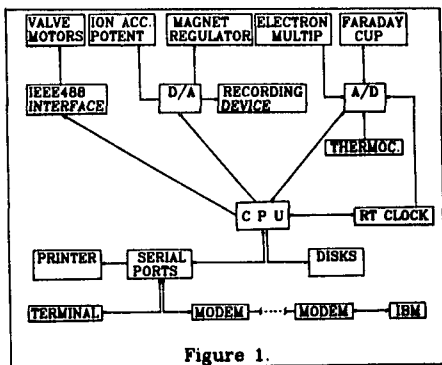


Figure 1.

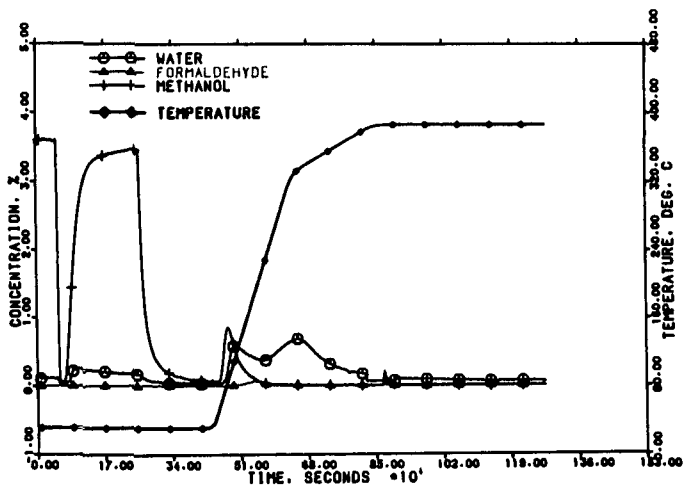


Figure 2.

CONTROLLING A TRIPLE QUADRUPOLE MASS SPECTROMETER WITH A MULTIPLE MICROPROCESSOR SYSTEM.

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A high performance and highly flexible data acquisition and control system was needed to further explore the characteristics of triple quadrupole mass spectrometry (TQMS) in our laboratory. A system of computer hardware was developed where several processors could operate in parallel. This system consists primarily of separate interfaces for transferring data, command and status information. The system was constructed using an Intel 8088 microprocessor acting as a system master and three Intel 8088 microprocessors acting as slaves. In such a distributed processing system each processor is assigned a specific task. Critical to the TQMS implementation is the separation of the tasks of data acquisition, ion path control, and peak finding into separate slave processors. The slave processors contain interfaces to all aspects of instrument performance: mass selection, lens potentials, collision gas pressures, etc. This modular approach leads to a system where each major section of the instrument control has its own dedicated intelligence. Figure 1 illustrates the interconnection of the master to the slave processors and the connection of the slave processors to the instrument. The horizontal arrows between the slave processors represent the status communication hardware used to synchronize the processors during scanning operations. The double ended arrows connecting the master to the various slaves indicates the path of data transfer where the master can directly access the memory in each slave. Finally, commands are transferred from the master to each slave through a first-in first-out (FIFO) buffer, depicted as a series of small boxes between the master and each slave in figure 1.

Software for the control system was written entirely in the programming language FORTH. Extensions were made to a standard FORTH system that allow the user to interact and program the system as a single unit rather than a series of isolated processors. FORTH routines can be developed and tested on the master processor (the only processor the user interacts with directly); thereafter, compiled FORTH code can then be downloaded into the appropriate slave processor. The master processor can then direct a slave to execute a particular routine by simply referencing the routine's name.

Figure 2 illustrates the increased throughput that can be achieved by the use of several processors in the place of a single processor. The data acquisition cycle can be divided into several separate tasks: setting of ion path potentials, acquisition of an individual data point, and data reduction in the form of peak finding algorithms. In a single processor environment each of these tasks is performed in series. In the multiprocessor environment these tasks are performed in separate processors allowing overlap of execution of the tasks as illustrated in the lower portion of figure 2. Parallel processing increases the amount of computing power per data point by a factor of two to three. In addition, different portions of the data acquisition cycle can be modified in one processor without affecting the code in place in the other processors.

This parallel processing system allows operations that are often implemented in hardware (for speed considerations) to be performed in software. For an instrument operating in the research environment the flexibility of a primarily software based system is a great benefit. In this implementation both the hardware and software become more modular, making it easier to implement and test different data acquisition, peak finding and scanning algorithms.

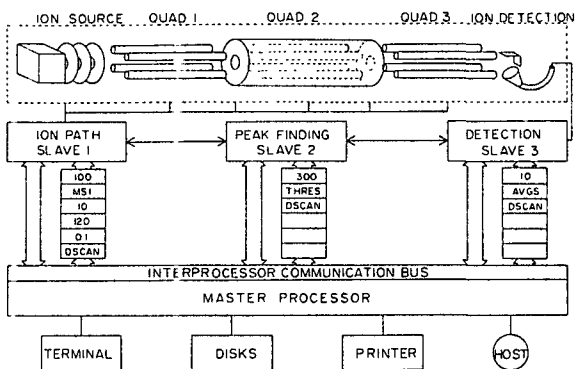


Figure 1

DATA ACQUISITION WITH A SINGLE MICROPROCESSOR SYSTEM



DATA ACQUISITION WITH A MULTIPLE MICROPROCESSOR SYSTEM

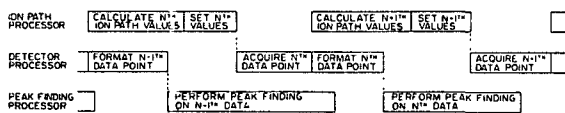


Figure 2

THE DEVELOPMENT AND PERFORMANCE OF A MULTI-PROCESSOR GC/MS DATA SYSTEM

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The data system associated with the Hewlett-Packard 5987 and 5996 GC/MS systems provides a multi-user, multi-tasking environment for the acquisition, evaluation, and presentation of mass spectral data. This product utilizes the Hewlett-Packard RTE-6VM operating system as the main, or host, computer. Multi-tasking is accomplished by priority timesharing and disk swapping of program modules present in one of several memory partitions. One large memory partition can be used for data evaluation by the RPN data processing environment. (1)

High speed data acquisition consistent with state of the art gas and liquid chromatography is provided by offloading the major responsibility for this acquisition to a satellite processor. (2,3) This "intelligent scanning interface" is designed around the Motorola 68000 based Hewlett-Packard Series 200 Desktop computer. The mass spectral data is double buffered and output via direct memory access (DMA) by the scanning interface to the host system. This data is received by a high priority program locked into one memory partition of the host RTE computer. The program ("D2TAC") writes the data to a mass storage device, using a large buffer to minimize disk I/O. This program has the primary responsibility for communicating with the scanning interface for data acquisition and instrument control. One other program ("GXGCC", where XX corresponds to the GC or instrument type) communicates with the scanning interface to invoke run table commands. Other programs (CSPRM for real time control, status, and display, RPN functions for evaluation of the current data) communicate indirectly to the scanning interface via blocks of system global area.

The modular design, and offloading the direct data acquisition to the scanning interface, results in a very low overhead for the data acquisition process. High data rates (less than 100 ms cycle times) can require 10 to 15 percent of the RTE host's memory cycles. In most cases, however, data acquisition generally uses five percent, or less, of the host's memory cycles. This scheme thus provides an effective multi-user, multi-tasking, and multi-instrument, GC/MS data system.

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2. Pearce, D.S., and Sakai, P.S., "A New Satellite Processor for GC/MS Data Acquisition and Control", Paper #589, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 1983.
3. Goodley, P.C., and Kenyon, C.N., "The Hewlett-Packard 5987A, A New GC/MS/DS Designed for Fast Eluting Capillary Peaks", Paper #366, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, 1983.

EFFICIENT USE OF CENTRAL MASS SPECTROMETRY FACILITIES. HIGH THROUGH-PUT, AUTOMATION, AND THE ROLE OF THE DATA SYSTEM. (A speculation to promote discussion). D.V. BOWEN, J. THORPE, D.V. CRO, Analytical Chemistry Department, Pfizer Central Research, Sandwich, Kent, CT13 9NJ, UK.

OBJECTIVE:

To provide a rapid mass spectrometry service to synthetic chemists, making the maximum use of capital equipment and releasing the skilled mass spectrometrists from routine samples.

DISCUSSION

The requirement that a mass spectrometry laboratory operates for at least 20 hours a day involves either shift work or preferably automatic sample handling, automatic operation of the mass spectrometer and automated interpretation of data.

The specific goals of such an operation should be:

1. to process 4 samples per hour at night.
2. to process 200 unattended samples per week.
3. to obtain E.I., positive C.I. and negative C.I. spectra for each sample.
4. to return all data on routine samples within 48 hours and on "urgent" samples within 12 hours.

Presently, the average time taken for an operator to run a sample, select, print and plot spectra is 13 minutes (range 6 - 18 minutes). During this time the operator is free for interpretation for 9 minutes (range 3 - 21 minutes). With a fully automated mass spectrometer, data system and interpretation system, a chemist will be able to log in a proposed structure and sample description at a computer terminal. The chemist will then present the sample, in solution, to the MS lab. The sample will then be placed into an automatic direct probe or LC/GC automated injector. The sample description will be called into the mass spectrometer and the sample run.

For both auto probe and belt type interfaces E.I., positive C.I. and negative C.I. spectra will be collected and representative spectra will be selected and flagged by the data system. The difference between the two sampling methods is that an auto probe gives controlled heating and therefore distillation of the sample into the source whereas the belt type interface gives overlapping spectra of all components. In the latter case the volatile impurities are lost during solvent stripping and the less volatile impurities may not be seen. Therefore the LC belt interface is more useful for pure compounds.

The automated interpretation could take place in one of three ways:

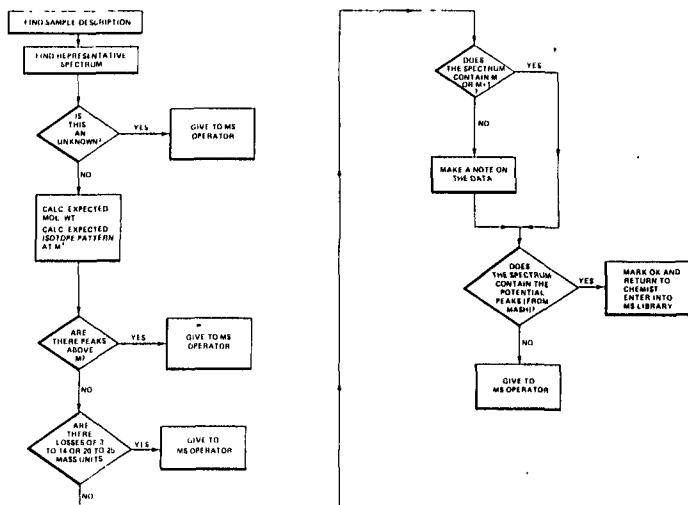
1. by library search - useful for known compounds.
2. by a self-training interpretative program (Cornell University STIRS program) - useful when the sample is a member of a known class of compounds.
3. by interpretation from the proposed structure (Kodak MASH program).

In the last case the computer breaks bonds in the compound to generate a spectrum of "potential m/z values". The computer then compares the observed and "potential" spectra making three tests:

1. most peaks in the observed spectrum must be in the potential spectrum.
2. many peaks in the potential spectrum must be in the observed spectrum.
3. there must not be peaks observed above the isotope cluster at the predicted molecular weight.

In conclusion, it is possible to automate a mass spectrometer to obtain spectra from "pure" samples. It is also possible to automate the interpretation of those data.

FLOW CHART



This flow chart shows how the computer will interpret the mass spectrum of a (supposedly) pure new compound of (supposedly) known structure.

MPB 23 | SPECIALIZED QUANTITATIVE AND RAPID
SCREENING SOFTWARE FOR DIRECT MIXTURE ANALYSIS BY MS/MS

Peter Gzowski, William R. Davidson and Takeo Sakuma,
SCIEX®, 55 Glen Cameron Road, Unit 202, Thornhill, Ontario,
L3T 1P2, Canada and Harry A. McLeod, Health Protection
Branch, Health and Welfare Canada, Ottawa, Ontario.

A software package, DESORB, has been developed for the TAGA® 6000 MS/MS system for the highly productive quantitative analysis of sample extracts for "target" compounds, as well as for the rapid screening of complex mixtures for low levels of contaminants. The program allows for the monitoring of eight parent ion/daughter ion pairs during the desorption cycle of the direct insertion probe. Each parent ion/daughter ion pair may be monitored using the optimum instrumental conditions for that particular fragmentation process as opposed to using a compromise set of parameters for all eight fragmentations. A two-stage thermal desorption temperature program provides flexibility in optimizing the desorption of a particular compound and can be used to allow some degree of separation of compounds during the desorption cycle.

Data analysis occurs immediately following the desorption cycle. Concentrations of the compounds of interest, branching ratios, and raw data are tabulated along with the desorption profiles for the eight ion pairs for a summary of the run. Sample turnaround time is generally less than one minute.

Quantitation may be achieved either from calibration curves, standard addition or internal standards. By entering the concentration of standards before desorption, a calibration curve may be obtained following a series of such desorptions. Functions are also available for averaging data, summarizing data, smoothing data and determining detection limits.

MPB 29 | AUTOMATED QUANTITATIVE DETERMINATION OF
TARGET COMPOUNDS IN GC/MS ANALYSIS; P. W. Ryan and
B. N. Colby; S-CUBED, Box 1620, La Jolla, CA 92038

A set of computer programs will be described which can be integrated into the INCOS/MSDS system to greatly increase the speed, convenience and effectiveness of quantitative target compound analyses. In designing this GC/MS data processing system, particular attention has been given to the essential delicate balance between high speed automated data processing and expert operator intervention. The programs provide diagnostic information to assist the operator in evaluating and adjusting the data reduction process, and they offer convenient opportunities to edit the partially reduced data where necessitated by ambiguous data or unusual circumstances.

Modifications to Facilitate Higher Mass Analysis on a Finnigan
MAT/Incos Mass Spectrometer System

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In recent years, increased emphasis has been placed on mass spectrometry for the analysis of labile nonvolatile higher molecular weight materials.¹ This is particularly true in the fields of biochemistry and polymer science. The advent of "soft" ionization/vaporization methods, especially field desorption and fast atom bombardment in the case of magnetic mass spectrometers, has greatly enhanced our ability to generate useful spectra at masses greater than 1000 u. This in turn has resulted in the need to develop facile techniques for computer acquisition and display of higher mass ions.

Many commercial mass spectrometer systems are still inadequately equipped to handle computerized data acquisition at masses ≥ 1000 u. We have modified the electronics of a Varian (now Finnigan) MAT 311A mass spectrometer to facilitate higher mass computerized data acquisition. At the same time we have modified the digital mass display for manual operation to give correct mass marking over the entire range of the magnet (1 to ~ 1500 u). It is felt that other users of similar equipment may wish to modify their systems to incorporate these improvements.

The MAT 311A was designed originally to operate in field stabilized mode only up to 1024 u (2^{10} in binary notation), despite the fact that the magnet can scan to much higher mass (~ 1500 u) at full 3kV accelerating voltage. Recently, Finnigan MAT introduced a modification to correct this deficiency.² This "Higher Mass Range" modification was written for the MAT 212/312 series instruments rather than for the MAT 311A. We found that our older circuitry required additional modifications to those specified in the Finnigan MAT publication. Readers who wish a circuit diagram and a detailed listing of the required changes may contact us for this information.

The net result of our modifications is that the digital mass display reads correctly over the full range of the magnet (~ 1 to 1500 u at 3kV accelerating voltage). The oscillograph mass marker also works properly over this range. Also, the Incos computer now can acquire data over essentially the full mass range in field mode. The computer is calibrated using a mixture of perfluorokerosene (PFK), tris(perfluoroheptyl)-s-triazine (mol wt 1185), and tris(perfluorononyl)-s-triazine (mol wt 1485). This mixture is introduced through the reference inlet held at 140°; ionization is by electron impact (70eV). A special reference mass table "MIX" has been set up for the calibration mixture. The Incos software allows one to extend the 3kV calibration to spectra acquired at lower accelerating voltages by editing the calibration table with routine "EDCT". EDCT multiplies all values in the 3kV calibration table by any appropriate factor. To illustrate the improved MAT 311A system, a mass spectrum of the calibration mixture is enclosed (Figure 1).

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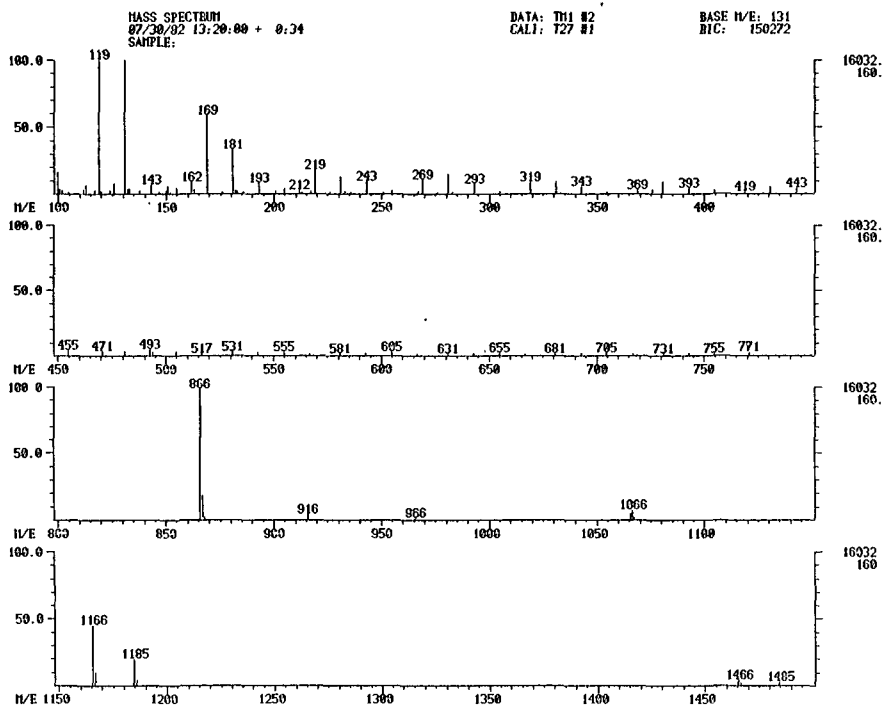


FIGURE 1
Electron Impact Mass Spectrum of Calibration Mixture

A NEW ALGORITHM FOR COMPUTING ISOTOPIC DISTRIBUTIONS

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The mass spectra of most molecules contain multiple peaks due to isotopic distributions of the constituent atoms. Examination of the relative intensities of these peaks is an aid in establishing the elemental composition of the ion and in validating the detection of certain compounds by GC/MS using MID. Many computer programs have been developed to calculate the isotopic pattern for an assumed chemical formula, but these programs are either limited to nominal mass calculations or they require substantial amounts of computer time.^{1,2,3} An algorithm has now been developed which can generate high resolution-high mass molecular or fragment ion distributions with very little computer time.

The efficiency of this algorithm is achieved by minimizing both the number of calculations for each composition and the number of compositions examined. The isotopic distribution for each element is calculated separately from the expansion of $(a_0 + a_1 + a_2 \dots)^m$. Each term has the form $A_{ijk} = \frac{m!}{i!j!k!} a_0^i a_1^j a_2^k$ where a_0 , a_1 and a_2 are the abundances of the isotopes, i , j and k are the number of atoms of each isotope and m is the total number of atoms of the element. It can be shown that $A_{i-1,j+1,k} = A_{ijk} \frac{a_1 i}{a_0 (j+1)}$ so that starting with the monoisotopic composition, $A_{m00} = a_0^m$, all remaining abundances can be calculated by simple multiplication and division without calculating any factorial or exponential terms. As the abundances are calculated the largest abundance is saved as AMAX and the total accumulated abundance is saved as TOTAL. A lower limit for the abundance, $AMIN = 10^{-4} * AMAX$, is updated each time a new AMAX is found. Compositions with abundance greater than AMIN are retained in memory and those below AMIN are discarded. When TOTAL reaches some limit, e.g. 0.9999, the calculations for the element are terminated. It is generally not necessary to calculate every possible isotopic composition for an element. After all the necessary compositions have been calculated, they are sorted in order of decreasing abundance and those with

abundance greater than AMIN are saved on disk for later use.

The molecular compositions are generated by combining the elemental compositions. Let $X_i Y_j Z_k$ represent the combination of the i^{th} composition of element X, the j^{th} composition of Y and the k^{th} composition of Z. $X_1 Y_1 Z_1$ is the most abundant molecular composition and has abundance IMAX. A lower limit, $IMIN = 10^{-4} * IMAX$, can be quickly calculated. While generating the possible combinations, if $X_i Y_j Z_k$ has abundance less than IMIN then $X_i Y_j Z_{k+1}$ need not be calculated and if $X_i Y_j$ is less than IMIN then no compositions with Z need to be calculated. As the compositions are calculated their contributions to a low resolution spectrum are also calculated.

This algorithm has been incorporated into a program written in FORTRAN for Data General Nova 4x computers. The information available from the program includes: the most abundant composition, the average mass of the ion cluster, the exact mass and abundance of the individual compositions, and a tabular listing and a bar graph display of the distribution of molecular ions for a specified chemical formula.

Acknowledgement: This work was supported by the Midwest Center for Mass Spectrometry, an NSF-supported regional instrumentation facility (Grant No. CHE 7818572).

1. Marilyn Brownawell and Joseph San Filippo, Jr. J. of Chem. Educ., 59, 663-665, (1982).
2. Edward Carberry and Bruce Mattson, program available from Quantum Chemistry Program Exchange, Indiana University, Chemistry Department, program No. 298.
3. J. Yergey, G. Hansen, D. Heller, C. Fenselau and R. Cotter, presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honolulu, HI, June 6-11, 1982.

SOFTWARE FOR THE STATISTICAL EVALUATION OF ACCURATE MASS SPECTRA FOR
MIXTURE ANALYSIS

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The use of accurate mass measurements in mass spectrometry is of significant value in the interpretation of spectra of mixtures due to the production of elemental compositions rather than nominal masses. Accurate masses may be derived from low resolution spectra, but high resolution is mandatory for the analysis of mixtures to eliminate unresolved multiplets which would produce incorrect accurate masses. However, in order to fully exploit the information in high resolution spectra, it is imperative that the quality of the measurements be characterized in detail. This would then provide error tolerance parameters that ensure inclusion of the correct elemental composition in the list of possible compositions for each accurate mass. This would also ensure that the list is no longer than the instrument performance warrants.

As the first phase of a study designed to develop new mass spectrometric methods for the analysis of complex mixtures, we have established a protocol for the detailed evaluation of the quality of mass measurements made at high resolution.

The first step in our evaluation scheme is the tuning of the mass spectrometer for a resolution of 10,000 in both the static, or peak switch mode, and dynamic, or scanning, mode using the characteristic ions of either perfluorokerosene (PFK) or perfluorotributylamine (PFTBA). Secondly, we acquire 200 or more high resolution spectra of a mixture of PFK and PFTBA over the entire mass range (50-750). This data set is then submitted to the mass measurement error statistics program, STAT.

STAT is a comprehensive statistics package designed specifically for high resolution mass spectra. The user specifies only the scan queue to be examined, test masses to be evaluated, and the error window in which to search for the test masses. The first part of STAT reads the scans from the disk and tabulates the measured mass, mass error, intensity, and peak width (number of points) at threshold for each of the test masses in each scan (see Table 1).

The second step in STAT is a tabulation of all the mass measurements errors as a function of mass and scan number. (Table 2) For each scan, the mean error, RMS error, and standard deviation of the errors are given to indicate the quality of that scan.

All the errors are summarized in a comprehensive table for the third part of STAT. Table 3 shows the listing of the evaluation mass, mean observed mass, mean error, standard deviation of the mean error, and the mean intensity for each test mass over the entire data set. The last column is the total number of scans in which the evaluation mass was found within the defined error window (usually 20 ppm). In Table 3, 200 scans were evaluated, with 8 of the 15 test masses from PFTBA shown.

Figure 1 shows the output of the last part of STAT. It consists of a plot of the errors in the form of a frequency histogram with one histogram plotted for each test mass and one for the overall composite error distribution. The histograms serve the purpose of giving the user a graphical display of the quality of the mass measurements by showing how the errors distribute around the mean error and the true error of 0 ppm. Positive and negative errors are shown in order to accentuate any systematic deviations in the distributions. With each histogram is output the Gaussian curve calculated from the mean error and the standard deviation, and the following parameters that describe the shape of the error histogram:

1. Skewness - degree of asymmetry about the mean. 0 is perfect.

2. Kurtosis - degree of "peakedness" of the distribution.
3. Chi-squared - measure of correspondence to a Gaussian curve.

The results of the STAT program enable the user to make quantitative evaluations of high resolution mass spectrometer performance. Some examples of applications of this statistical scheme are shown in Figures 2 and 3, where we show the composite histograms for different instruments and different scan rates at a resolution of 10,000. In addition to these fundamental uses, we have used this software to establish the mass measurement accuracy of a fast-scanning (3 sec/decade), high resolution (>10,000) instrument for analysis of limited quantities (which permit only 1 or 2 scans) of unique mutagenic fractions containing completely unknown compounds; this application will be presented in a subsequent publication.

Table 1

SCAN NUMBER	173	226	PEAKS	MISSED	0	PEAKS
EVALUATION	MASS	INTENSITY	THRESHOLD	ERROR	OBSERVED	MASS
	MASS	(X)	PK. WIDTH	(PPM)		
113	99699	25	61	25	-0.28	113.99699
175	99350	9	62	21	-2.99	175.99297
225	99031	5	58	21	1.66	225.99068
313	98393	3	69	21	-0.26	313.98385
375	98074	5	98	21	-0.44	375.98057
425	97755	18	26	35	0.57	425.97779
501	97117	22	74	29	-3.33	501.96950
613	96497	22	42	25	3.01	613.96654

Table 2

MASS	131	132	133
113.99699	0.10	1.17	4.33
175.99350	1.18	-0.31	-0.61
225.99031	-0.55	0.05	0.35
313.98393	0.16	3.56	5.34
375.98074	0.39	-0.09	4.32
425.97755	0.98	0.15	5.04
501.97117	2.15	2.39	-2.62
613.96497	-1.76	0.21	-1.46
RMS	2.15	1.50	2.47
MEAN	0.85	0.40	1.31
S.O.	2.04	1.50	2.53

Table 3

EVALUATION	MASS	MEAN	DEV	SD	INTENSITY	FOUND
	MASS	MASS	PPM	PPM	MASS	
113	99699	113.99675	0.57	1.70	5620	200
175	99350	175.99338	0.71	1.95	1903	200
225	99031	225.99027	-0.17	2.02	1066	200
313	98393	313.98436	1.37	2.88	919	200
375	98074	375.98092	0.48	2.68	1205	200
425	97755	425.97864	2.55	2.09	3609	200
501	97117	501.97110	0.13	1.59	5297	200
613	96497	613.96537	0.86	2.27	5722	200

Figure 2

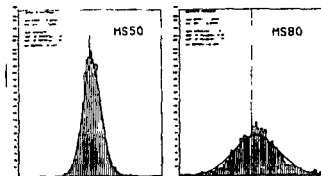


Figure 1

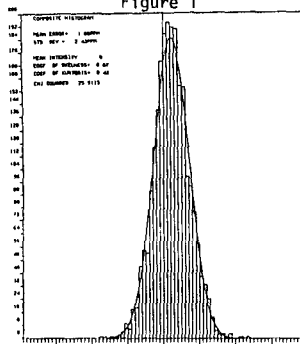
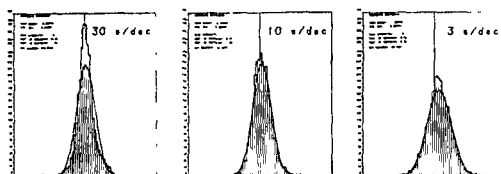


Figure 3



SIMPLE HIGH PRECISION MASS CALIBRATION/DIAGNOSTIC ROUTINES FOR QUADRUPOLE MS/MS
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Thornhill, Ontario, Canada, L3T 1P2

MASS CALIBRATION

A mass calibration function (MCAL) has been developed to operate with the TAGA® 6000 MS/MS Data System which offers an automatically sequenced calibration of Quadrupole 1 followed by Quadrupole 3. The design features of this software module are:

1. The user chooses any calibration compound suited to the ion source installed on the MS/MS system.
2. The number of calibration masses is operator chosen, ranging between a minimum of 2 masses and a maximum of 12 masses.
3. The instrument is fully controlled through the data system. As with all instrument parameters, the MCAL user has the flexibility of setting individual measurement controls such as the measurement time at each mass position and the threshold signal level. MCAL presets the mass scale increment for each step of the scan at .05 amu.
4. The maximum of the mass spectral peak is taken as the calibration data point. The calculation of the peak maximum is based on averaged intensities over a 1.6 amu window. The displayed spectral information is shown in a 4 amu window.
5. At the end of acquisition of the mass calibration data, the operator is provided with a split screen graphics display showing four normalized peak profiles, together with printout of ion intensity, peak width and peak shift from previous calibration.

The very simple user to software dialog is shown in Figure 1 together with an example of a Statement Table containing MCAL and using the TAGA COMMAND LANGUAGE. All MS/MS Scan Modes plus parameter changes and functions (such as MCAL and RUN) can be executed from the Statement Table. Figure 2 shows an actual example of a mass calibration of Quadrupole 1. Note that small intensity peaks will show more statistical variation than large peaks but are nevertheless suitable for use with this design of mass calibration. Note also that a perfect constant peak width set-up is not required nor is a high resolution necessary to find a good value of the peak maximum. This means that the operator can execute a mass calibration without excessive attention to tune-up or stability of signal from the calibration compound. For operator convenience and efficiency, the same parameter can be used for MCAL as used for detection and analysis of organics present in trace quantities.

The mass scale of each of the TAGA® 6000 quadrupole mass filters corresponds to the RF voltage of the power supply, which is computer-driven, through a high quality 16-bit DAC. In the ideal case, Mass DAC Value vs. Mass is a straight line passing through zero. In the actual case, the electrical hardware varies from unit to unit and the mass calibration compensates for small non-linearities by defining a series of straight line segments, shown graphically in Figure 3. Ultra-fine scan with averaging is achieved by setting STEP to .009155 amu, the increment associated with a single DAC unit.

DIAGNOSTICS

A number of diagnostic capabilities particularly suited to MS/MS are available, examples of which are given below.

Example One: The DPK function allows a fast look at any six parent-daughter pairs, prior to a data collection run. Following a simple entry of pairs of mass numbers, the operator is presented with a display similar to that shown in Figure 2. The dynamic signal range covered is $>10^6$, while a normalized display using preset 4 amu windows aids the operator to assess the information quickly. Without the DPK function, the user would have to set-up and run lines 10 to 50 of the Statement Table shown in Figure 1.

Example Two: The operator can design a diagnostic routine and store it in the user's library. In Figure 4 the declustering of reactant ions from the Atmospheric Pressure Chemical Ionization (APCI) Source is reviewed as a function of the voltage on a lens element. The parameter to be varied is designated as IP, has numerical values C1, C2, C3 etc. and is plotted on the X axis.

Example Three: Real-time tune-up of Quadrupole 1 and Quadrupole 3 is shown in Figure 5. The operator interrupts for parameter change (in this case, resolution) at any time. A circular buffer records the six most recent changes. The screen may be erased from a programmed key on the terminal. A logarithmic intensity scale is appropriate for this type of tune-up. Note that in some of the spectra of Figure 5, the valley of the peak is three orders of magnitude below the peak maximum.

```
TAGA? MCL 3,73,121,196,214,256,297,314,391
SAVE MCL CALIBRATION? (Y/N) _
```

```
TAGA? RUN 60,70
```

```
QUAD 1 SCRN      QUAD 3 SCRN
START END        START END
CT=ALN(ENH) VS=LUN(EN) TL
10 70 53 57 (YU=4000000) GR=TR
30 91 71 70 (YU=2000000) GR=TR
40 196 187 111 (YU=20000) GR=BL
50 196 67 71 (YU=20000) GR=BR
60 MCL 1,73,121,196,214,256,297,314,391
70 MCL 3,73,121,196,214,256,297,314,391
```

Figure 1 Operator to Software Dialog and Statement Table containing MCL, Using TAGA COMMAND LANGUAGE

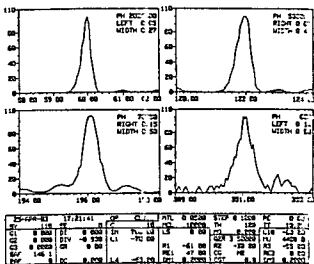


Figure 2 Mass Calibration Output - Quadrupole 1

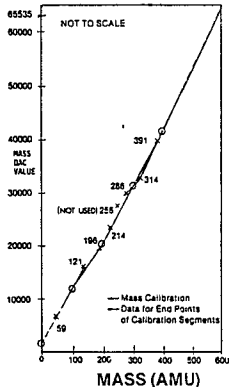


Figure 3 Calibration Line Segments used to drive the Mass DAC

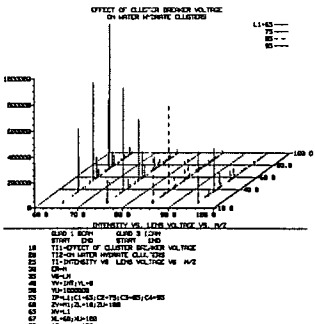


Figure 4 Review of declustering of resonant ions from APCL Source

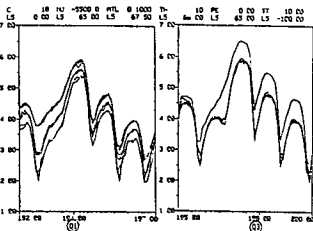


Figure 5. Real-time tune-up

QUOTIENT=SPECTRA, AIDING THE COMPARISON OF MASS SPECTRA AND THE EVALUATION OF SPECTRA SERIES.

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Definitions: The QUOTIENT-SPECTRUM of two spectra A and B is presented as "spectrum" in which the "peaks" at certain mass numbers represent the quotients $Q_m = a_m/b_m$ of the intensities in the spectra A and B after a normalization which centralizes the quotients around 1. The quotients Q_m are cut-off below 1/10 and above 10. A logarithmic scaling is chosen for the quotient spectrum setting $Q_m=10$ at the 100% level, $Q_m=1$ at 50% and $Q_m=1/10$ at 5%.

Features of Quotient Spectra: The most frequent deviations between mass spectral patterns arise from pressure changes during scan or from the influence of source- and instrument parameters. Both effects are continual changes resulting in some sort of "skewed" spectra. For source temperature and pressure effects this proved to be true at least approximately with the exception of molecular ions and ions from decompositions or (formally) decomposition-like fragmentations.

In consequence, the intensities in a quotient spectrum follow simple curves with slight curvature. By an iteration including a suitable test for outliers a BEST-FITTING PARABOLA is determined and the SAMPLE VARIANCE of the quotients relative to the parabola is then a MEASURE OF "IDENTITY" which is independent from the mentioned typical deviations. Therefore we use properties derived from quotient spectra to extend our library search SISCOM for similar compounds by an additional identity-ranking.

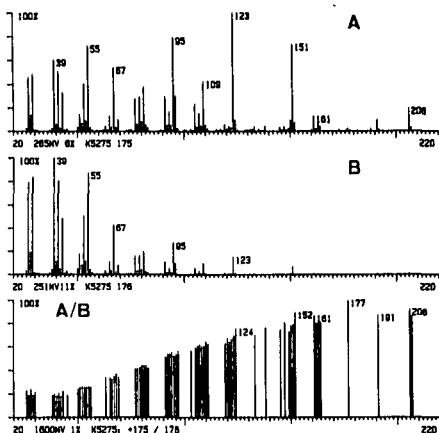


Fig.1 is an example of two spectra of one component taken before and after a GC-peak maximum. The shape of the quotient spectrum allows an easier and safer recognition of the "identity" than the original spectra. A slight modification allows to apply this type of identification to a reference spectrum and a mixture:

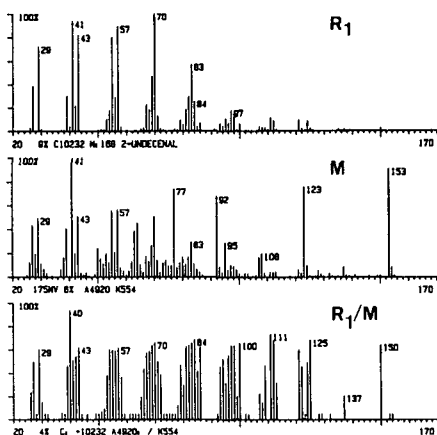


Fig.2 shows spectra of a mixture (M) and one of the components (R_1) together with the quotient spectrum R_1/M . The set of large quotients corresponds to the characteristic and almost unique peaks of this component, the quotients at 5% correspond to the respective peaks of the second component. Quotients between these belong to ions common to both components. In this type of application the parabola is fitted to the set of large quotients only.

Evaluation of GC/MS spectra series: Mass chromatograms, maximizing in different spectra of one GC-peak, can be due to unresolved components or pressure changes during the scan. Before an overlap correction can be carried out skewed spectra of one component must be excluded first because an erroneous overlap correction of such spectra results in wrong spectra.

Assessment of Duplicate Reference Spectra: Because quotient spectra are very sensitive to contaminations and irregularities they are well suited to find out reference spectra among duplicates which are suspect of being bad or wrong and even the correctness of small peaks in spectra is assessable if more than one duplicate exists.

Further applications yet under development are corrections of differently skewed spectra and mixtures of more than two components.

EXPLOITATION OF SSMS PHOTOPLATES
BY DIGITAL IMAGE PROCESSING

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INTRODUCTION

A spark source mass spectrometry spectrum, collected on photoplate brings to the analyst a large number of informations, the exploitation of which is composed of four major steps for quantization of species included in the analysed material :

- photographic plate digitalization
- ionic species identification
- photographic detector laws fitting
- concentration computation of identified species.

EQUIPEMENT

Digitalization and processing are performed on two distinct stations which exchange information through removable cartridge disks.

A first microcomputer ensures digitalization of the 15 exposures, all along the useful part of the photoplate. It monitors a high performance micro-densitometer (resolution = 1 micron, speed = 50 mm/sec).

The three parts of the processing are performed through a second micro-computer in connexion with a color graphic monitor, which is the most important terminal of this station.

DISPLAYED IMAGES (See illustration - next page)

The image displayed to the operator is composed of three subimages :

The 15 exposures of the acquired spectrum are visualized on the upper third of the screen, in a color range adapted to the dynamics of the image (optical density). The plate is viewed from above, in a mass range selected by the operator by dialogue.

A reference spectrum is displayed on the middle third of the screen, on the same mass scale as the analysed spectrum. It shows thin rays the color, height and position of which depend on the identity, natural abundance and position of simple ionic kinds that the mass spectrograph may transfer (MS 7 AEI).

The bottom third is reserved for drawing, on request, optical density profiles corresponding to the displayed photoplate section.

IDENTIFICATION (GRAPHIC ASPECT)

Ionic kinds identification is performed by comparison between patterns to be analysed with those presented on the reference spectrum.

....//..

The software manipulates image very fast (zooming, shifting ...), bringing the analyst quickness and flexibility to provide identification (pattern recognition, vertex research ...).

DETECTOR LAW DETERMINATION

The photographic detector response curves are built on the graphic display according to the law introduced by Hull. The man and computer dialogue entirely let the analyst fit the parameters of the law to experimental conditions.

CONCENTRATION - COMPUTATION

The analyst keys in the list of isotopes to be used for impurities concentration computation. The optical density at the top of corresponding lines (automatic location) is converted into ionic density through response laws. Computed concentrations are printed, and stored on disk.

CONCLUSION

This SSMS photoplates exploitation process brings both quickness and flexibility.

Quickness : a complete processing takes less than half an hour, including the 10 minutes for digitalization which does not require the analyst's presence.

Flexibility : the analyst is always master of the work (the computer is in the chemist's service).

In the form of optical density values stored in file, information is not debased by ageing as photoplates are. All intermediary results are saved on disk. The analyst can resume exploitation of a spectrum already processed, and benefit from previous results. From now on, all transfert function from the sample to be analysed to the image file of the photoplate, can be studied in an image and signal processing perspective.

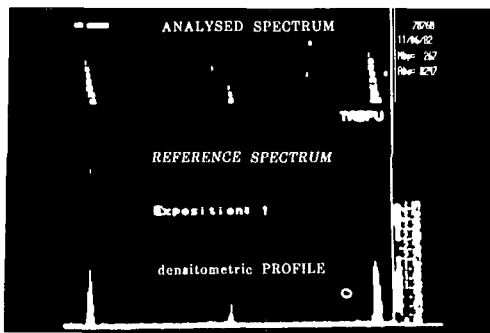


Image displayed on the graphic color monitor

UPGRADING A LARGE SIZE QUADRUPOLE GC/MS TO A TRIPLE QUADRUPOLE GC/MS/MS

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An R1010 GC/MS has been successfully upgraded to a triple quadrupole MS/MS by adding a quadrupole collision chamber followed by a second analyzer with its associated electronics and an additional diffusion pump.

It has not been necessary to modify the basic options of the R1010 (GC, solid probe, DCI, LC/MS inlet, 1500 amu or multiplier) in order to implement the upgrade. In addition, the same order of magnitude insensitivity is retained when one analyzer is used in either the positive or negative ion mode.

In order to test our system a standard "daughter ion" experiment was carried out by focusing one ion through the first analyzer, decomposing it in the collision chamber and scanning the second analyzer for the daughter fragments. The data was acquired using the SIDAR data system.

The critical parameters for collision activated decomposition (CAD), namely collision energy and collision chamber pressure, have been investigated. Our pumping system allows 10^{-1} Torr inside the collision chamber. The maximum collision energy used is ± 50 eV. After calibration and test runs on the calibration compound FC-43, as well as cyclohexane, a well known reference compound DMMPA¹ was subjected to MS/MS analysis.

The EI spectrum of DMMPA is shown in Figure 1.

Mass 164 was selected as the parent ion for obtaining the CAD spectrum. The analog display for this spectrum (Figure 2) shows unit mass resolution. In spite of the fact that M/Z 164 is not the base peak, and that unit mass resolution was used on both analyzers, excellent sensitivity was obtained. This is due to the fact that loss in sensitivity of CAD generated spectra is minimized when using a large size quadrupole.

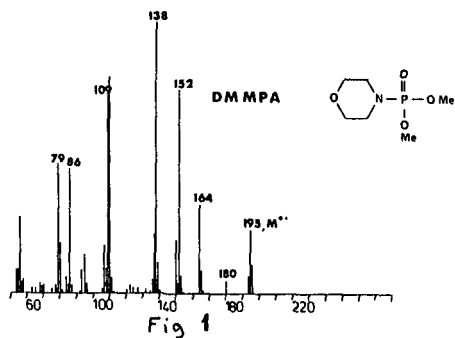
A known peptide was also subjected to MS/MS analysis using our DCI probe. Due to the inherent difficulty in obtaining molecular ions for peptides, the DCI probe was used. Figure 3 shows the CAD spectrum for the analyzed peptide. An intense MH⁺ was obtained at M/Z 628. In addition, daughter ions were obtained yielding information on the sequencing of this peptide.

Finally, the CAD spectrum of Iron (III) meso-tetraphenyl porphyrin chloride was obtained (Figure 4). It is important to note that although low energy collisions were used, it was possible to obtain a CAD spectrum for this high molecular weight compound.

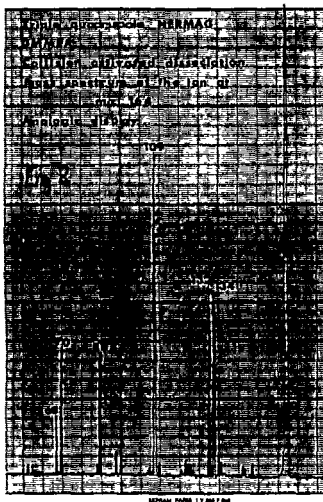
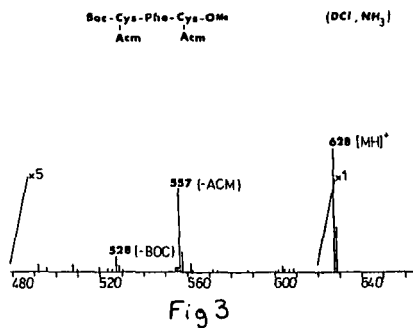
In conclusion, we have shown that it is possible to upgrade a standard GC-MS using a large quadrupole, without any alteration of its performance. Furthermore, excellent CAD spectra were obtained with unit mass resolution and minimized loss insensitivity.

¹ P.H. DAWSON et al ORGANIC MASS SPECTROMETRY 17 212 (1982)

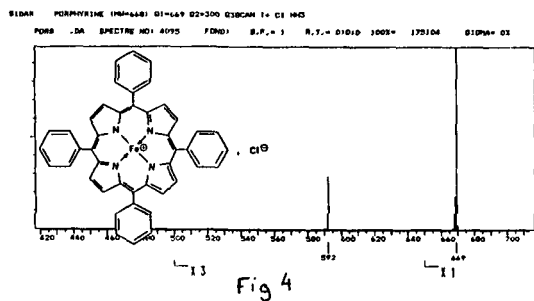
El (70eV) mass spectrum of DMMPA



Collision activated dissociation mass spectrum of the $[MH]^+$ ion, m/z 628, from



IRON(III) MESO-TETRAPHENYL PORPHYRIN, CHLORIDE



MS/MS AND THERMAL DESORPTION/IONIZATION. THE IDEAL COMBINATION?*

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It has been stated by numerous researchers that the "ideal" ionization method for use with MS/MS would produce one and only one ion for each component in the sample, with that ion being structurally representative of the neutral precursor. The advantages of such an ionization method are two-fold. First, by producing only one ion per component you are sure that the ion is representative of a component in the sample and not a fragment ion from a higher mass ion. Second, sensitivity will be increased for the MS/MS experiment since all the ion current for a given component will be concentrated in one ion and not dispersed among several fragment ions.

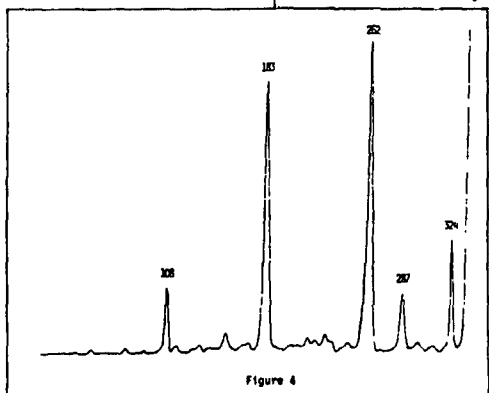
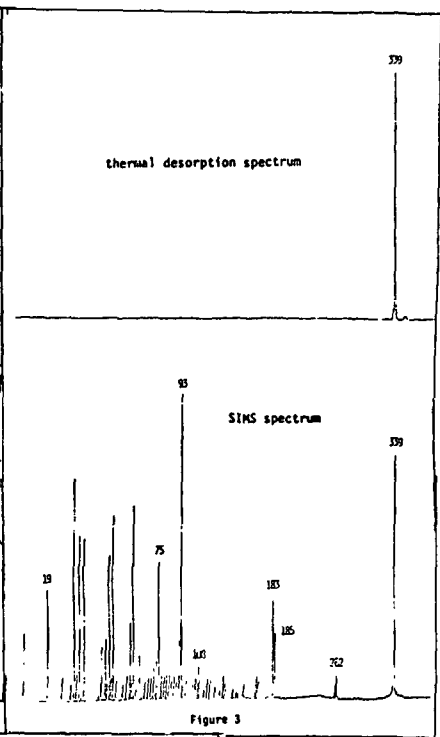
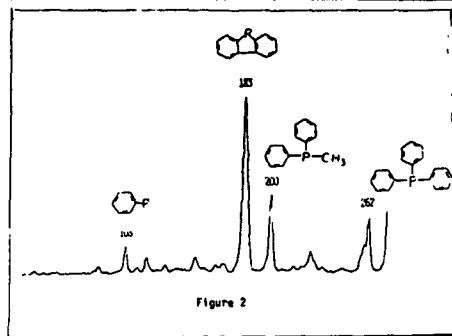
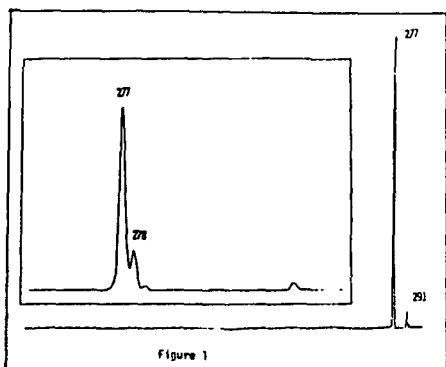
For compounds amenable to thermal desorption/ionization, the above conditions are met under appropriate control of the experiment. Intact cations for quaternary ammonium, phosphonium and other organic salts can be desorbed thermally, and at appropriate (i.e., relatively low) temperatures only the intact cation is observed. In addition, contrary to popular belief, the intact salt molecule can be evaporated. This can then be ionized at high filament temperatures to give, again, the intact cation.

Figure 1 shows the thermal desorption mass spectrum of triphenylmethylphosphonium bromide. Besides the intact cation at m/z 277, only a small peak due to an impurity of the ethyl analog is observed. Figure 2 shows the MS/MS spectrum of m/z 277. The major peaks are very characteristic of the structure of the cation with 183 being a common peak seen in the mass spectra and MS/MS spectra of multi-phenyl substituted phosphorous compounds.

Figure 3 compares the mass spectra of tetraphenylphosphonium bromide obtained by thermal desorption and SIMS. Structurally significant fragments can be seen in the SIMS spectra and, in addition, an abundance of "chemical noise" due to the glycerol matrix is evident. The MS/MS spectrum of the thermally desorbed intact cation of tetraphenylphosphonium bromide is shown in Figure 4. In addition to the structurally significant peaks, a peak at m/z 324 corresponding to methyl loss is observed. While such fragmentations are unusual, they are not to be unexpected and will complicate interpretation of MS/MS spectra.

Thermal desorption/ionization seems to be very close to the "ideal" ionization mode for use with MS/MS. Conversely, MS/MS is almost a necessity with thermal desorption if structural information is desired. Much more work needs still to be done to find the range of application of thermal desorption/ionization, but for the compounds now known to be amenable to such ionization, the combination of thermal desorption/ionization with MS/MS does indeed seem to be "ideal".

*Research sponsored by the U. S. Department of Energy, Division of Basic Energy Sciences, under Contract W-7405-eng-26 with the Union Carbide Corporation.



MPC 3 | INITIAL EXPERIENCES WITH THE ZAB-3F MASS SPECTROMETER; J. M. Gilliam and J. L. Occolowitz; Lilly Research Laboratories, Indianapolis, Indiana 46285

The geometry of the ZAB-3F (B₁EB₂) mass spectrometer together with the modes of ionization available on our instrument (EI, CI, and FAB) result in the ability to perform various MS/MS experiments on a wide variety of precursor ions. For MS/MS experiments the two most notable modes of operation are:

- a. Preselection with magnet B₁ at moderate resolution, followed by mass analysis of ions resulting from collisions in the second FFR using a linked scan of the electrostatic analyser and magnet B₂ and
- b. Preselection with B₁ and E, at moderate to high resolution, followed by mass analysis of ions resulting from collisions in the third FFR using a scan of B₂. In this mode resolution for ions entering B₂ can be enhanced by lowering the collision energy and reaccelerating after collision.

For this presentation we will compare the MS/MS resolutions and sensitivities obtainable in each mode with each other and with those obtainable from other instrumentation. We will also discuss the variation of resolution, sensitivity, and fragmentation with ion collision energy used in the second mode.

MPC 9 | USE OF A COLLISION CELL AS A "CHEMICAL FILTER" IN FAB MASS SPECTROMETRY AND OTHER APPLICATIONS. D.I. Carroll and E.C. Horning, Institute for Lipid Research, Baylor College of Medicine, Houston, TX 77030.

The use of a collision cell as a reaction chamber to serve as a "chemical filter" to remove MH⁺ ions or adduct ions associated with weakly basic molecules, while transmitting MH⁺ ions from strong gas phase bases, was studied experimentally with a triple quadrupole mass spectrometer. In a series of experiments using tetrahydrofuran (THF) as the proton acceptor [proton affinity (PA) 196 kcal/mol] in nitrogen, it was found that the transmission of ions was reduced by only about 10-20% as the collision cell pressure rose to 20-30 millitorr and that equilibration occurred at these pressures. With the first quadrupole operated in the RF only total transmission mode, MH⁺ ions for methanol (PA 182 kcal/mol) or glycerol and N,N-diethylmethylamine (PA 226 kcal/mol) were sent through the THF reaction cell. At low cell pressures, 1-10 millitorr, the products were those expected from collision induced dissociation reactions. At 20 millitorr, ions from methanol or glycerol were no longer present, but MH⁺ ions from the amine were still transmitted. The decrease in total amine ion current under these conditions was about 15%. In effect, the THF reaction chamber acted as a "chemical filter" to remove ions of compounds with basicity below that of THF.

Many current applications of FAB ionization procedures involve strong gas phase bases (for example, peptides and nucleosides). It is expected that matrix ions and noise can be eliminated from FAB spectra by use of a reaction chamber sited before the mass analyzer and operated at 20 millitorr with appropriate reagents in nitrogen.

AN E-Q SPECTROMETER FOR INVESTIGATING DISSOCIATION OF IONS.

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Our knowledge of the properties of organic and other ions has been greatly enhanced in recent years by studying their dissociation in field-free regions of sector mass spectrometers. This work has been hindered in many instances because fragment-ion peaks are broad and fragment-ion spectra can thus only be measured at low resolution. The broadness arises because of the translational energy released when an excited polyatomic ion dissociates which is considerably amplified in the laboratory frame of reference if the velocity of the parent ions is high. Whilst this allows accurate measurement of peak shapes from which valuable information on the distribution of translational energies released can be determined it can also give rise to overlapping peaks and loss of information on some of the dissociation reactions.

In order to overcome this problem we have designed and built a new type of spectrometer with which ion-kinetic-energy peak shapes can be recorded with high accuracy and which also allows fragment-ion spectra of pre-selected parent-ion species to be determined with unit-mass resolution. Its main components are an ion source, an electric sector and quadrupole mass filter. The source and electric sector are refurbished parts of an MS9 spectrometer and the quadrupole mass filter is the model MM12-12S manufactured by VG Analytical Ltd. The source is operated at 5 kV and unimolecular or collision-induced dissociation occurring in the first field-free region can be studied. By scanning the voltages applied to the electric-sector plates the fragment ions can be transmitted in turn through the sector. Y and Z deflectors then steer the beam and a Z focusing Einzel lens focuses it into a deceleration region. For the quadrupole to work satisfactorily as a mass filter it must operate on slow-moving ions, i.e. having less than 15 eV translational energy. In all the work with the system, therefore, a voltage V_Q is applied to the quadrupole which is derived from a programmable 5 kV supply driven by the same reference voltage as that used to drive the electric-sector supply. V_Q is adjusted so that it just prevents the beam from entering the quadrupole; a 15 V offset is then introduced so that ions having 15 eV translational energy enter the quadrupole. Any ions transmitted by the electric sector have a translational energy which is determined by the voltage applied to the electric-sector plates. The system just described ensures that ions leaving the sector always have their translational energies reduced to 15 eV before they enter the quadrupole.

Various scanning procedures are used to obtain the information listed below.

(a) Mass Spectrum

With the electric sector set at a value E say, all stable ions leaving the source are transmitted by the electric sector. Because the quadrupole is appropriately biased all the ions have their energy reduced to 15 eV before entering the quadrupole. By scanning its mass range the quadrupole transmits each ion species in turn and a mass spectrum is obtained.

(b) Fragment-ion spectrum resulting from dissociation of ions of a pre-selected species

If the dissociation, either unimolecular or collision-induced, of an ionic species of mass m_1 is to be studied, the spectrometer is set up so that m_1 ions are transmitted by the quadrupole when the electric-sector voltage is set at E . The mass setting and electric-sector voltage are then scanned linearly to zero in the same time. Daughter ions m_2^+ resulting from dissociation of m_1^+ ions will be transmitted through the sector over a

range of voltages around $E^1 = m_2 E / m_1$. The quadrupole, however, will only transmit m_2^+ ions at the centre of this range and to either side of the centre depending on the resolution of the quadrupole which is at least unit-mass. Thus, as scanning proceeds, a spectrum of all fragment ions formed from the pre-selected parent ion species m_1^+ will be obtained. The spectrum will have unit-mass resolution and a linear mass scale as a function of time.

(c) Spectrum of all parent ions which dissociate to give the same species of daughter ion

To obtain this spectrum, the quadrupole is set to transmit pre-selected daughter ions m_d^+ and the setting remains unaltered. The electric-sector voltage is scanned linearly from E to zero. Peaks due to the transmission of m_d^+ ions will appear at certain electric-sector voltages E^1 which correspond to various parents emitted from the source which dissociate in the first field-free region. Knowing the electric-sector voltages E^1 at which the peaks occur allows the calculation of the masses of the parents through the relationship $m_p = E m_d / E^1$.

(d) Spectrum of all parent ions that result in the loss of a neutral fragment of pre-selected mass

Let the neutral fragment considered in this type of scan have a mass N . A daughter ion is transmitted by the electric sector at a voltage

$$E^1 = E m_d / (m_d + N) = E / (1 + N / m_d)$$

The quadrupole is scanned so that the mass transmitted varies linearly with time. The electric-sector voltage E^1 must, however, be scanned to satisfy the above relationship. Knowing the peak positions on the m_d scale allows the identification of the parent ions which lose a neutral fragment N in dissociation reactions.

(e) Ion-kinetic-energy fragment-ion peaks for chosen dissociation reactions undistorted by ion currents from other reactions

If a known dissociation reaction is to be studied the quadrupole is set to transmit the daughter ions of mass m_d and, if the parent ions have mass m_p , the electric-sector voltage is scanned in the region $E^1 = E m_d / m_p$ to give the ion-kinetic-energy fragment-ion peak. Analysis of the peak shape gives information on the translational energy released for the specific reaction studied.

This procedure allows all the peaks in the ion-kinetic-energy spectrum to be deconvoluted from one another and has been used successfully to produce resolved spectra from samples including phenetole, n-butylbenzene and m-chlorophenol.

MODIFICATION OF AN AEI MS-9 INTO A HYBRID MS/MS

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Applications of Mass Spectrometry/Mass Spectrometry (MS/MS) for qualitative and quantitative analysis of complex sample matrices have experienced a rapid growth during the past several years. This technique has been used to solve environmentally significant problems, and more recently, analyses of drugs and drug metabolites in biological fluids.

To explore the utility of MS/MS, an AEI MS-9 was modified into a hybrid MS/MS. The modifications included the design and fabrication of a collision cell, deceleration lenses, focusing lenses, and a vacuum housing in which these components, as well as a quadrupole mass filter were installed. These modifications were placed after the magnetic sector of the original MS-9, resulting in an instrument of EBQ geometry. In order to install these modifications it was necessary to elevate the tube unit 18 inches from the floor. Additional modifications included the addition of a solid state magnet power supply, protection circuitry, and the electronics necessary to control the quadrupole mass filter.

The resulting EBQ instrument provides a relatively high parent ion resolution (5000, 10% valley) thus allowing for elimination of possible interferences. Furthermore, it allows rapid scanning of the third sector of unit mass resolution. In addition, this instrument is equipped with an SRI chemical ionization source which is capable of performing in an electron impact or chemical ionization mode.

A FIELD IONIZATION MS/MS INSTRUMENT

By

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INTRODUCTION

The purpose of this project was to design, assemble, and demonstrate a field ionization mass spectrometer/mass spectrometer (FIMS/MS) system. An SRI volcano style field ionization source was used to obtain maximum sensitivity. In order to minimize cost, an existing quadrupole mass filter and 90 degree magnetic sector were combined into an FIMS/MS instrument with QB geometry.

INSTRUMENT DESIGN

With the available components, both QB and BQ configurations were possible. The QB geometry was chosen for the following reasons:

1. The ion source is operated near ground potential simplifying sample introduction from a variety of inlets such as leak valves, gas chromatographs, and solids probes.
2. The ion/neutral collision energy can be varied over a wide range while maintaining very high transmission efficiency through the second MS.
3. Ions are generally accelerated to higher energy as they move through the instrument from the ion source to the final detector, making it easier to keep the beam small and well focused.

A scale drawing of the completed QB FIMS/MS is shown in Figure 1. Ions produced by the SRI field ionization source are focused and steered into the Extranuclear 3/4 inch quadrupole rod assembly. Sharp focusing and precise positioning of the ion beam is essential for obtaining high transmission of high mass ions through the quadrupole.

Ions exiting the quadrupole are extracted by an electrostatic octapole lense which both compresses the beam diameter and helps to restore it to a circular cross section. Following acceleration to the selected collision energy, the ion beam enters the first of two sets of dual electrostatic quadrupole lenses. Here it is compressed horizontally and extended vertically into a ribbon-like cross section to improve the overlap with the neutral gas jet and to increase its transmission through the slits of the magnetic sector. Beam steering is also provided to assure that the beam is exactly aligned with the subsequent ion optics.

The collision cell is actually composed of three independent regions which can be mechanically moved into alignment with the ion beam. The center region contains a neutral gas jet fabricated from a short length of 0.007 inch ID stainless steel tubing. Large slots were cut in the top and bottom of this region to allow excess collision gas to escape. When this region is aligned with the ion beam, the estimated conductance to the collision region diffusion pump is 30 l/s. By moving the gas jet across the ion beam while monitoring the signal from a fragment ion, it is possible to demonstrate that about one-half of the fragments are formed as a result of collisions with gas in the jet and one-half are caused by collisions with gas molecules which are inside the collision cell but not in the jet itself.

The collision cell contains a second region which encloses a deflector plate for directing the ion beam into an off-axis Channeltron ion detector. The third region of the collision cell is for experiments with novel methods of fragmentation of the mass selected molecular ion beam. Experiments with electron impact fragmentation have been started during the last few weeks.

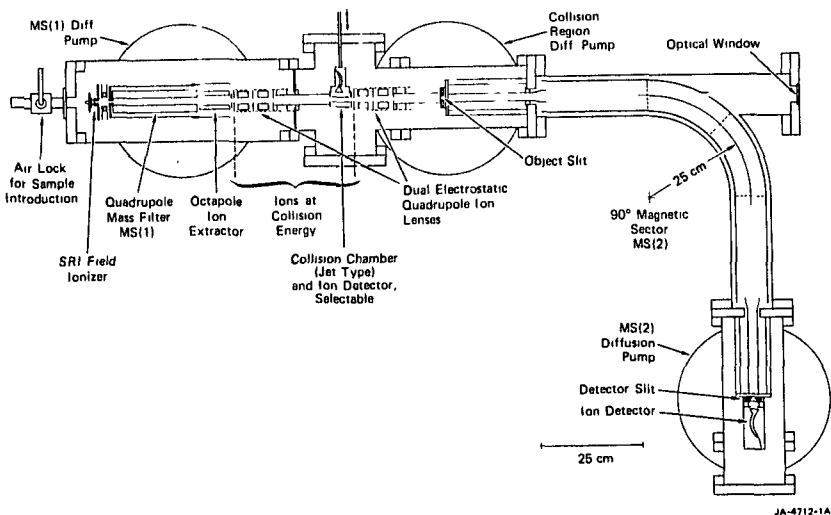
Following the collision chamber, the ions are accelerated to the final analysis energy through a series of three slits, each 0.04 inches wide. The ion beam is then refocused onto the entrance slit of the 90 degree magnetic sector using the second set of dual electrostatic quadrupole lenses (Figure 5). Ion energy for unfragmented molecular ions is variable up to 5 kV but has been most often set at 3 kV. Because the entire vacuum system is at ground potential, it was necessary to completely enclose the ion flight path with an electrically isolated shield biased as the appropriate potential. The liner for the flight tube is constructed of 0.019 inch stainless steel in four separate sections which can be inserted into the flight tube from one end. A side arm on the magnetic sector flight tube is fitted with an optical quality window which makes it possible to use a small He-Ne laser to facilitate the mechanical alignment of the entire ion optical train.

Ions leaving the magnetic sector pass through the variable width detector slit and directly into the continuous dynode (Channeltron) multiplier. The multiplier is normally operated in the pulse counting mode with the output a ground potential.

SENSITIVITY

Because the overall transmission efficiency of the FIMS/MS ion optical system is high, the sensitivity of the instrument is almost as great as our FIMS instruments. Measured overall ionization efficiency is one ion per million molecules for perylene molecular ion at m/z 252. In other words, one mass resolved molecular ion is detected by the second MS for each one million molecules placed on the solids sample probe when both mass analyzers are operated at resolutions of better than 250.

In single reaction monitoring, the quadrupole is set to transmit the molecular ion of interest and the magnetic sector is tuned to a characteristic fragment ion for the specific compound. For a compound of molecular weight 200 which has a fragment ion with an intensity of one percent of the molecular ion signal, a sample of 330 μg is required to yield a signal of one thousand ion counts at the detector of the second MS.



SRI FIMS/MS (QB Type)

DESIGN OF A THRESHOLD PHOTOELECTRON-SECONDARY ION COINCIDENCE SPECTROMETER WITH A TWO-CHAMBER SOURCE

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Information on the energetics of gaseous ions below their dissociation thresholds is necessary to interpret fragmentation patterns quantitatively, particularly when fragmentation is preceded by isomerization whose threshold energy is below the fragmentation onset.

One way of investigating structures of ions below their thresholds for dissociation is to utilize ion-molecule reactions which are specific to structure. For example (1), the acyclic isomers of benzene react by charge exchange with 2-propyl iodide but give no detectable condensation products:



This is reversed for $C_6H_6^+$ derived from benzene, where reaction [2] is the only significant mechanism.

Ion-molecule reactions are very helpful in the measurement of reaction cross-section in state-to-state reactions (2). Ion-molecule reactions are particularly suited for such investigations since primary ions with various internal energies can be readily prepared by ionization (3). The previous techniques used in such studies, however, had considerable ambiguities on the knowledge of relative population of the states of the reactant ions produced. The threshold electron-secondary ion coincidence technique can overcome this problem and makes it possible to measure state-selected reaction cross-sections (4). Moreover, ion-molecule reactions are sensitive to structure, and so this technique can aid in the determination of structures of molecular ions below their unimolecular dissociation thresholds.

We have therefore designed a threshold photoelectron-secondary ion coincidence spectrometer to investigate chemical dynamics of ion-molecule reactions and the structures of gaseous ions below their dissociation thresholds. Two main types of experiments are planned: (a) The measurement of relative reaction probability as a function of ion internal energy at a fixed collision energy (E_c); relative cross-sections can be calculated from the ratio of peak areas in the coincidence time-of-flight (TOF) spectra. (b) Elucidation of collision energy dependency of ion-molecule reaction probability, (σ versus E).

Figure 1 shows the diagram of our apparatus. It consists of a threshold photoelectron energy analyzer and a time-of-flight mass spectrometer with a two-chamber source. The selection of zero kinetic energy electrons is accomplished by the combination of a collimated hole structure (CHS) and a Bessel Box analyzer (BBA), which serves to reduce the effect of nonzero kinetic energy electrons transmitted through the CHS (5). The BBA is adopted as the additional energy selector because it is compact, easily constructed and has good collection characteristics.

Primary ions are produced by monochromatic photons, are extracted, and enter the second or reaction region. Secondary ions resulting from collision with the target gas in the second chamber are extracted, separated by their time-of-flight characteristics and detected by the channeltron multiplier. Threshold photoelectrons ejected in the same events pass through the CHS and the BBA and are detected by a separate channeltron multiplier. The electron and ion signals initiate the start and stop pulses, respectively, of a Time-to-Pulse-Height Converter (TPHC). The output of the TPHC is sorted in a Multichannel Pulse Height Analyzer (MCA) which thus produces a TOF mass spectrum.

The spectra expected for the reaction:

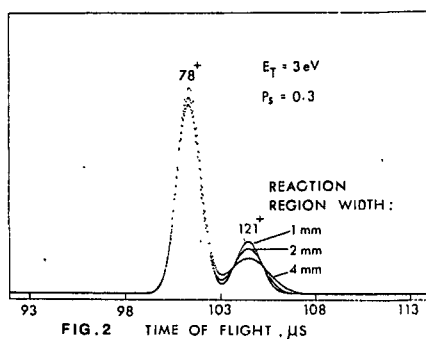
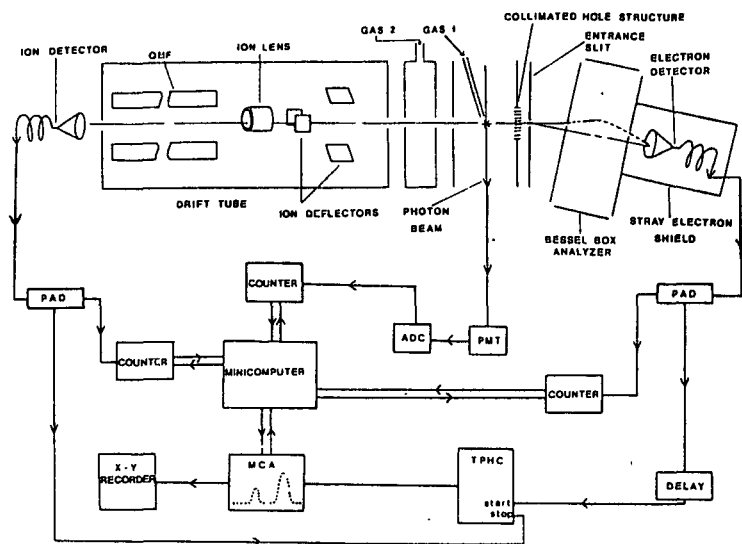


were simulated on the basis of the spectator model. Figure 2 demonstrates the effect of ion-molecule reaction region width on the shapes of the primary and secondary ions' TOF peak shapes. Calculation was done using a collision energy of 3 eV and a 30% probability of primary to secondary ion conversion by ion-molecule reaction.

Acknowledgments: We thank Dr. Theo van Kalleveen and Dr. J.P. Gilman for many helpful discussions, and the Department of Energy for support under contract number DE-AC02-76ER02567.

References: (1) M.L. Gross, D.H. Russel, R.J. Aerni, and S.A. Bronczyk, *J. Am. Chem. Soc.*, **99**, 3603 (1977). (2) (a) R.D. Levine and R.B. Bernstein, *Molecular Reaction Dynamics*. Oxford: Clarendon Press, 1974; (b) M.A.D. Fluendy and K.P. Lawley, *Chemical Applications of Molecular Beam Scattering*. London: Chapman and Hall, 1973. (3) P.L. LeBreton, A.D. Williamson, and J.L. Beuchamp, *J. Chem. Phys.*, **62**, 1623 (1975). (4) (a) T. Baer, P.T. Murray, and L. Squires, *ibid.*, **68**, 4901 (1978); (b) I. Koyano and K. Tanaka, *ibid.*, **72**, 4858 (1980). (5) T. Hsieh, J.P. Gilman, M.J. Weiss, G.G. Meisels and P.M. Hierl, *Int. J. Mass Spectrom. Ion Phys.*, **36**, 317 (1980).

FIG. 1 TPESICO INSTRUMENT WITH A TWO-CHAMBER SOURCE



TANDEM DOUBLE-FOCUSING MASS SPECTROMETRY; I. J. Amster, M. A. Baldwin, S. L. Cohen, P. O. Danis, D. C. McGilvery, C. J. Proctor, and F. W. McLafferty, Chemistry Dept., Cornell Univ., Ithaca, NY 14853

A variety of improvements to this instrument at Cornell (JACS, 1980, 102, 3360; 1983, 105, 1654) have given increased sensitivity, resolution, and ease of operation, especially for high mass ions. The new magnet can mass analyze 7 keV ions of mass 15,000 ($\text{Cs}_{58}\text{I}_{57}^+$). Linked scanning of the second electrostatic analyzer and magnet has demonstrated a resolution of 1500 in MS-II, allowing the separation of the isobaric ions C_4H_9^+ and $\text{C}_3\text{H}_5\text{O}^+$.

This MS/MS instrument has proven to be of particular value for the identification of individual components in impure samples, separating the molecular parent ion of the components in MS-I to obtain the CAD spectrum of the "pure" component in MS-II. This method is also useful with fast atom bombardment ionization to distinguish sample peaks from those caused by artifacts. The change in FAB peak abundances during bombardment can be caused by intermolecular reactions in the sample, as illustrated by CAD spectra of peaks above $(\text{M} + \text{H})^+$ produced by FAB of cyanocobalamin. CAD of FAB-produced high molecular weight compounds has great potential for structure elucidation and substructure recognition. CAD spectra of the 1329^+ ions from cyanocobalamin, hydroxocobalamin, methylcobalamin and coenzyme B_{12} are all identical within experimental error. CAD data is also shown for peptides such as bradykinin (M.W. 1060), lysyl bradykinin (M.W. 1188), and human gastrin (M.W. 2095).

QUANTITATIVE MS/MS : DETERMINATION OF TRACE COMPOUNDS AND PROFILING OF HOMOLOGUES IN BIOLOGICAL MATRICES BY B^2E LINKED SCANS

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Usually MS/MS (MIKES and B/E linked scans) is used in testing mixtures for the presence of one special compound. However, very often an interesting compound or even a series of homologue compounds must be determined quantitatively. The recently developed $B^2E = \text{constant}$ linked scan [1,2] offers new possibilities for quantitations. Due to its much higher precursor ion resolution than the B^2/E scan quantitation may be based on an internal standard, e.g. a deuterium labelled analogue. Therefore only very simple sample workup procedures will be necessary.

The principle is shown with a model compound (Chloro-methylbenzoate), analyzed in a MAT CH5-DF mass spectrometer, equipped with a high precision digital linked scan unit MSP 8103.

Two examples of testing complex mixtures are presented:

- The quantitative determination of caffeine in different soft drinks (100 mg/litre) and
- the quantitative profiling of biogenic amines in an american red wine (2-20 mg/litre)

Literature: R.K. Boyd, C.J. Porter and J.H. Beynon

1)Org. Mass Spectrom. 16,490 (1981)

2)Int. J. Mass Spectrom. Ion Phys. 44, 199 (1982)

Low Energy Collision Induced Dissociation of Some Simple Negative Ions Studied
Using a Triple Quadrupole

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Division of Physics
National Research Council of Canada
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There have been a number of fundamental studies reported from this laboratory¹ on the low energy collision induced dissociation of complex positive ions such as the protonated water clusters, n-butylbenzene, benzene and protonated ethyl alcohol. There has been less fundamental work on negative ions but there are a series of measurements on simple negative ions at low collision energies using a tandem magnetic sector instrument (see ref. 2 and references therein). This provides an interesting comparison for the triple quadrupole experiments.

Our work on protonated ethyl alcohol³, suggested an inverse relationship between the observed most probable scattering angle and the ratio of the collision energy to the threshold energy, but the interpretation is not unambiguous because the amount of energy used up as internal excitation energy is not precisely known. The dissociation of O_2^- into O^- in collision with argon provides an unambiguous case since the products are atomic.

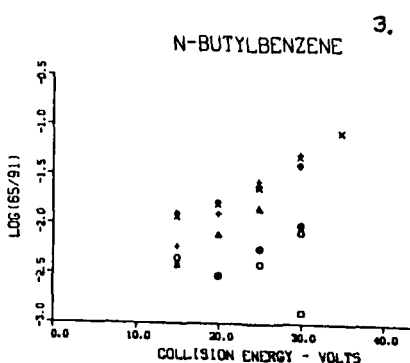
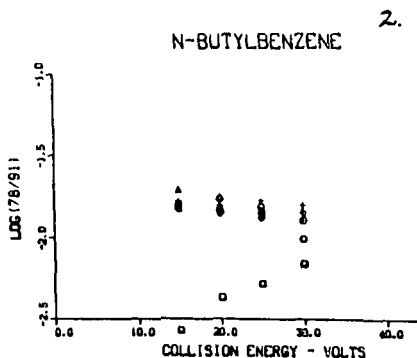
Figure 1 shows measurements made in a Sciex Inc. Taga 6000 triple quadrupole of the dissociation of O_2^- at various centre-of-mass collision energies. The absolute cross-section is estimated from the calculated target density. The cross-section begins to rise at the thermochemical threshold of 4.1 eV and reaches a sharp maximum near 10.5 eV. This is in contrast to complex polyatomic ions where we have found the cross-section to plateau at higher energies. A statistical theory for dissociation producing three outgoing particles⁴ suggests a low above threshold (E_0) where the cross-section is given by

$$\sigma = A(E-E_0)^n/E$$

where n is approximately 2. Figure 2 gives the cross-section data replotted as $(\sigma E)^{0.5}$ vE showing good agreement with the theory and the thermochemical threshold. ($n = 2.2$ gives slightly improved agreement.)

Daughter ion energies can be interpreted in terms of the scattering angles in the collision.¹ Figure 3 shows the normalised scattering angles as a function of the ratio of collision energy to threshold energy. Near threshold, the most probable scattering angle is large, and fairly violent collisions are required. At higher energies, glancing collisions become more effective. The maximum in the cross-section occurs for a scattering angle near 90° . At a given collision energy, the spread of scattering angles is relatively narrow. Similar measurements have been made on the dissociation of O_3^- , NO_2^- and CO_3^- and will be fully reported elsewhere.

1. P.H. Dawson and D.J. Douglas, Int. J. Mass Spectrom. Ion Phys. **47**, 121 (1983)
2. C. Lifshitz, R.L.C. Wu, T.O. Tiernan and D.T. Terwilliger, J. Chem. Phys. **68**, 247 (1978)
3. P.H. Dawson, Int. J. Mass Spectrom. Ion Phys. (1983)
4. C. Rebeck and R.D. Levine, J. Chem. Phys. **58**, 3942 (1973)



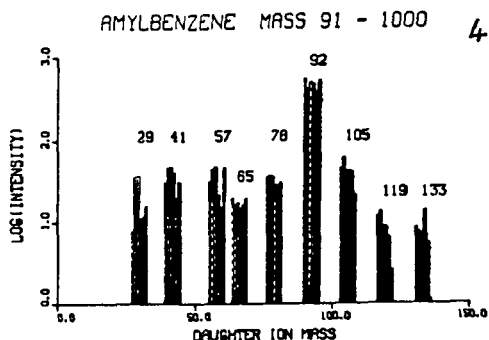
PROPOSED PROCEDURE FOR SETTING A STANDARD CONDITION FOR THE GENERATION OF TRANSFERABLE LIBRARY SPECTRA FOR TRIPLE QUADRUPOLES

Parent Ion: Amylbenzene, $C_{11}H_{16}^+$, M.W. = 148

Collision Gas: Argon

PROCEDURE

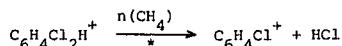
- (a) Select a nominal collision energy of 25 eV
(a.1) Make QUAD 3 dc level (R3) less than (or =) QUAD 2
- (b) Adjust the argon flow until $m/z = 65$ is 2% of $m/z = 91$
- (c) Decrease collision energy by ~5 volts
Adjust collision energy until $m/z = 92$ is 45% of $m/z = 91$ (~20 eV)
Make sure 91 and 92 are well resolved
- (d) Increase collision energy by 5 volts above that of step (c) and re-adjust argon flow until $m/z = 65$ is 2% of $m/z = 91$
- (e) Repeat steps (c) and (d) until no further re-adjustment is required. Finish with step (d) so that the final collision energy is the higher of the two values used. Record target thickness.
- (f) As a check of instrument performance the $m/z = 105$ to $m/z = 91$ ratio should now be ~4-6%. This is not very dependent on target thickness or collision energy.



HIGH PRESSURE COLLISIONAL ACTIVATION MASS SPECTROMETRY: REACTIONS OF FRAGMENT IONS

Karl Blom and Burnaby Munson, Department of Chemistry, University of Delaware,
Newark, Delaware 19711

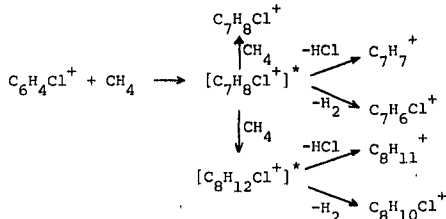
Ions produced under high pressure or chemical ionization (CI) conditions can be collisionally activated and dissociated within the high pressure ion source by applying relatively large electric fields across the source. The resulting fragment ions may react with the high pressure reagent gas, producing new ions before extraction from the ion source. We recently described a multi-region ion source for studying the processes occurring under high pressure collisional activation (HPCA) conditions.^{1,2} In the 2-region configuration, Fig. 1(a), sample ions are produced under low field conditions in region 1 and are then collisionally activated by larger fields in region 2. Figure 1(b) shows the 2-region CH₄ HPCA mass spectrum of m-dichlorobenzene at a CH₄ pressure of 0.4 torr and a field strength in region 2 of 120 V/cm. The major decomposition product is C₆H₄Cl⁺ from the fragmentation process:



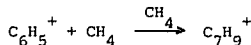
The C₇H₇⁺ in Fig. 1(b) results from the rapid reaction of C₆H₄Cl⁺ with the high pressure of CH₄ in region 2. The relative abundance of C₇H₇⁺ increases and the relative abundance of C₆H₄Cl⁺ decreases with increasing CH₄ pressure establishing the subsequent reaction:



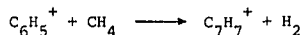
In the 3-region configuration of the multi-region source, Fig. 1(c), fragment ions produced by collisional activation in region 2 react with the reagent gas in a region of low field strength, region 3, before leaving the source. Figure 1(d) shows the 3-region CH₄ HPCA mass spectrum of m-dichlorobenzene at a CH₄ pressure of 0.4 torr and a field strength in region 2 of 120 V/cm. Comparison of Figs. 1(b) and (d) clearly establishes that the C₆H₄Cl⁺ produced in region 2 has reacted with the high pressure of CH₄ in region 3 to produce several new ions. The reaction sequence in region 3 is:



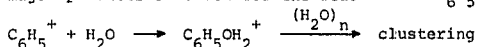
Phenyl ions, C₆H₅⁺, were prepared by high pressure collisionally activated dissociation of C₆H₅ClH⁺ or C₆H₅Cl⁺ in region 2 of the 3-region source and allowed to react with a number of gases in region 3. The C₆H₅⁺ did not react rapidly with N₂ or O₂ in region 3. The phenyl ion reacted rapidly with CH₄ in region 3 to produce C₇H₉⁺ by a collisionally stabilized addition reaction,



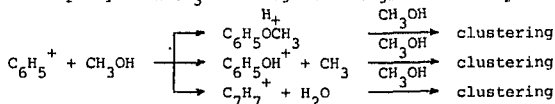
and C₇H₇⁺,



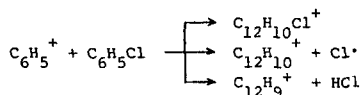
Addition ions were the major products observed for the reaction of C₆H₅⁺ with H₂O.



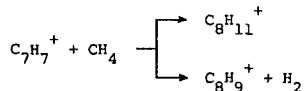
The phenyl ion reacted rapidly with CH₃OH in region 3 to give several products.



The phenyl ion also reacted rapidly with C₆H₅Cl.



The reactions of fragment ions are useful in characterizing their structures. The major fragment ion in the 2-region CH_4 HPCA mass spectrum of p-chlorotoluene is C_7H_7^+ , presumably the p-methylphenyl ion. The p-methylphenyl ion reacts rapidly with CH_4 :



The major product from the exothermic proton transfer from CH_5^+ and C_2H_5^+ to benzylpropanoate is C_7H_7^+ , either a benzyl or tropylium ion. This C_7H_7^+ ion does not react rapidly with CH_4 . A major fragment ion in the 2-region CH_4 HPCA mass spectrum of toluene is C_7H_7^+ ; the structure of this ion cannot be assigned a priori. However, this C_7H_7^+ does not react rapidly with CH_4 ; therefore, it does not have the structure of the reactive p-methylphenyl ion.

With high pressure collisional activation it is possible to study the chemistry of ions not otherwise easily produced under high pressure conditions. The chemistry of isomeric fragment ions can be useful in characterizing their structures.

1. K. Blom, B. Munson, presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics; Honolulu, Hawaii, June 6-11, 1982.
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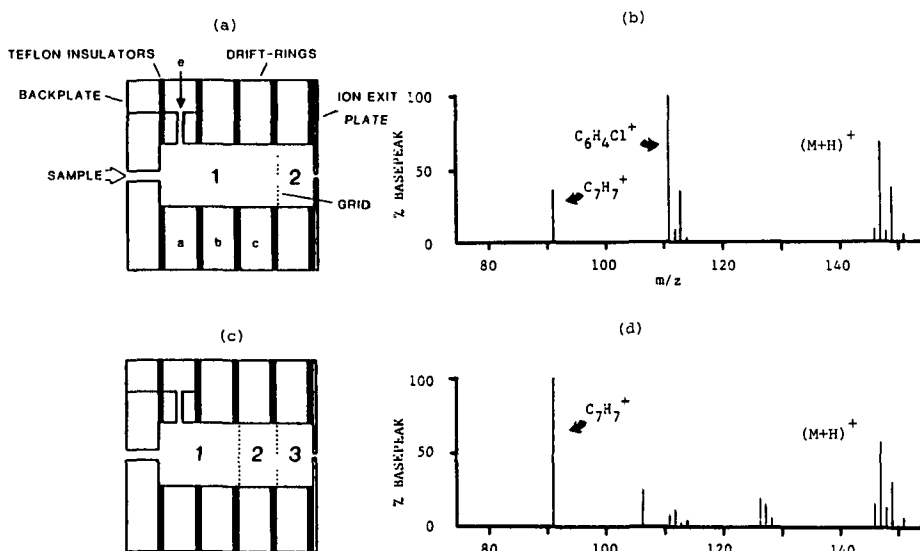
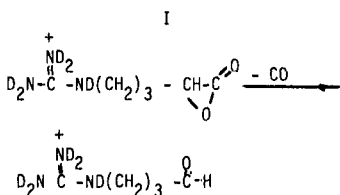


Figure 1: CH_4 HPCA Mass Spectra of m-Dichlorobenzene.

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The surprising transition of the (M+H) ion, $175^+ - 130^+$ (seen in the mass spectrum) shows a metastable which corresponds to the transition $183^+ - 135^+$ of the octadeuterated ion indicating loss of 3 deuterium labels. Interference from the metastable transitions $175^+ - 129^+$ and $175^+ - 131^+$ is apparent from the metastable spectrum of the 177^+ (A+2) ion. These are suppressed in the collision spectrum of 177^+ so that the natural abundance of ^{18}O and ^{13}C permit identifications of this loss as $CONH_3$ (or $COND_3$) from the MS/MS spectrum of 177^+ . A mechanism which yields this loss is



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In a second application of SIMS-MS/MS, isotope ratios are measured from the collision spectrum using secondary ions such as NaRbCl^+ to determine the isotopic ratio of $^{87}\text{Rb}/^{85}\text{Rb}$. The ion of mass 145⁺ consists of isobaric $\text{Na}^{87}\text{Rb}^{35}\text{Cl}^+$ and $\text{Na}^{85}\text{Rb}^{37}\text{Cl}^+$ species. Rb isotopic ratio is given by

$$\frac{[^{87}\text{Rb}]}{[^{85}\text{Rb}]} = \left(\frac{P_{87}}{P_{85}} + \left(\frac{[^{37}\text{Cl}]}{[^{35}\text{Cl}]} \right) \right)$$

where P indicates peak height, and the brackets are the natural abundances. Since $[^{35}\text{Cl}]/[^{37}\text{Cl}]$ is well known, the Rb ratio is easily and accurately determined. The advantages of this approach include the absolute removal of ion source effects (such as fractionation) and enhancement of the intensity of the less abundant isotope by suitable choice of the counterisotope. For the case of Rb, the preliminary efforts showed a precision of .068%.

Acknowledgment

Research sponsored by the U. S. Department of Energy, Division of Basic Energy Sciences, under Contract W-7405-eng-26 with the Union Carbide Corporation.

Particle Induced Mass Spectrometry/Mass Spectrometry of Alkaloids. L. Kelner, H. M. Fales and Z. Bian, NIH, Bethesda, MD 20205.

A particle bombardment ionization system (SIMS or FAB) has been coupled to a tandem quadrupole mass spectrometer (1,2) in order to analyze various biological compounds. The target was bombarded by Ar ions or neutrals produced in a specially designed ion/neutral gun with a very low energy spread (> 1 eV) equipped with a Wien type velocity filter. The technique provides quasimolecular ions which can be selectively fragmented in the collision cell (CID - collision induced dissociation) for structural information and for analysis of mixtures. The successful application of MS/MS to analysis of pyrrolizidine alkaloids using chemical ionization has been reported recently (3). Significant advantages of the MS/MS technique compared with LC/MS and GC/MS exist in terms of speed, sensitivity, and the ability to measure the alkaloids together as a chemical class. Some alkaloids have also been examined by MS/MS coupled with liquid ion evaporation which is analogous to the field desorption technique (4).

In general we find that the SIMS method, using a glycerol matrix, provides intense MH^+ ions as in chemical ionization. In contrast to the latter, however, certain ions are much less prominent. Thus crinine, haemanthamine and lycorine show intense loss of water, water and/or methanol, and 2 waters respectively in ordinary C.I. even with the very low energy $C_4H_9^+$ ion derived from isobutane as a reagent gas. However, in SIMS (glycerol) such losses are minimal, suggesting that the MH^+ ions are formed with very little excess energy. The reasons for this are not clear but comparison of the SIMS experiment with C.I. using glycerol as a reagent gas would be interesting. The short residence time of the MH^+ ions in SIMS may be an important factor.

In SIMS/CID the MH^+ ions of these alkaloids still show less water and methanol loss relative to C.I. and in one case the spectrum more nearly resembles the EI spectrum. Thus in haemanthamine important fragment ions appear in both types of spectra at m/z 181, 226, 227, 269(270). However, quite different fragmentation patterns appear in CID with the other three alkaloids, although some common ions appear in both spectra. This aspect clearly merits further investigation but it is perhaps not unreasonable to expect different cleavage from an MH^+ and an M^+ ion.

We also analyzed various mixtures of alkaloids by SIMS/MS. As one example, a mixture of crinine and haemanthamine was dissolved in a glycerol matrix prior to being placed on the multi-specimen target manipulator (2). Intense MH^+ ions were observed at m/z 272 and 302.

Combining particle bombardment for ionization with analysis by MS/MS provides obvious advantages in biological specimens.

In these studies we initially used ion energies of 2-3 eV to produce CID spectra. Even though this was enough to produce intense CID spectra of glycerol, PEG, arginine, etc., it was not sufficient to fragment these particularly stable alkaloids. By biasing the target to higher positive voltage, higher energy secondary ions are admitted first into the mass analyzer and then to the collision chamber. At ion energies around 25-30 eV reasonable CID spectra resulted.

We have also observed a difference in the energy distribution of the sample ions and the matrix. For example, the energy distribution of secondary ions of MH^+ for crinine (m/z 272) and G_3H^+ for glycerol (m/z 277) differ in the positions of their maxima by about 2 eV(5). This means that when the energy analyzer is positioned in the front of the quadrupole, it can be preferentially focused (energetically) on the ion of interest at the same time suppressing the interference ion. We feel that this might be of some value in certain applications of SIMS and FAB, as well as SIMS/MS and FABMS/MS.

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GC-MS DETERMINATION OF HERBICIDES IN WATER.

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The demand for precise and accurate determination of trace (ppb and ppt) concentrations of organic compounds in the environment has increased at a large extent in the last years. In many instances, preanalytical steps for sample concentration from the original environmental matrices such as soil, water or air, are extremely important and their reliability must be of the same grade of the analytical procedure.

Following a method applied to pesticides (1), a preconcentration technique is set up that using chromatographic extraction, allows sample size reduction by transferring the herbicides from water to an organic solvent with a factor enrichment of more than 10^3 .

The identification and quantitative analysis of the compounds of interest is made by GC-MS in the SIM mode and this allows to overcome the lack of separation of some compounds.

A DANI 3900 gas chromatograph is coupled via a jet separator to a VG 70-70H mass spectrometer equipped with a microprocessed eight channel Multiple Ion Detector (MID). The molecular ion of n-hexylbenzene, that bleeds continuously into the ion source, is used as "lock mass". The total cycle time is kept at a value of 0.9 sec. and the channel time at 0.1 sec. for the peaks monitored.

A GC glass column 2 m long, 2 mm i.d. packed with Supelcoport 100-120 mesh coated with 1.5 % SP2250 + 1.95 % SP2401 is used. This column results very good for herbicides and pesticides, but the triazines are not separated. Operating conditions are: T: 200 °C isothermal, carrier: extrapure Helium for GC-MS operation, Nitrogen for ECD operation, flow rate: 40 mL/min in both instances. A chromatogram of the herbicides using ECD as detector is reported in the lower part of Figure 1. The dynamic resolving power of the mass spectrometer is kept at a value of 3300 (10 % valley definition).

In these conditions, the second decimal figure is certain so that hydrocarbons fragments are excluded from the peak monitored and those belonging to chlorinated pesticides as well. Because of the possible presence of pesticides in the matrix containing herbicides, this feature makes the method highly selective. The GC column has been chosen with the aim of obtaining the best resolution within the shortest analysis time. In fact, most of the herbicides tested are separated but the s-Triazines give a single peak, namely n. 5 in the ECD chromatogram of Figure 1. The three compounds, atrazine, simazine and propazine are identified by SIM.

The MID programme is made in such a way that every compound is identified by two mass fragmentograms, one monitoring the molecular ion, the other one a structurally significant fragment ion.

It has to be noted that the relative abundance of the ions does not reflect exactly the one in the mass spectra shown in Figure 2. This is due to the fact that the accelerating voltage is different for ions of different m/e ratio to be focussed, so that the sensitivity in the various channels is not the same. However, the intensities are constant for a given MID program, so that a further identification parameter is given by the ratio of the peak areas. On the quantitative point of view, we have already shown (2) that the mass spectrometer can be used a quantitative detector in a better and more specific way than the electron capture detector.

For what calibration curves are concerned, a higher linear range is observed for the mass spectrometer, as it could be expected. Atrazine, having one chlorine atom only in the molecule yields a fairly linear calibration curve with ECD. Other compounds with a higher number of Cl atoms present a worse situation, and in this instances the mass spectrometer presents an extremely higher performance.

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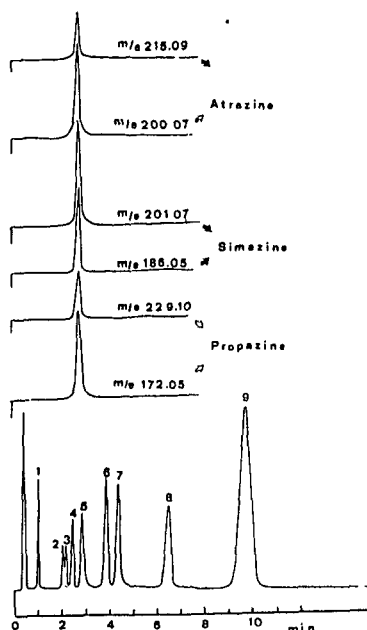


FIGURE 1 ECD chromatogram of the herbicides examined and Selected Ion Monitor signal for the compounds indicated
Peak identification:
1) Diclobenil, 2) Trifluralin, 3) CIPC, 4) 2,40 ME, 5) Atrazine, Propazine, Simazine, 6) 2,4,5T ME, 7) Aldrin, 8) DCPA, 9) Silvex ME

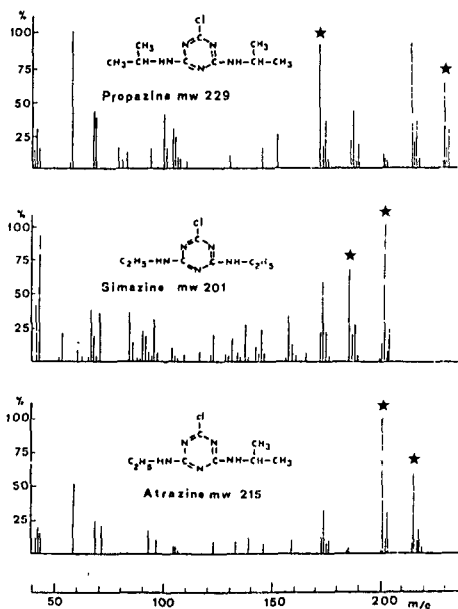


FIGURE 2. Mass spectra of three Triazines that yield a single peak a the GC column
Peaks with asterisks are chosen for S.I.M.

QUALITATIVE AND QUANTITATIVE DETERMINATIONS OF AROCLOR FORMULATIONS

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Examples of single ion and total ion reconstructed chromatograms of a sample containing Aroclors 1242, 1248, 1254 and 1260 are shown in Fig. 1. Peaks labeled in these chromatograms represent mono- (Fig. 1A), di- (1B), tri- (1C) tetra- (1D), penta- (1E), hexa- (1F) and hepta- (1G) chlorinated biphenyl components adopted for data reduction. The areas of the 40 single ion peaks or peak aggregates (Fig. 1) obtained from one set of reference Aroclors and test samples were used for multiple regression analysis as formulated below:

$$Y_1 = X_{1,1242}C_{1242} + X_{1,1248}C_{1248} + X_{1,1254}C_{1254} + X_{1,1260}C_{1260}$$

$$Y_2 = X_{2,1242}C_{1242} + X_{2,1248}C_{1248} + X_{2,1254}C_{1254} + X_{2,1260}C_{1260}$$

$$Y_{40} = X_{40,1242}C_{1242} + X_{40,1248}C_{1248} + X_{40,1254}C_{1254} + X_{40,1260}C_{1260}$$

where Y_n ($n = 1$ to 40) is the intensity of peak n observed in the test sample. $X_{n,m}$ ($m = 1242, 1248, 1254$ or 1260) is the intensity of peak n observed in unit concentration of reference Aroclor m . C_m is the concentration of Aroclor m in the test sample.

Table I lists regression analysis results obtained from sample experiments. When expected values are known, they are also included in the Table (in parentheses) for comparison.

Test samples 5328 and 5330 were prepared by mixing reference Aroclors 1242, 1248, 1254 and 1260 from which their chromatographic patterns were established. Data obtained from sample 5328 correlate very well with the reference Aroclors to give high coefficients of determination ($> 99\%$) and small constant terms. Aroclor concentrations thus obtained also agree well with the theoretical values shown in parentheses. Sample 5330 was a hexane-methylene chloride extract of an aqueous sample known to contain only Aroclors 1248 and 1254. Accordingly, regression analyses were performed based only on these two Aroclors. As above, good results were obtained. However, a better test of the method was to perform the regression analysis on this test sample data based on all four Aroclors. Stepwise regression analysis of 5330 indicated that Aroclor 1260 data could not meet the 0.50 significance level for entry into the model. The use of Aroclor 1242 data did not improve the "goodness of fit" significantly, and resulted in a low concentration of Aroclor 1242 with much higher concentrations of Aroclors 1248 and 1254 (shown in brackets). Based on these results we conclude that Aroclors 1260 and 1242 are not significant components in this sample. A visual inspection of the chromatographic pattern would not necessarily make this conclusion apparent. It is also interesting to examine the quantitative results obtained from the regression analysis of 5330. The fact that an almost identical sum of Aroclor concentration is obtained from the use of two (Aroclors 1248 and 1254) or four (Aroclors 1242, 1248, 1254 and 1260) variables certainly will satisfy most analytical situations in which only total PCB concentration is needed.

Sample 5332, a hexane-acetone extract, is a sediment sample spiked in a different laboratory with a known amount of Aroclor 1242 of unknown lot and unknown sample history. Aroclor 1242 was the only reference that met the 0.5 significance level for entry into the stepwise model of the multiple regression analysis. However, considering the low value of the coefficient of determination, one may have difficulty in deciding whether PCBs present are actually Aroclor 1242 or a nonspecific combination of PCBs.

Extract from field sediment sample 5009 (unknown history) gave lower coefficients of determination. This indicates a deviation of chromatographic patterns from that of Aroclor references, presumably as a result of differential partial degradation of PCBs. This data along with that obtained from 5332 lead to an interesting question -- at what value of coefficient of determination should one consider the test sample as a specific combination of Aroclors, rather than a nonspecific mixture of PCBs? Obviously, PCB metabolism and degradation patterns have to be systematically studied before a reliable conclusion can be reached.

FIG 1

SAMPLE RECONSTRUCTED GC/MS ION CHROMATOGRAMS

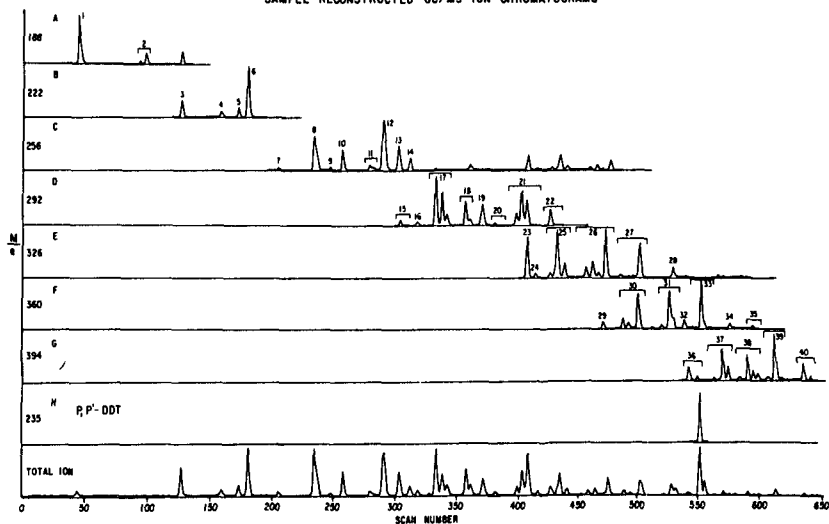


TABLE I RESULTS OF MULTIPLE REGRESSION ANALYSIS

Sample No.	Sample Description	Aroclor Concentration/ppm				Coef. of detm/%	Const term
		1242	1248	1254	1260		
5328*	Control mixture	41.1 (44.4)*	98.2 (104)	121 (152)	53.9 (47.4)	314 (348)	99.6 -17
5330*	Control mixture (extract from an aqueous sample)	[9.44]*	161 [147] (163)	185 [192] (238)	346 [348] (401)	99.3 [99.5]	16 [-4.2]
5332 ^E	Sediment extract	23.6 (39.4)				55.8	40
5009 ^E	Field sediment		35.6	72.7		108	87.8 -2.7

*Aroclors in these samples and Aroclors in standards are from the same lot.

*Numbers inside parentheses are expected values.

*Numbers in brackets are results obtained from regression on four Aroclors.

^EAroclors in these samples and Aroclors in standards are from different lots.

ENVIRONMENTAL FATES OF COMBUSTION-GENERATED CHLORINATED DIOXINS AND FURANS

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Recent reports have shown that the combustion of municipal wastes, chemical wastes, and coal may be a major source of polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) found in the environment. It has even been postulated that PCDD and PCDF are formed in all combustion processes and that they have been with us since the advent of fire [R. R. Bumb et al., SCIENCE 210, 385 (1980)]. In this work, we set out to answer three questions regarding PCDD and PCDF: (a) Is combustion a major source of dioxins and furans? (b) Are PCDD and PCDF ubiquitous in the environment? (c) Have PCDD and PCDF always been ubiquitous?

To address these questions, we measured PCDD and PCDF in samples from ultimate environmental sinks such as lacustrine sediments. Sediment samples from several locations in the Saginaw River and Bay and southern Lake Huron were studied. Several kinds of information can be determined by analyzing sediments. First, PCDD and PCDF profiles found in sediments can be compared with possible sources to determine major inputs. Second, the decrease of PCDD and PCDF with distance can be used to locate sources. At remote locations, atmospheric deposition is the primary mode of transport to sediments and by analyzing such sites, we can determine the extent of movement in the environment.

Another valuable source of information is the analysis of sediment cores. Sediments can be sampled to preserve the historical input of materials. Sections of cores are analyzed and dated by radioisotopic techniques. In this manner the historical input of dioxins and furans can be determined. This gives information about anthropogenic vs. natural sources of these compounds. A very sharp time-dependent change may even identify a specific source.

Experimental

Sediments were extracted first with isopropanol for 24 hours and then with methylene chloride for an additional 24 hours. Cleanup included silica and alumina fractionation. Analyses were obtained by negative chemical ionization GC/MS with methane as the reagent gas. Quantitation was achieved by comparing various PCDD and PCDF to the internal standard ³⁷Cl₈-octachlorodioxin (OCDD), with correction for relative response factors. The limit of detection was 0.2 pg for OCDD.

Results

Combustion-generated dioxins and furans were found in sediment samples in an urban area as well as in remote sites. PCDD and PCDF were found at about 100 ppt at the remote sites. These data indicate that PCDD and PCDF are stable in the environment and, indeed, may be ubiquitous. The PCDD and PCDF isomer profiles were similar in all sediment samples, including the most remote. These profiles closely followed those found in combustion samples, suggesting that combustion is the major source of these compounds. An urban to remote transect showed that the concentration decreases as the distance from combustion sources increases; this indicates the anthropogenic nature of these compounds.

When PCDD and PCDF were measured in sediment cores, an interesting trend was found. The depth-concentration profiles in these cores showed that the input of PCDD and PCDF increased dramatically at a depth corresponding to approximately 1940 and remained high to the present time. In Figure 1, the time-dependent concentration profiles of OCDD and H₇CDF in one Lake Huron core are compared to the trends for United States' coal production and production of chlorinated aromatics for that time period. It is clear that coal use has not been a major source of PCDD and PCDF. However, the sedimentary historical record agrees well with the production of chlorinated aromatic compounds. This suggests that chlorinated aromatics are the precursors of PCDD and PCDF, and these compounds are present in combustion fuels. Low or undetectable levels of PCDD and PCDF at depths corresponding to times before 1940 show that PCDD and PCDF were not with us since the advent of fire.

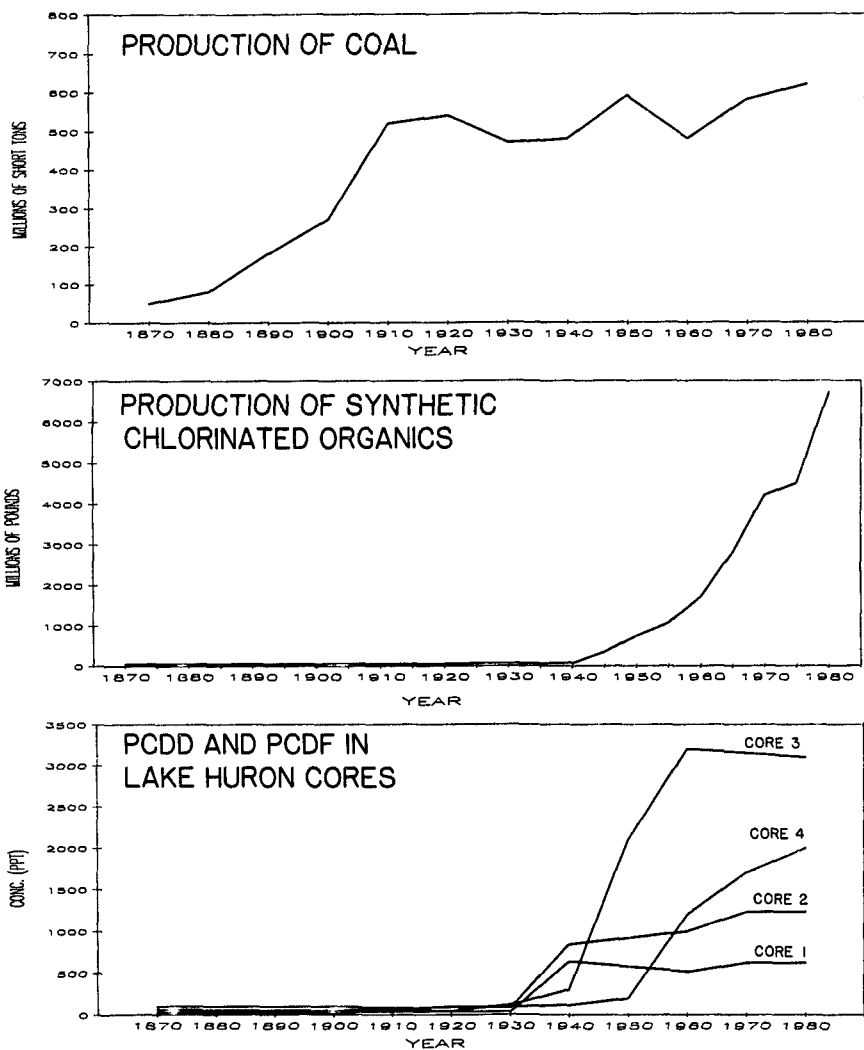


Figure 1. U.S. production of coal and chloro-aromatics compared to the concentration of PCDD and PCDF in Lake Huron Core 1 as a function of time.

QUANTITATIVE ANALYSES OF ENVIRONMENTAL SAMPLES FOR CHLORINATED DIBENZO-p-DIOXINS
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Chlorinated dibenzo-p-dioxins (CDDs) comprise a series of 75 chlorinated congeners some of which have been found to be extraordinarily toxic in laboratory animal tests and in vitro enzymatic induction assays. The CDDs are apparently widely distributed in the environment and the origins of these environmental contaminants include use and application of chloro-phenol-derived pesticides and chemicals, incineration processes, and faulty disposal of chemical wastes (arising from a variety of haloaromatic manufacturing processes). Global concern over CDDs in the environment has been prompted by large-scale accidents/incidents such as those which have occurred during application of Herbicide Orange in Southeast Asia, the industrial accidents at Seveso, Italy (1976); Nitro, Virginia (1949); Love Canal, N.Y. (1979); and most recently in Verona and Times Beach, Missouri. In most of the incidents cited above, specialized analytical methodology was developed and applied in order to detect, quantify and monitor the CDDs in the environment affected by the accident/incident. In view of the apparent toxic hazard posed by certain of the CDDs and the probability that certain CDDs arise primarily from only specific sources, the detection schemes employed to assess episodes of CDDs contamination should not only be sensitive ($<1 \times 10^{-8}$ g/g) but also specific (the 2,3,7,8-TCDD isomer is of greatest concern and frequently must be quantitated in the presence of high concentrations of other CDDs including the 21 other TCDD isomers as well as other chlorocarbon contaminants). The analytical technology which has been developed and applied in this laboratory to investigate CDDs present in soil samples is described below and representative results are presented.

Missouri USA Soils.-At present, the extent of TCDD contamination in the environment at various sites in the State of Missouri is being investigated by USEPA. An analytical protocol has been developed and is being applied for determining 2,3,7,8-TCDD in these samples which entails sample extraction, elution chromatographic clean-up of sample extracts, and finally GCMS quantitation of, presumably, the 2,3,7,8-TCDD isomer. In this laboratory a study to assess the relative efficiencies of various extraction schemes has been accomplished and the data are presented in Table 1. The two schemes included a simple extraction using disposable glass bottles and a more elaborate Soxhlet extraction. As can be seen, the bottle extraction scheme using hexane:methanol compared favorably with a 16-hr. Soxhlet extraction employing benzene. The details of the clean-up procedure will be published elsewhere. Typically, the TCDDs in these soil extracts are quantitated by gas chromatography (employing an SP2340 column)-low resolution mass spectrometry. The mass spectrometer is operated in the multiple ion monitoring mode. Since the gas chromatographic separation and quantitation of the 2,3,7,8-TCDD isomer is of great importance, the SP2340 column has been carefully evaluated in this laboratory and Figures 1-3 illustrate results obtained in quantitating 2,3,7,8-TCDD using this column. Figure 1 illustrates results obtained for a standard containing twelve TCDD isomers. Note that under these conditions DDE (included only as an internal standard, typically DDE is removed by sample clean-up procedures) coelutes with the 1,3,6,8-TCDD isomer, however the 2,3,7,8-isomer is resolved from the 1,2,3,4 and 1,2,7,8- isomers. Figure 2 illustrates the performance of the column following injection of aliquots of 37 sample extracts, a 5µl injection of triethylamine (recommended for passivation of columns contaminated with polar residues) and an additional 19 sample extracts. Note that 2,3,7,8-TCDD and 1,2,3,4-TCDD are not resolved. Following a second triethylamine injection and bake-out, the twelve TCDD isomer calibration mixture was again analyzed and the results obtained are shown in Figure 3. Note that the elution order of the 2,3,7,8- and 1,2,3,4- TCDD isomers is reversed and the 2,3,7,8- and 1,2,7,8- TCDD isomers are not resolved. One concludes that the isomer specificity of this particular SP2340 column is markedly affected by injection of sample extracts. Clearly in this case the column efficiency for separating isomers has degraded; in addition the possibility that column efficiency changes abruptly during a particular GCMS analysis must be acknowledged. This phenomenon and solutions thereto (perhaps inclusion of additional ^{13}C -labelled isomer internal standards) are currently being investigated.

In conclusion, it should be pointed out that overall these procedures being implemented for screening large numbers of Missouri soil samples for 2,3,7,8-TCDD content appear to be efficacious based upon the fact that in this laboratory the recoveries of the ^{37}Cl -2,3,7,8-TCDD internal standard have averaged >70% for several hundred samples analyzed.

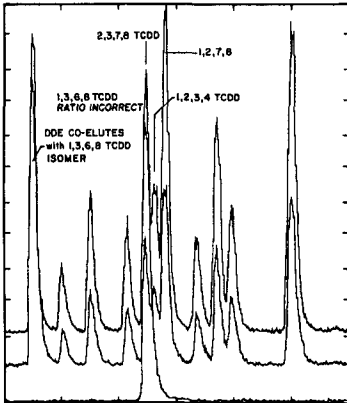


Figure 1. Results of HRGC/MS Analysis of Twelve TCDD Isomer Calibration Mixture Using Newly Installed SP2340 Column

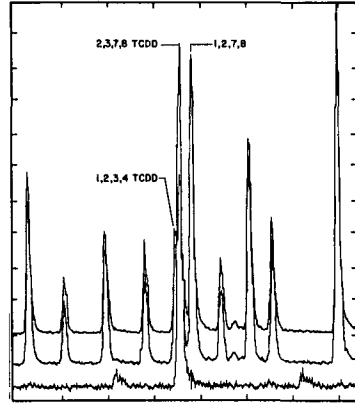


Figure 2. Results of HRGC/MS Analysis of Twelve TCDD Isomer Calibration Mixture Following Analysis Extracts and Initial Deactivation.

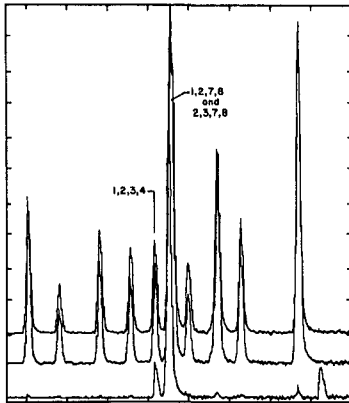


Figure 3. Results of HRGC/MS Analysis of Twelve TCDD Isomer Calibration Mixture Following Further A Second Deactivation.

CONCENTRATION OF 2,3,7,8-TCDD IN ALIQUOTS OF A MISSOURI SOIL SAMPLE FROM CONTAMINATED HORSE ARENAS AS DETERMINED BY APPLYING SEVERAL DIFFERENT EXTRACTION PROCEDURES

MSD SAMPLE NO.	EXTRACTION PROCEDURE APPLIED	CONCENTRATION OF 2,3,7,8-TCDD (µg/g)
DMA-30E	WET SOIL, BOTTLE EXTRACTED BY AGITATING WITH HEXANE-METHANOL (4:1) FOR 3.5 HRS	27
DMA-30G	WET SOIL BLENDED WITH SODIUM SULFATE (1:1) BOTTLE EXTRACTED BY AGITATING WITH HEXANE FOR 3.5 HRS	10
DMA-30H	AIR-DRIED SOIL SCHMULET EXTRACTED WITH BENZENE FOR 16 HRS	27
DMA-30I	AIR-DRIED SOIL BOTTLE EXTRACTED BY AGITATING WITH HEXANE FOR 3.5 HRS	23
DMA-30J	AIR-DRIED SOIL MIXED WITH SODIUM SULFATE AND BOTTLE EXTRACTED BY AGITATING WITH HEXANE FOR 3.5 HRS	21
DMA-30K	AIR-DRIED SOIL SCHMULET EXTRACTED WITH TOLUENE FOR 16 HRS	24
DMA-30L	AIR-DRIED SOIL BOTTLE EXTRACTED BY ULTRASONICATING SOIL WITH HEXANE AND MILD HEATING, FOLLOWED BY AGITATING FOR 3.5 HRS	21
DMA-30M	WET SOIL BLENDED WITH SODIUM SULFATE (1:1) AND SCHMULET EXTRACTED FOR 16 HRS	26
DMA-30N	WET SOIL BLENDED WITH SODIUM SULFATE (1:1) AND BOTTLE EXTRACTED BY AGITATING WITH HEXANE FOR 3.5 HRS	14

*ON BASIS OF DRY WEIGHT OF SOILS IN SEPARATE EXPERIMENTS THIS SOIL WAS FOUND TO CONTAIN 18% WATER.

Table 1

MPC 19 Comparison of Methods for the Determination of PCDD's and PCDF'S in Transformer Oil and Transformer Oil Soot

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12201

Pyrolysis of PCB insulating fluid is known to form polychlorinated dibenzofurans (PCDF's). Since transformer insulating fluids may also contain chlorobenzenes the formation of chlorinated dibenzo-p-dioxins (PCDD's) including the extremely toxic 2,3,7,8 isomer is expected when these fluids are pyrolyzed. A fire in a basement transformer of a large office building in New York State contaminated the building with a heavy soot when the soot was transported throughout the building by the air handling system. The characterization of these pyrolysis products and monitoring of the subsequent building clean-up has involved a variety of trace analytical techniques. In addition some interesting chemical aspects of the fire has been observed.

Initial analysis of the transformer soot for 2,3,7,8-TCDD and 2,3,7,8-TCDF was done by HRGC/HRMS following an extensive isomer specific preparation procedure employing HPLC. The results of these analyses showed high levels of 2,3,7,8-TCDF (200 ppm) and 2,3,7,8-TCDD (2.8 ppm). A more complete characterization of the soot was required to establish the toxicity of the soot, to compare with samples taken after the building clean-up and to check a cell keratinization assay. Soot samples from each floor were subject to an extraction and clean-up procedure which isolates planar chlorinated aromatic compounds in one extract. This extract was then analyzed by HRGC/HRMS. GC conditions were chosen so as to coelute all the isomers with the same number of chlorine atoms (Figure A). By acquiring data over the entire mass range information on all the PCDF's and PCDD's could be obtained during an experiment. Complete HR mass spectra lends confidence to the identification of all components of interest (Figure B-mass spectrum of penta CDF). Quantitation was done by comparison of the intensity of the native PCDF's to an isotopically labelled internal standard. The results indicated that the ninth floor was most contaminated. The results correlated well with the cell keratinization assay. PCDF's were most prevalent in the soot and polychloronaphthalenes (PCN's) were identified as second most prevalent contaminant.

The clean-up of the building has been monitored using HRGC/HRMS analysis of air samples collected from cleaned floors. An SP-2330 capillary column was used to establish isomer specificity and HPLC was not employed. (Figure C-1,4,7,8,-2,3,7,8-and 1,2,3,4,-TCDD). Elimination of the HPLC steps increased recoveries, allowed for automated sample preparation and low limits of detection, i.e. .2 pg/m³. The air sample study indicated that most of the contamination is present either in the gas phase or on very small particles.

In the interests of developing a rapid scanning procedure for PCDD's and PCDF's we have incorporated circuitry into the Kratos MS-50 to rapidly and simultaneously monitor the collisionally induced loss of COCl in both the native and labelled PCDD's and PCDF's. The loss of COCl is unique since PCB's and PCN's do not contain oxygen. This instrumental technique combined with a simple clean-up procedure could be used to rapidly screen transformer oils and soots for PCDD's and PCDF's.

MASS CHROMATOGRAMS OF PCDF MOLECULAR IONS

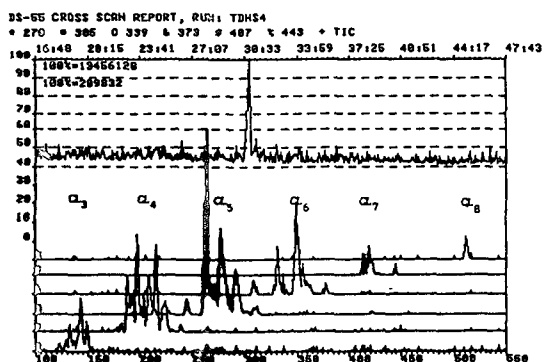


Fig A

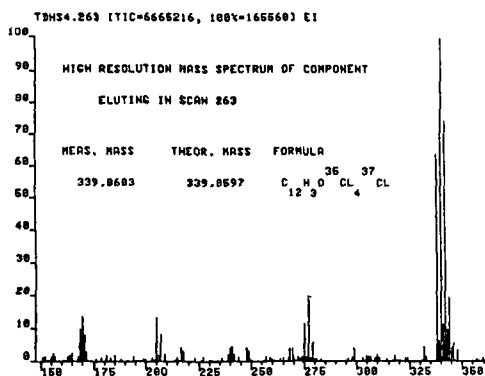


Fig B

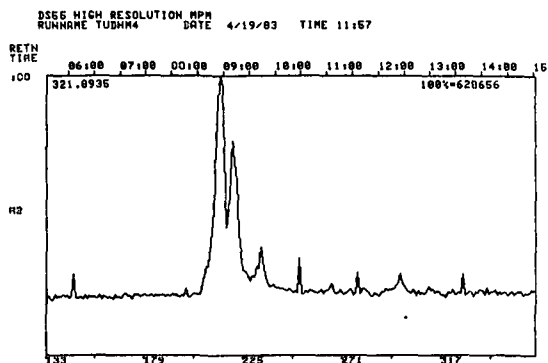


Fig C

Analysis for Tetrachlorodibenzofurans

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An analytical method in current use for determination of tetrachlorodibenzofurans (TCDFs) is described. The method is based on high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS) detection techniques. The internal standard used for determination of sample preparation efficiency, analytical criteria used for confirmation of TCDF isomers, potential interference compounds encountered, and the findings generated for various matrices, e.g., chemical waste, fly ash, and fish are discussed. These discussions will show:

- ° The analytical methodology (1) is adequate for quantitative determination of ppt to ppm levels of TCDFs in various matrices.
- ° The labeled ($^{37}\text{Cl}_4$ or $^{13}\text{C}_{12}$) 2,3,7,8-TCDD can be used for reliable and accurate determination of analytical methodology efficiency achieved for TCDFs. Also, the incorporation of both labeled TCDD and OCDD can be used for determining the efficiency for tetra thru octa CDDs and CDFs if a modified version of the extraction and clean-up procedure is used (available upon request).
- ° Hexachlorodiphenylethers have been identified in sediments, chemical waste, fish, etc. They can be easily mistaken for TCDFs in EI selected ion monitoring analysis because the major fragment resulting from loss of Cl_2 is identical to TCDF (same exact mass, elemental composition and 4 chlorine isotope ratio). Also, their HRGC retention times are similar to those exhibited by TCDFs.
- ° The analytical criteria used for confirmation of TCDFs in this laboratory;
 - a. HRGC-HRMS retention time window based on a mixture of 28 TCDF isomers derived from fly ash and 2,3,7,8-TCDF analytical standard.
 - b. Molecular ion chlorine isotope ratio.
 - c. HRGC-HRMS confirmation of exact masses that correspond to elemental compositions of TCDF.
 - d. COCL loss indicative of TCDF structure.
 - e. HRGC-HRMS analyses that confirm the absence of hexachlorodiphenylethers, for example, the simultaneous analysis of the exact masses of m^+ and $m+2$ of TCDF and hexachlorodiphenylether (m/z 304/306 and m/z 374/376).
 - f. HRGC-HRMS responses for m/z 304/306 must be greater than 2.5x noise level.
- ° Quantification of TCDFs is based upon the concentration of 2,3,7,8-TCDF analytical standard.
- ° The insufficient number of labeled and native TCDF isomers available for reference purpose prohibits full utilization of analytical capabilities. This also applies to the higher chlorinated CDFs.

Reference

(1) R. L. Harless, et al Analytical Chemistry, July 1980 pp. 1239-1245

Disclaimer - "This is an abstract of a proposed presentation and does not necessarily reflect EPA policy."

MPC-21: GC/MS analysis of bacterial metabolites of biphenyl and deuterated (d_{10}) biphenyl.

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INTRODUCTION

Polychlorinated biphenyls have been extensively used since the 1930's for many applications based on their dielectric properties and their chemical and thermal stability. However, as it has been recognized since 1966, their slow biodegradability makes them some of the most persistent pollutants in the global ecosystem, including animals and humans. While at first these substances were believed to be essentially non-degradable, numerous studies have shown that PCB's are metabolized by mammalian and micro-organisms into hydroxylated compounds. In the mammalian models, it has been shown that the metabolic conversions are initiated by the hepatic monooxygenase enzymes leading to the formation of intermediates arene oxides. These in turn may isomerize to phenols and react with various nucleophiles including cellular macromolecules.

In the course of a research program on the development of bacterial strains capable 1) to imitate closely mammalian metabolism and 2) to degrade PCB's into non toxic or non mutagenic compounds, biphenyl was used as a model substrate in order to determine the catabolic properties of a gram negative bacterial strain (B-206) towards organic pollutants containing the biphenyl nucleus.

EXPERIMENTAL

The bacterial cultures (containing NaNO_3 as the nitrogen source) were incubated for 96 and 144 h. The cells were harvested by centrifugation and the aqueous phase extracted with dichloromethane. The aqueous layers were then acidified ($\text{pH} = 3$) and re-extracted with the organic solvent. The structures of the metabolites were determined by GC/MS analysis of the derivatized (BSTFA/ACN; deuterated BSA/ACN; diazomethane) bacterial extracts. Deuterated biphenyl (BPd_{10}) was also used as a substrate for microbial transformation in order to ascertain the proposed structures of the metabolites and to determine the biodegradation pathways of BPC by strain B-206.

RESULTS

The results show that biphenyl is converted in a number of acidic compounds such as benzoic acid, isomers of 2-hydroxy 6-oxo-6 phenyl hexa-2,4-dienoic acid, 2-hydroxy 6-oxo-6 phenyl hexanoic acid and other short chain acids. These acids could be formed by the oxidative meta cleavage of an intermediate 1,2-biphenyldiol that could not be detected in the bacterial cultures. The mass spectra of the TMS derivatives of 2-hydroxy 6-oxo-6-phenyl hexa-2,4-dienoic acids are very similar and are characterized by the following ions: m/z 362 (M)⁺, 347 ($\text{M}-\text{CH}_3$) and 245 ($\text{M}-\text{COOTMS}$; base peak)⁺.

Although 2-hydroxy 6-oxo-6-phenyl hexanoic acid TMS derivative was not chromatographically resolved from the peak corresponding to one of the isomeric unsaturated acids, its structure could be easily determined on the basis of its characteristic ions at m/z 366 (M)⁺, 351 ($\text{M}-15$)⁺, 249 ($\text{M}-\text{COOTMS}$)⁺, 147 [$(\text{CH}_3)_2\text{Si}-\text{O}-\text{Si}(\text{CH}_3)_3$]⁺ and 129 [$\text{CH}_2=\text{CH}-\text{CH}=\text{O}-\text{TMS}$]⁺ and by comparison with the mass spectrum of its p-chlorinated analog.

We have also observed the formation of other important metabolites. The mass spectra of their TMS derivatives are consistent with the structures of biphenyl containing hydroxy and nitroso functional groups. The mass spectra of the TMS and TMS- d_{10} derivatives of these nitroso compounds are also presented.

MPC-22: GC/MS identification of new bacterial ring-hydroxylation and ring-dechlorination metabolites of DDT.

Robert Massé, Diane Lalanne and François Messier, Institut National de la Recherche Scientifique (INRS-Santé), Montréal H1N 3M5, Canada and Michel Sylvestre, Institut Armand Frappier, Université du Québec, Ville de Laval H7N 4Z3, Canada.

INTRODUCTION

It has been shown that the major routes of DDT biotransformation in bacterial as well as in mammalian organisms are through specific and selective dechlorination, oxydation and decarboxylation reactions on its 1,1,1-trichloroethane side chain (1). It can be seen from various studies that p-chlorine ring substitution makes DDT highly resistant to microbial degradation. Only one report indicates that DDT ring-cleavage can occur to yield p-chlorophenylacetate (2). In a previous communication, we reported the formation of isomeric phenolic metabolites during the biotransformation of 4-chlorobiphenyl by bacterial strain B-206 (3). Exposure of DDT to gram negative strain B-206 in the presence of NaNO_3 for several days lead to the formation of ring-hydroxylated and ring-dechlorinated metabolites.

EXPERIMENTAL

Bacterial strain B-206 was grown in the presence of DDT as sole carbon source for various periods of time (1 to 15 days) as previously described (3). The incubation mixtures were extracted at pH 7 and pH 3 with ethyl acetate. The organic extract were trimethylsilylated with BSTFA-acetonitrile (80:20, v/v) and $[\text{C}_6\text{H}_6]$ BSA-acetonitrile and analyzed by combined high resolution gas chromatography-low resolution mass spectrometry. Chromatographic separation was carried out using a Perkin-Elmer Sigma 3 gas chromatograph equipped with a 30 m SE-30 fused silica capillary column. The column temperature was programmed from 120°C at 80°C/min up to 200°C and at 3°C/min up to 280°C. Electron impact mass spectra were recorded on a Kratos MS-25 mass spectrometer at 70 eV ionization potential. The scanning speed was 1 sec/decade, source temperature was set at 250°C and trap current was 100 μA .

RESULTS AND DISCUSSION

The metabolic profiles obtained by GC/MS analysis of the bacterial extracts reveal that DDT is transformed by strain B-206 via two different metabolic pathways. First, the trichloroethane side chain is dechlorinated to DDD [1,1-dichloro-2,2-bis(p-chlorophenyl) ethane] M^+ 318, DDE [1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene] M^+ 316 and DDMU [1-chloro-2,2-bis(p-chlorophenyl)ethylene] M^+ 282. In parallel, specific enzymatic hydroxylations of the chlorinated aromatic rings with concomitant elimination of one chlorine atom lead to the formation of new hydroxylated and dechlorinated bacterial metabolites of DDT.

The structural identification of the hydroxylated metabolites was determined by the highly characteristic fragmentation patterns of their corresponding trimethylsilyl ether derivatives. The major metabolite, which accumulated in the bacterial cultures, was identified as 1,1,1 trichloro 2[2'-hydroxy-4'-chlorophenyl]-2-[4'-hydroxyphenyl] ethane. The mass spectrum of its TMS derivative shows a molecular ion at m/z 494 with a characteristic isotopic ion cluster, indicating the presence of four chlorine atoms. Other diagnostically important ions are observed at m/z 377 ($\text{M}-\text{CCl}_3$), m/z 335 ($\text{M}-159$), m/z 300 (m/z 335 - Cl) and m/z 289 ($\text{M}-\text{CCl}_3-\text{OTMS}+\text{H}$). The TMS derivatives of three other minor metabolites, bearing aromatic hydroxyl substituents at positions 2' and 4' and having a 1,1-dichloroethane (M^+ at m/z 460), 1,1-dichloroethylene [M^+ at m/z 458] and 1-chloroethylene [M^+ at m/z 424] side chain, were also detected. The mass spectra of TMS derivatives of the metabolites containing a chlorinated ethylene chain are characterized by the loss of 123 a.m.u. [$\text{M}-15$ from the 4'-OTMS group - Me SiCl] and 158 a.m.u. [$\text{M}-123-\text{Cl}$], whereas those containing a chlorinated ethane chain are dominated by the loss of Cl . In addition the bacterial biotransformation of DDT also leads to the formation of the monohydroxylated derivatives of DDT, DDE and DDMU with molecular ions (TMS) at m/z 440, 404 and 370 respectively.

vely. Finally, a minor metabolite of DDT the mass spectrum of which exhibited a molecular ion at m/z 528 and a prominent ion at m/z 411 (M-117) was identified as 2',2''dihydroxy DDT. These highly characteristic fragmentation patterns were thus used to establish the orientation of the aromatic hydroxylation by strain B-206. Comparison of the mass spectra as TMS and [$^{2}H_6$] TMS derivatives was used to determine the structure of these newly discovered DDT bacterial metabolites. Thus, in view of the ability of strain B-206 to hydroxylate and dechlorinate DDT aromatic rings, a new route for the ultimate breakdown of environmental DDT and halogenated aromatic hydrocarbons should be developed in a near future.

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2. D.D. Focht and M. Alexander, Science, 170:91 (1970).
3. M. Sylvestre, R. Massé, F. Messier et al., Appl. Environ. Microbiol., 44:871 (1982).

MPC-23: Metabolism of 4-chlorobiphenyl by the *Achromobacter* strain B-218 and *Bacillus brevis* strain B-257.

François Messier, Marie-France Lévesque and Robert Massé, Institut National de la Recherche Scientifique (INRS-Santé), Montréal H1N 3M5, Canada and Michel Sylvestre, Institut Armand Frappier, Ville de Laval H7N 4Z3, Canada.

INTRODUCTION

The polychlorinated biphenyls (PCB's) are recognized as one of the most ubiquitous and persistent environmental pollutants in the ecosystem. They have been used extensively and, as a result of massive production and improper disposal, they have become widely distributed in soil, water, food, wild life and humans. We have undertaken studies on the microbial biodegradation of PCB's in order to develop bacterial strains with the ability to transform PCB's into non toxic metabolites for man and animals. Among the bacterial strains studied, two strains isolated from activated sludge (*Bacillus brevis* B-257 and *Achromobacter* B-218) exhibit an interesting biodegradation potential toward chlorinated biphenyls. In a preliminary study, 4-chlorobiphenyl (4-CB) was chosen as a model substrate. This paper presents the metabolic profiles obtained by GC/MS analysis of the bacterial extracts.

EXPERIMENTAL

Both strains were grown with 4-CB for predetermined periods of time at 29°C in a shaking incubator under a CO₂ atmosphere (10%). The cells were removed by centrifugation and the supernatant extracted at pH = 7 and 3 with ethyl acetate. The ethyl acetate organic phases were evaporated and the residue derivatized and analysed by GC/MS. A Kratos MS-25 mass spectrometer was used in combination with a Perkin-Elmer Sigma 3 gas chromatograph equipped with a 30 m DB-1 capillary column. The GC/MS parameters are: injection mode: splitless; injector temperature: 250°C; column temperature: 140°C for 5 min, then programmed to 280°C at 5°C/min; source temperature: 250°C; electron energy: 70 eV; accelerating voltage: 3 kV and trap current: 100 µA. The mass spectra were recorded in the electron impact mode at a scanning speed of 1 sec/decade.

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry analysis of the bacterial extracts shows that 4-chlorobiphenyl afforded similar qualitative and quantitative metabolic profiles when incubated with strains B-218 and B-257. The major bacterial metabolite produced by both strains was 4-chlorobenzoic acid, which results from the extensive oxidative degradation of the non-chlorinated aromatic ring of 4-CB. Several other acidic metabolites were detected: a) 4-[p-chlorophenyl]-4-oxo-butanoic acid (base peak at m/z 139, resulting from the cleavage of the C₃-C₄ bond), b) 6-chlorophenyl-6-hydroxy-hexanoic acid, the TMS derivative mass spectrum of which is characterized by an intense ion at m/z 213 (Cl-CH₂-CH=OTMS), c) three isomeric diunsaturated acids which were identified as 2-hydroxy-6-p-chlorophenyl-6-oxo-hexa-2,4-dienoic acid. Their corresponding TMS derivatives were chromatographically resolved. However, the isomeric acids could not be differentiated by their respective mass spectrum which were very similar with a molecular ion at m/z 396 and a prominent ion at m/z 279 which is the result of the elimination of the -COOTMS group. In addition, the TMS derivatives of two other isomeric metabolites showing a molecular ion at m/z 456 and M-15 ion at m/z 441 were also detected. [²H₉] TMS labeling and diazomethane derivatization, strongly suggested that the metabolites had one -COOH group and two hydroxyl functions located on a chlorinated phenyl nucleus. Finally, by comparison with the mass spectra of other acidic metabolites, the bacterial metabolites were assigned the structure of 5-[p-chloro-2',3'-dihydroxy phenyl]-penta-2,4-dienoic acid.

In conclusion, both bacterial strains B-218 and B-257 were shown to possess the capability to degrade 4-chlorobiphenyl mainly into 4-chlorobenzoic acid. The biodegradation process includes a wide variety of intermediate acidic metabolites, some of which have been isolated and identified.

MPC 24 | IMPROVED SENSITIVITY AND SPECIFICITY FOR
PCB ANALYSIS BY THERMAL DESORPTION/CAPILLARY GC/MS;
A. Schkuta, J.L. Stauffer, P.L. Levins; Arthur D. Little,
Inc., Cambridge, MA 02140.

The use of a commercially-available sample concentrator coupled to high resolution capillary chromatography/mass spectrometry offers significant improvement over the standard EPA method for PCB analysis. Large sample volumes (typically 80 μ L) can be used to give detection limits that are comparable to other GC detectors, while maintaining the unambiguous identification inherently possible by mass spectrometry.

Results will be shown that demonstrate that PCBs can be readily identified and quantified in complex sample matrices that produce considerable sample interferences when other types of detectors are used. This method eliminates false-positive determinations, and detects PCBs from combustion products and complex mixtures that may not be recognized as such when using other GC detectors, where similarity to standard Arochlor mixtures is the major identification criteria.

The sample size used for analysis is not limited by volume considerations, but is rather a function of the complexity of the sample matrix. The dynamic range of sample volume used (5 to 100 μ L are routinely used) allows for similar sample preparation of both "clean" and "dirty" samples. Use of the concentrator also extends column life and reduces ion source contamination.

Quality control and sample preparation of complex organic mixtures will also be discussed.

THE ANALYSIS OF AMDRO® FIRE ANT INSECTICIDE (AC 217,300) RESIDUES IN PASTURE GRASS AND CROP SAMPLES BY GAS CHROMATOGRAPHY-NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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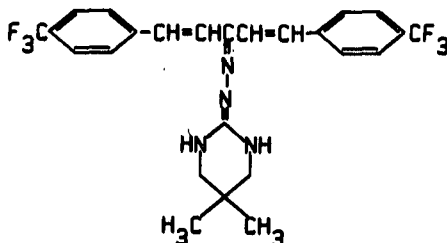
Introduction

AMDRO fire ant insecticide, the active ingredient of which is AC 217,300, is an amidinohydrazone type of insecticide. AMDRO fire ant insecticide is conditionally registered in the U.S. as an insecticide for the control of fire ants, harvester ants, and big headed ants. Residues of AC 217,300 in pasture grass and other crop samples can be determined by HPLC with detection at 300 nm following extraction of the analyte from the commodity and clean up on an XAD-2 column. Confirmation of the HPLC peak as AC 217,300 can be accomplished by GC-ECD.

Objective

To explore the utility of gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICIMS) for the analysis of AC 217,300 residues in crude extracts of pasture grass and other crop samples.

Structure



Experimental

Analyses of AC 217,300 residues in pasture grass and crop samples using multiple ion detection (MID) employed the following analytical conditions:

1. GC-MS Instrumentation	Finnigan 4000
2. Data System	INCOs 2300
3. Column	3% OV-1 on 60/80 Gas Chrom Q (4' X 2 mm ID)
4. Carrier-Reagent Gas	CH ₄
5. Column Oven Temperature	280°C
6. Injection Port Temperature	300°C
7. Interface Temperature	300°C
8. Source Temperature	350°C
9. Methane Flow Rate	15 ml/min
10. Source Pressure	400 microns
11. Conversion Dynodes	+ 3.0 Kv
12. Electron Multiplier	- 1.0 Kv
13. Preamplifier Range	10 ⁻⁸ amp/volt
14. Ions Monitored	m/z 494 ⁻ , 492 ⁻

Conclusions

A method of analysis for residue levels (0.05 ppm) of AC 217,300 in pasture grass and crop samples has been developed using gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICIMS). Using methane as the GC carrier gas-CI reagent gas, NICIMS generates almost solely the M⁺ ion of AC 217,300 at m/z 494. By applying multiple ion detection (MID) techniques, GC-NICIMS demonstrated a linear response range of 40 pg to 400 pg. Based on an injection aliquot corresponding to 2 mg of the commodity, this response range is equivalent to 0.02 ppm to 0.20 ppm of AC 217,300 in the commodity.

Sample work up prior to GC-NICIMS analysis is minimal and requires only extracting 20 grams of the commodity with 200 ml of CH₂Cl₂, drying the extract with Na₂SO₄, and stripping the extract to a final volume of 50 ml. Control extracts of the commodities analyzed show apparent AC 217,300 residues of less than 0.002 ppm. The good recoveries obtained on fortified samples (Table I) suggest that satisfactory quantitation can be achieved by bracketing external standards. Instances of low recoveries, especially from oily commodities such as whole green soybeans with pods, can be overcome by use of an CH₃CN/hexane partitioning step prior to GC-NICIMS analysis or by use of a carbon-13 labeled internal standard. The analysis of crude extracts by this analytical methodology is feasible because of the high electron capture cross-section of AC 217,300, the high molecular weight of AC 217,300, the mass specificity of NICIMS, and the absence of interferences with the AC 217,300 response from extracts of control commodities.

Table I: Recovery Data for AC 217,300 from Commodities

Commodity	ppm Control	Avg. % Recovery 0.05 ppm Fort. Control (1)	Avg. % Recovery 0.05 ppm Fort. Sample	Avg. % Recovery 0.1 ppm Fort. Sample
Bahia Grass	0.001	91.2	80.9	79.6
Alfalfa	<0.001	81.2	60.4	50.1
Soybean Foliage	<0.001	85.4	70.9	71.7
Broccoli	<0.002	87.8	80.3	80.5
Corn Silage	0.001	99.4	90.6	85.9
Leaf Lettuce	0.001	98.1	119.6	105.4
Whole Green Soybeans with Pods	0.002	61.2	NA (2)	NA (2)

(1) This solution was prepared by spiking 10 ml of the 50 ml control extract with 200 ng of AC 217,300. The resulting solution was equivalent to the extract obtained from the commodity fortified at 0.05 ppm with AC 217,300 if 100% extractability was achieved.

(2) NA = Not analyzed.

Characterization of Formaldehyde Oligomers by Capillary GC/Ammonia CI. Millington, D. S., Utterback, D. and Gold, A. R. Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, NC 27514.

In solution with water and methanol, formaldehyde forms a complex mixture of oligomers consisting mostly of a series of polyoxymethylene glycols, $\text{HO}(\text{CH}_2\text{O})_n\text{H}$, and their corresponding monomethyl ethers, $\text{MeO}(\text{CH}_2\text{O})_n\text{H}$ (1).

Analysis of these mixtures has hitherto been frustrated by the lack of a satisfactory method for the separation and definitive characterization of the individual components. The oligomers can be stabilized for GC/MS analysis by formation of TMS derivatives (2), which unfortunately do not exhibit molecular or differentiating fragment ions in their EI mass spectra. There was no significant improvement in our hands using CI with either of the commonly used reactant gases methane or isobutane.

In contrast, the TMS derivatives formed stable adducts with ammonium ion when ammonia was used as reactant gas, enabling their definitive characterization without difficulty (3). On most types of non-polar GC column, including a 30 m capillary with chemically bonded OV-1 equivalent liquid phase, oligomers of the type $\text{MeO}(\text{CH}_2\text{O})_{n+1}\text{TMS}$ overlap almost exactly with those corresponding to $\text{TMSO}(\text{CH}_2\text{O})_n\text{TMS}$, but the two series types were satisfactorily resolved on a 30 m capillary coated with SE-54 equivalent liquid phase (J&W Scientific DB-5N, 1μ thickness) (Figure 1).

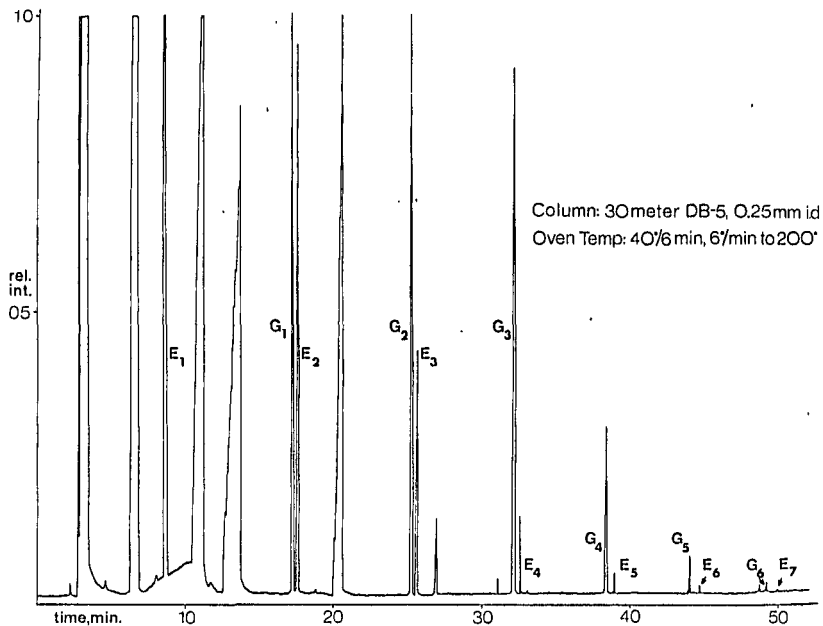


Figure 1. Resolution of formaldehyde oligomers as TMS derivatives on a 30 m DB-5 capillary GC column. Series G: polyoxymethylene glycols $n=1-6$; series E: glycol monomethyl ethers $n=1-7$.

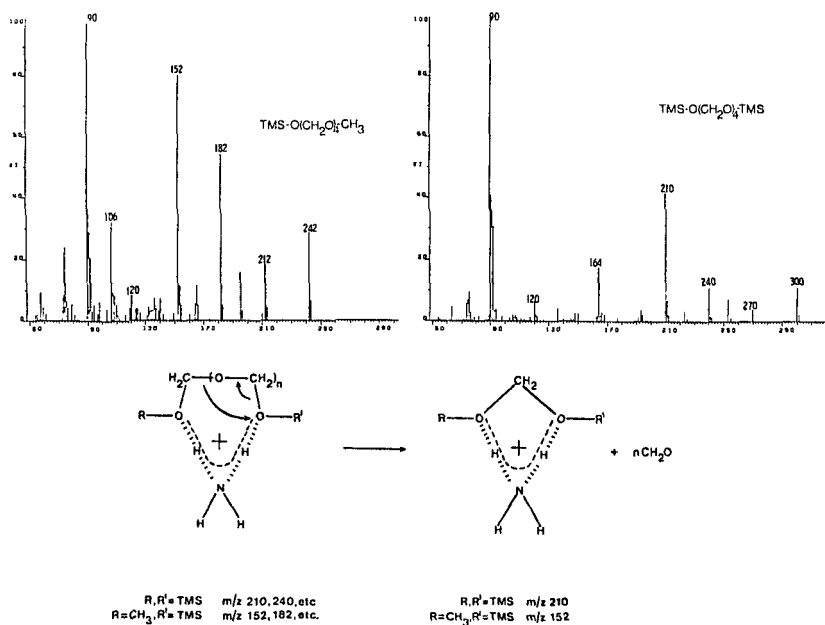


Figure 2. Examples of ammonia CI spectra from formaldehyde oligomers; corresponding to peaks E₄ and G₄.

Representative mass spectra from each series (Figure 2) are consistent with elimination of formaldehyde units from the (M + NH₄) adducts. Accurate mass measurements, performed by peak matching at 8,000 resolution during CI/MS using a VG-7070F double focusing spectrometer, support the proposed structures and postulated mechanism (Figure 2) to account for the observed spectra.

The ability to resolve and identify the formaldehyde oligomers will facilitate the total analysis of formaldehyde solutions and the headspace above the solutions.

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Analysis of non-Consent Decree Organic Pollutants in Consent Decree Survey Samples. J. T. Bursey, Analytical Sciences Division, Research Triangle Institute, Research Triangle Park, NC 27709 and W. M. Shackelford, USEPA, Athens Environmental Research Laboratory, Athens, GA 30613

As a result of the June 1976 Consent Decree (1,2), EPA agreed to prescribe the best available treatment (BAT) for industrial wastes in 21 industrial categories. The chemical analysis program to support this work involved the analysis of over 4000 samples from 46 industrial categories and subcategories. Addressed specifically were 129 priority pollutants (114 organics, 13 metals, cyanide, asbestos). After the screening analysis for the 114 organics, magnetic tapes containing the raw GC/MS data for each sample were sent to EPA's Athens Environmental Research Laboratory along with the corresponding extract(s). There, computerized analysis of the GC/MS data was performed to determine compounds not on the 114-compound list. After computer analysis at the Athens laboratory, selected extracts were reanalyzed at the Research Triangle Institute to confirm the computer identifications. Although the computer program at Athens Environmental Research Laboratory provides an indication of a "level of confidence" for the compound identifications, a higher level of confidence than simple comparison of a mass spectrum with a mass spectral data base was desired. Thus, in the initial portion of the program, the following steps were performed: 1) the extract was reanalyzed under capillary gas chromatography conditions; 2) authentic standards of identified compounds were obtained and analyzed under the same conditions used for capillary GC analysis of sample extracts, to obtain response factors and relative retention time data; and 3) the extracts were spiked with the authentic standards of the compounds of interest to confirm identity (coelution of standard and tentatively identified compound with no change in the mass spectrum).

In the second phase of the reanalysis, capillary analysis was used on industrial effluent extracts for which computerized analysis had not identified spectra that were observed several times. Two questions were examined: 1) could the spectrum still be observed under the new set of analysis conditions? and 2) could the compound now be identified by a computerized library search with capillary resolution? The answer to the first question, in the vast majority of the approximately fifty cases studied, was that a similar or identical spectrum was observed in the reanalysis. The answer to the second question was an unequivocal no; the compound identification was not dependent upon conditions of better chromatographic resolution.

Efforts to identify the compounds of interest included capillary gas chromatography with accurate mass measurement to $\pm 5 \times 10^{-3}$ daltons, usually as the first step, and based upon the results of this measurement, further decisions were made. Chemical ionization (positive or negative mode) was used to add information about the nature of the compound of interest, using accurate mass measurement on the M+1 species. An additional characterization method (a coupled technique) employed a Unacon extract concentrator, a capillary gas chromatograph, and a Fourier transform infrared spectrometer. Although the sensitivity of the Fourier transform infrared spectrometer was, in general, inferior to the capillary GC/MS by one or more orders of magnitude, the use of the extract concentrator appeared to aid in overcoming this deficiency to yield valuable structural information.

The work was supported by EPA Contract No. 68-03-2867.

- 1) Settlement Agreement, NRDC et al. vs. EPA, U.S. District Court for the District of Columbia, June 7, 1976.
- 2) Joint Modification of Settlement Agreement, U.S. District Court for the District of Columbia, March 9, 1979.

Results of Tuning a Double Focusing
Mass Spectrometer using DFTPP

by

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Reference compounds have been used in mass spectrometric analysis for many years. Spectra in the API Project 44 collection included the ratio of pairs of peaks in either *n*-butane or *n*-hexadecane as an indication of tuning. Many of those spectra were obtained on 180° magnetic instruments of essentially the same design and this control was adequate. Sporadic attempts at that time to standardize 90° instrument performance with that of 180° instruments were generally unsuccessful. With the explosive proliferation since 1971 of quadrupole mass spectrometers as a consequence of their use for computerized GC/MS identifications, it again has become highly desirable to standardize performance among many types of instruments. The increasing use of GC/MS for the qualitative/quantitative analysis of solid waste samples has made standardization even more important.

Eichelberger et al.¹ chose a reference compound, decafluorotriphenyl phosphine (DFTPP), that gave reasonable peaks over the mass range of general interest in GC/MS, and they selected intensity ranges at a number of masses that would indicate instrument performance was satisfactory. Performance criteria were based largely on well-tuned Finnigan 1015 quadrupoles. It has been possible to tune more recent quadrupoles to meet the DFTPP criteria either directly or by mathematical processing of a measured spectrum; however, magnetic instruments frequently cannot be tuned to meet the base peak specification.

In the present study, six parameters that were expected to affect the cracking pattern of DFTPP were chosen for a statistical study. Source temperature, ionizing current, electron multiplier voltage, quantity of DFTPP, accelerating potential, and the mass at which source tuning was optimized were varied in a Latin Square design. The relative abundances of criterion ions of DFTPP were measured as responses. Data analysis indicated both that not all responses were sensitive to the same variables and that several of the variables interacted strongly.

Based on the batch inlet system study, a set of conditions was selected and demonstrated that satisfied the DFTPP criteria in all respects. The same conditions were evaluated with a GC inlet, and it was found that GC oven conditions had to be adjusted to maintain the DFTPP cracking pattern criteria. Using these final conditions, spectra were obtained of tricosane and methyl hexadecanoate. Comparison of these spectra with those obtained with the traditional tuning of the CH5-DF, indicated that the cracking pattern from the instrument under DFTPP conditions approximated that from a quadrupole mass spectrometer. The ratio of the molecular ion of tricosane (m/z 324) to the 57 peak for the DFTPP tuning was an order of magnitude less than for the normal tuning; for the methyl hexadecanoate comparison, the ratio of the molecular ion (m/z = 270) to the 74 peak was decreased by a factor of 5.

Because of the importance of molecular ion information in either spectra matching or spectra interpreting, it is undesirable to bias instrumental conditions against sensitivity at higher masses. It is concluded, therefore, that the present specifications, which were written primarily for quadrupole instruments, are achievable, but are not appropriate to tuning the CH5-DF/GC for trace analysis.

NOTE: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

¹ Eichelberger, J. W., L. E. Harris, and W. L. Budde, "Reference Compound to Calibrate Ion Abundance Measurements in Gas Chromatography-Mass Spectrometry Systems," Anal. Chem., 47, 995 (1975).

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ROLE OF MASS SPECTROSCOPIC CONFIRMATION FOR ROUTINE
METHODS IN ENVIRONMENTAL ANALYSIS

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Introduction. The analysis of environmental samples for toxic residues with routine methods may be complicated by interferences, if complex matrices are involved. Certain environmental and biological samples are so complex that the absence of interferences must be demonstrated by a second analytical technique. Confirmatory studies by gas chromatography/mass spectrometry (GC/MS) have the requisite specificity to assure that the target compound is present. Mass spectrometry has the sensitivity to allow split-duplicate analysis with both routine and confirmatory methods. The studies described in this presentation reflect our experiences in supporting a routine chromatographic laboratory engaged in trace organic analysis.

Case 1. In conducting an investigation of suspected worker exposure to several agricultural pesticides, we were requested to determine concentrations of Iorsban, DEF (tributyl phosphorotrithioate), disulfoton, and pydrin in various biological and environmental samples. Gas chromatography with flame photometric detection was chosen as the routine method. Mass spectrometric confirmation was requested for selected positive samples. A VG ZAB-2F GC/MS system in full scan, low resolution mode with a VG 2250 data system was used for confirmation.

In the GC/MS analysis of several samples thought to contain disulfoton, a peak eluting near the retention time of the analytical standard was observed, but the mass spectrum was inconsistent with the target pesticide. Apparently, this compound constituted an interference under conditions used for the routine method, being observed by the element-specific detector and exhibiting the appropriate retention time.

Adequate confirmation for the other pesticides was obtained by using this GC/MS approach.

Case 2. Another investigation concerned possible worker exposure to marijuana dust from a storage facility in legal offices. The approach we used was to analyze for Δ^9 -tetrahydrocannabinol (THC) in various dust samples and to use this compound as a marker for the presence of marijuana. A routine high performance liquid chromatographic (HPLC) method with electrochemical detection indicated a number of positive samples, some from the marijuana storage rooms and some samples from neighboring (non-storage) rooms.

Several different analytical techniques, including thin layer chromatography and electron capture gas chromatography, were examined in attempts to provide confirmatory data. The complex sample matrix made such confirmation impossible. The application of capillary GC/MS with the VG ZAB in the selected ion recording mode was successful in qualitative confirmation. Only those dust samples taken from the drug storage room yielded positive GC/MS results for THC. No neighboring rooms were judged to be positive, contrary to the conclusions from the routine method. On the basis of the large number of false positives, the HPLC routine method was judged to be unsuitable for this application.

Case 3. A third investigation in residue analysis concerned individuals with a history of exposure to environmental DDT. The conventional approach has been extraction of serum, sample cleanup by column chromatography, followed by analysis by electron capture gas chromatography. The individuals studied showed significantly increased serum levels of the DDE isomers (metabolites of DDT). A selected subset of sample extracts was submitted for mass spectrometric confirmation.

A Finnigan 4000 GC/MS system with the INCOS 2300 data system was used in a selected ion recording mode for the problem. All samples from this subset were confirmed for the presence of both p,p'-DDE and o,p'-DDE.

Confirmation of p,p'-DDE was established by meeting the following analytical criteria.

1. Retention time was within 3 seconds of the authentic standard.
2. Five ions monitored ($m/z = 176, 246, 248, 316, \text{ and } 318$) maximize within one scan of each other.
3. Isotope ratios were within $\pm 4\%$ of the mean value observed for the authentic standard.

Confirmation of o,p'-DDE was conducted with somewhat relaxed criteria because of lower compound concentrations.

1. Retention time observed within 2 seconds of the standard.
2. Four ions monitored ($m/z = 246, 248, 316, \text{ and } 318$) maximize within two scans of each other.
3. Isotope ratios were within $\pm 25\%$ of the mean observed for the standard.

Closing. As these studies demonstrate, the presence of interferences is a legitimate concern when a complex sample matrix is involved. The situation will continue to be a problem as unconventional detector systems are applied to environmental analysis. Certainly, method development should include a demonstration of specificity with actual test samples. Mass spectrometry is an ideal second analytical technique for validation data obtained by routine methods.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health and Human Services.

TOA 1 | ION-MOLECULE REACTIONS OF PHOSPHORUS-CONTAINING IONS: IMPLICATIONS FOR INTERSTELLAR DETECTION.
V. G. Anicich and W. T. Huntress, Jr., Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109 and L. R. Thorne, Sandia National Labs, Livermore, CA 94550

The chemistry of P^+ and PH^+ ions with CO_2 , H_2O , O_2 , N_2 , CO , and HCN has been examined using ion cyclotron resonance spectroscopy. In addition we have studied the reactions of the PCH_2^+ , PCH_3^+ , PNH_2^+ , PNH_3^+ , PO^+ , HPO^+ , and H_2PO^+ ions with H_2 and CO . This complements our previous studies of the PH_n^+ ions with PH_3 , NH_3 , and CH_4 (where $n = 0$ to 4). We have utilized this information together with a chemical model to predict which products might be expected from ion-molecule chemistry in dense interstellar clouds. The model predicts that the majority of the phosphorus will be in the form of the PO molecule at chemical equilibrium. We expect this information will be useful to the radio astronomers looking for interstellar molecules containing phosphorus.

This research was performed at the Jet Propulsion Laboratory under contract with The National Aeronautics and Space Administration.

TOA 2 | UNIMOLECULAR AND BIMOLECULAR REACTIONS IN THE $C_6H_6^+$ SYSTEM: EXPERIMENT AND THEORY; Martin F. Jarrold, Winfried Wagner-Redeker, Nicholas Kirchner, Andreas J. Illies and Michael T. Bowers; Department of Chemistry, University of California, Santa Barbara, CA 93106.

$C_6H_6^+$ collision complexes have been made by ion-molecule reactions using the reagents $l-C_4H_4^+/C_2H_2$, $c-C_4H_4^+/C_2H_2$, $C_2H_2^+/l-C_4H_4$, and $C_6H_4^+/H_2$. Absolute rate constants, product distributions and isotopic scrambling have been measured on a tandem ICR spectrometer. Kinetic energy distributions of the products of the metastable reactions forming $C_6H_5^+/H$, $C_6H_4^+/H_2$, $C_4H_4^+/C_2H_2$ and $C_3H_3^+/C_3H_3$ from electron impact on benzene have been measured on a high-resolution reverse geometry mass spectrometer as a function of ion source temperature. Photoion photoelectron coincidence methods have been used to measure absolute unimolecular rate constants and branching ratios as a function of energy in $C_6H_6^+$ ions formed from a number of C_6H_6 neutral isomers [data from the literature]. A statistical theory model has been developed based on the transition state switching concept and applied to the experimental data. Mechanistic information is obtained from the comparison.

TOA 3 | COMPARATIVE STUDY OF THE REACTIVITY OF STATE
SELECTED IONS USING A TANDEM/TRIPLE MASS SPECTROMETER*

Imre Szabo, Chemistry Centre, P.O.Box 740, S-220 07 Lund,
Sweden

The aim of this paper is to show that in tandem and triple mass spectrometers it is possible a) to produce continuous beams of ions in selected electronic states with various degrees of vibrational excitation and b) to compare the reactivity of state selected ions.

In a tandem mass spectrometer of perpendicular type the sample is ionized by a beam of slow incident ions with known recombination energy¹. The internal energy of the molecular ions formed can be estimated and altered by ionizing with different incident ions in succession. Consecutive ion-molecule reactions can be studied as a function of this energy.

A triple mass spectrometer consists of three mass spectrometers in series with a mass and energy selected ion beam in each. In a triple instrument a molecular or a fragment ion produced in the second mass spectrometer is used to ionize a "test-gas" in the ion source of the third mass spectrometer. This allows us to compare the reactivity of one particular ion originating from the same or different molecules at different values of internal energy of the parent molecular ion. It is shown that CO^+ , CH_2^+ and C_2H_2^+ can be produced in different electronic states which cause different reactions with molecules of the same "test-gas".

1. E. Lindholm, in "Ion-Molecule Reactions" (editor J.L. Franklin), Vol. 2 p. 457, Plenum Press, New York, 1972.
 2. I. Szabo, in "Advances in Mass Spectrometry" (editor N.R. Daly), Vol. 7, p. 258, Heyden, London, 1978.
- * Work supported by the Swedish Natural Science Research Council.

TOA 4 | THE TEMPERATURE DEPENDENCE OF THE COLLISION
EFFICIENCIES OF EXOERGIC ION-MOLECULE REACTIONS; Michael
Henchman, Dept. of Chemistry, Brandeis University, Waltham,
MA 02254.

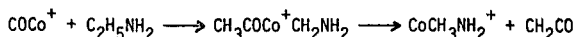
Considered are the collision efficiencies η of exoergic ion-molecule reactions at temperatures where the intermediate may be expected to behave ergodically. The approach considers only the energetic difference between reactants and products, ignoring structure. The kinetic behavior is determined by the reduced temperature $T_R = -RT/\Delta E^\ddagger$.

Analysis produces the general results: for $T_R \ll 1$, $\eta \sim 1$ and $d\eta/dT \sim 0$, and for $T_R > \sim 1$, $\eta < 1$ and $d\eta/dT < 0$. Predictions will be compared with experimental data for particular reactions.

GAS PHASE ORGANOMETALLIC CHEMISTRY: LIGAND EFFECTS; J. Allison, S.K. Huang, M. Lombarski, S. McElvany, B. Radecki, A. Tsarbopoulos, Department of Chemistry, Michigan State University, East Lansing, MI 48824

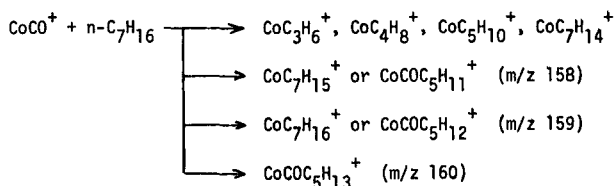
Ion/molecule reactions between metal ions (e.g. Co^+) with organic molecules have yielded insights into the basic processes of organometallic chemistry. When metal ions (M^+) are generated from electron impact on organometallic compounds, the reactions of ions of the type ML_n^+ can also be studied. The term "ligand effects" can be an all-encompassing classification for reactions of a metal ion with organic molecules which vary as the number and types of ligands on the metal vary.

Comparison of the Chemistry of M^+ and ML^+ . The presence of a ligand can have profound effects on the chemistry of a metal ion. For example, Co^+ reacts with many amines to form the product $\text{CoCH}_3\text{NH}_2^+$. This product is also observed as an ion/molecule reaction product in a mixture of $\text{Co}(\text{CO})_3\text{NO}$ and ethylamine. This would correspond to loss of CH_2 : which is unlikely. In this mixture, however, the precursor was not Co^+ but CoCO^+ .



In this case, CO is actively involved in the reaction. CoCO^+ inserts into the C-C bond, i.e., CO is not merely a "spectator". A number of reactions of this type have been observed.

A unique reactivity alteration occurs when one CO is added to Co^+ in its reaction with *n*-heptane. Co^+ forms 7 products in its reaction with *n*-heptane. Insertion into each C-C bond occurs, as well as insertion into C-H. In all cases, products are of the type $\text{Co}(\text{C}_n\text{H}_{2n})^+$ or $\text{Co}(\text{C}_n\text{H}_{2n-2})^+$. CoCO^+ forms many of the same products with *n*-heptane, and some unusual products.



The products at *m/z* 158 and 160 are unique because they correspond to a radical loss as opposed to an alkane (or H_2) loss. CID results on the products 158, 159 and 160 are reported.

Comparison of the Chemistry of ML_1^+ and ML_2^+ . The type of ligand bound to the metal can affect the reactivity. For example, both Co^+ and $CoCO^+$ react with all primary, secondary and tertiary amines. $CoNO^+$, in contrast, is unreactive. The NO ligand may "deactivate" the metal by donating significant electron density to the metal, and by complexing two metal orbitals.

Comparison of the Chemistry of ML^+ and ML_n^+ . In the case of metal carbonyls, as the number (n) of CO's on metal centers (e.g., Fe^+ , Cr^+) increases, reactions observed change from "molecular rearrangements" (for $n = 0, 1$) to ligand substitution reactions ($n > 1$). With bulky reactant molecules such as crown ethers, the number of ligands bound to a transition metal profoundly affect reactivity. The $M(CO)_n^+$ ($M = Fe^+, Cr^+$) ions where $n \geq 3$ are completely unreactive. This has not been observed for smaller molecules. Presumably if more than 3 CO's are attached to a metal ion, these ligands can prevent crown ethers from getting close enough to the metal for significant orbital overlap.

Reactions of Group Ib Metals and Selected Organics in the Wake of a Spark

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Spark source mass spectrometry (SSMS) has been used extensively for a number of years in the area of trace elemental analysis (1). The technique produces atomic ions by causing a high voltage radio frequency breakdown between two electrodes which contain the sample of interest. The spark discharge can therefore be used as a source of metal ions.

The reactions of metal ions with organic neutrals in mass spectrometry has only recently become the focus of extensive research. Such experiments have been performed in ion cyclotron resonance (2), secondary ion mass spectrometry (3), an ion beam apparatus (4), field ionization and field desorption (5), as well as several other techniques. This presentation reports the initial results obtained in the reactions of metal ions with organic neutrals in SSMS.

An AEI MS 702 spark source mass spectrometer has been modified to allow for the controlled addition of organic vapors into the source region of the instrument to pressures on the order of 1×10^{-4} torr. The ions produced by the sparking of the electrodes containing the metal of interest react with the organic vapors present to produce organometallic ions. The product ions may include the intact metal ion/organic neutral adduct as well as fragment or rearrangement products.

The reactions of Cu^+ and Ag^+ with methyl acetate formed similar products whose formulas were confirmed by reaction of the metal ions with $\text{CD}_3\text{COOCH}_3$ and $\text{CH}_3\text{COOCD}_3$. The products include MCH^+ , MCH_2^+ , MCH_3^+ , MOH^+ , MH_2O^+ , MCO^+ , MCH_2O^+ , MCH_3O^+ , and, in the case of Ag^+ , $\text{MC}_3\text{H}_8\text{O}_2^+$. Similar results are seen upon the reaction of these metal ions with ethyl acetate with the major differences being the formation of MC_2H_5^+ here as well as a larger extent of formation of the MH_2O^+ species and an increase in the relative abundance of Ag^+ /ethyl acetate adduct in comparison to the Ag^+ /methyl acetate adduct described above. These results are quite different from the results obtained with a Cu^+ methyl or ethyl acetate system as studied in ion cyclotron resonance (2a).

The formation of the intact metal ion adducts observed with silver ions but not with copper ions is ascribed to the differences in their excited state energy levels. The lowest levels of Ag^+ are of much higher energy than those of Cu^+ and therefore much lower population of silver ions in their excited states than with Cu^+ and therefore there will not be a large excess of energy in the collision complexes containing the silver ions as may exist with Cu^+ . This argument requires that Au^+ must behave more similarly to Cu^+ than to Ag^+ since its lower energy levels are closer in energy to those of Cu^+ . This prediction is borne out by the reactions of Cu^+ , Ag^+ , and Au^+ with 2,4-pentanedione in which the silver ion forms the intact adduct ion as its largest product ion whereas this ion is not observed with either Cu^+ or Au^+ .

The reactions of Cu^+ , Ag^+ , and Au^+ with cyclohexene show a similar pattern in which Ag^+ forms the intact adduct ion whereas Cu^+ and Au^+ do not. Ions which may be formed by a decyclotrimerization reaction in which the cyclohexene is fragmented into one acetylene and two ethylene molecules with one of these fragments attached to the metal ions is observed. Similar results are obtained for the reactions of these three metal ions with benzene in that the silver ion forms an intact adduct ion and a decyclotrimerization to produce three acetylene molecules with one attached to a metal ion is observed for Cu^+ and Au^+ . There is also evidence for the insertion of Au^+ into C-H bonds in that an AuC_6H_4^+ is formed.

References

- (1) See, for example, A. J. Ahearn (Ed.), *Trace Analysis by Mass Spectrometry*, Academic Press, New York, 1972.
- (2) See, for example, (a) R. C. Burnier, G. D. Byrd, B. S. Freiser, *Anal. Chem.*, 52 (1980) 1641. (b) R. W. Jones, R. H. Staley, *J. Am. Chem. Soc.*, 102, (1980) 3794. (c) J. Allison, D. P. Ridge, *J. Am. Chem. Soc.*, 103 (1981) 4998.

- (3) See, for example, R. J. Day, S. E. Unger, R. G. Cooks, *J. Am. Chem. Soc.*, 101 (1979) 499.
- (4) See, for example, P. B. Armentrout, J. L. Beauchamp, *J. Am. Chem. Soc.*, 103 (1981) 784.
- (5) See, for example, F. W. Röllgen, H. R. Schulten, *Org. Mass Spectrom.*, 10 (1975) 660.

TIME-SLICED STUDIES OF ION-MOLECULE REACTIONS
USING THE CAPILLARITRON ION SOURCE

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The capillaritron ion source (Phrasor Scientific, Duarte, Ca.) creates an intense, somewhat directional ion beam from a microdischarge located at the end of a 25 μ m hole in a tungsten cone, through which the gas of choice is flowing and applied to which is a positive high voltage. For this work, a capillaritron ion source has been used as the ion source of a double focusing reverse geometry mass spectrometer (VG ZAB-2F).

If it is accepted that ions are initially created at one point, then ions created by subsequent reaction at any distance from the starting point will have an energy deficiency determined by the electric field potential at the reaction point. By adjusting the applied voltage, such ions may be brought into the ZAB energy window, with the required offset precisely equaling the energy deficiency.

By recording the mass spectra obtained at each offset voltage and correcting for variations in the total ion current (TIC) (i.e., plotting spectra as %TIC), a picture of the spatial variation of the ion populations begins to emerge. In favorable cases, it is obvious from these plots that certain ions react with neutrals in the gas jet to produce new ions. Thus, the ion-molecule reactions are defined by these plots.

Since the applied voltage can, by calculation and calibration, be related to the time between initial ion formation and the formation of the product ion, time-sliced ion-molecule reaction studies can be performed with this ion source.

A number of gaseous reaction systems have been studied, including air, air/ethanol, air/toluene, and methane. The presence of known reaction pathways (e.g., $N^+ + O_2 \rightarrow NO^+$ and $CH_4^+ + CH_4 \rightarrow CH_5^+$) can be seen in the graphs, as well as other reaction channels.

Graphical representation of the results, deduced reaction pathways, and some estimates of reaction rates will be discussed, as well as some details of the calculations required and a discussion of factors complicating the analysis.

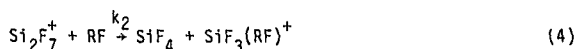
ION/MOLECULE REACTIONS OF SiF_4

W. D. Reents, Jr. and A. M. Majsce
Bell Laboratories
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The reactions of ions derived from SiF_4 with the neutral molecule are limited:



where k_1 is found to be 2.9×10^{-10} cc/molec-sec. Reactions may be divided into two types: fluoride abstraction (3) and trifluorosilyl cation (SiF_3^+) transfer (4).



Exploitation of these reactions using a Fourier Transform Mass Spectrometer coupled with its capability for exact mass measurement provide a means for identification of the impurity compounds (cf. Table I). This is extremely useful since reference compounds for chromatographic or infrared analyses are not commercially available.

Quantification is impossible without the reference compounds. A semi-quantitative technique is possible which again exploits the ion chemistry. For reactions (2) and (4) the rate of formation of the product ions are

$$\frac{\partial(\text{Si}_2\text{F}_7^+)}{\partial t} = k_1 [\text{SiF}_4^+] [\text{SiF}_4] \quad (5)$$

$$\frac{\partial(\text{SiF}_3\text{RF}^+)}{\partial t} = k_2 [\text{Si}_2\text{F}_7^+] [\text{RF}] \quad (6)$$

Dividing equation 6 by equation 5 yields

$$\frac{\frac{\partial(\text{SiF}_3\text{RF}^+)}{\partial t}}{\frac{\partial(\text{Si}_2\text{F}_7^+)}{\partial t}} = \frac{k_2}{k_1} \frac{[\text{Si}_2\text{F}_7^+]}{[\text{SiF}_4^+]} \frac{[\text{RF}]}{[\text{SiF}_4]}$$

If we assume that trifluorosilyl cation transfer reactions have equal rate constants (i.e., $k_1 = k_2$) and the concentration of trifluorosilyl cations is constant (i.e., $[\text{Si}_2\text{F}_7^+] = [\text{SiF}_4^+]$), then the concentration of the neutral molecule may be estimated by the measurement of an appropriate initial slope:

$$\frac{\frac{\partial(\text{SiF}_3\text{RF}^+)}{\partial t}}{\frac{\partial(\text{Si}_2\text{F}_7^+)}{\partial t}} \approx \frac{[\text{RF}]}{[\text{SiF}_4]}$$

Although this is not rigorously correct, this method does provide estimates for the impurity concentrations which are currently unavailable. The results for a commercial sample of SiF_4 is presented in Table I.

TABLE I
IMPURITY LEVELS IN COMMERCIAL SiF_4

<u>COMPOUND</u>	<u>CONCENTRATION (%)</u>	<u>COMPOUND</u>	<u>CONCENTRATION (%)</u>
SiF_4	97.5	$\text{Si}_2\text{F}_5\text{HO}$	0.28
SiF_3H	1.2	SiF_2H_2	0.24
$\text{Si}_2\text{F}_6\text{O}$	0.73	$\text{Si}_3\text{F}_8\text{O}_2$	0.08

TOA 9 | REACTIVITIES AND STRUCTURES OF $C_5H_5^+$ IONS AND THEIR RELATIONSHIP TO SOOT FORMATION; F. W. Brill, T. J. Buckley, and J. R. Eyler; Department of Chemistry, University of Florida, Gainesville, Florida 32611 and S. G. Lias and P. J. Ausloos; National Bureau of Standards, Washington, D.C. 20234.

Ion-molecule reactions have been proposed as important in the process of soot nucleation in fuel-rich flames (1). We have begun (2,3) a program of measuring the rates of ion-molecule reactions involving known flame ions and neutrals, and of using a variety of mass spectrometric techniques to determine the structures of the various isomeric ions which may be involved in these reactions. Early work concentrated on $C_3H_3^+$ ions, but studies have now been extended to $C_5H_5^+$. Several isomeric $C_5H_5^+$ ions can be formed by electron impact on neutral precursors, and we have determined their ion-molecule reaction rate coefficients with a number of neutrals found in flames, and have carried out both experimental and theoretical studies to help determine which of many possible structures should be assigned to each isomer. Structure and reactivity results will be presented and their relevance to ionic soot formation mechanisms discussed.

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TOA 10 | THE TEMPERATURE DEPENDENCE OF THE RATE OF SIMPLE CLUSTERING REACTIONS: $N_2^+ + N_2 + M \rightarrow N_4^+ + M$;

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The temperature dependence of the rate constant for clustering of N_2^+ with N_2 has received much attention recently and is the center of some controversy. The available literature will be critically reviewed. We have recently constructed a drift-type high pressure ion source with co-axial electron beam that is temperature variable over the range 80-500 K. The title reaction has been studied over this range for $M=He$ (80-190 K), $M=Ne$ (80-312 K) and $M=N_2$ (265-455 K) at ionizing electron energies just above threshold (16 eV). At these energies all N_2^+ ions are formed at the electron entrance slit essentially 100% in $v=0$. A plot of the natural log of the measured third order rate constant versus temperature is linear in all cases (correlation coefficient 0.99). The rate constant can thus be written in the form

$$k = CT^{-m}$$

yielding $m=1.74$, 1.52 and 1.53 for $M=N_2$, Ne and He, respectively. The values of k at $T=300$ K are 6.1 , 1.4 and $0.9 \times 10^{-29} \text{ cm}^6 \text{ s}^{-1}$ for $M=N_2$, Ne and He, respectively. A statistical phase space theory model for the reaction has been developed. Comparisons between our results, theory, and literature values will be made.

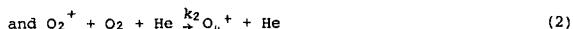
THE TEMPERATURE DEPENDENCES OF THE $N_2^+ + N_2 \rightarrow N_4^+$
AND $O_2^+ + O_2 \rightarrow O_4^+$ ASSOCIATION REACTIONS

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INTRODUCTION

The temperature dependences of three body association reactions have been investigated for many years in attempts to elucidate the mechanisms of ion-molecule association. Because of limitations in the experimental techniques, such studies have usually been carried out over limited temperature ranges (1). The variation with temperature of the three-body rate coefficients, k , is usually represented in terms of a power law, i.e. $k \propto T^{-n}$ (see below). Evidence is now becoming available from the body of data on such reactions which suggests that measurements over limited temperature ranges result in derived temperature dependences of k which are erroneously large (i.e. excessively large indices n). Clearly there is a need for studies over wider temperature ranges. Two experimental techniques which are particularly valuable in this respect are the variable-temperature Selected Ion Flow Tube (SIFT) developed at Birmingham (2,3) and the variable temperature ion Drift Tube (DT) developed at the Max-Planck Institut für Kernphysik in Heidelberg (4). The temperature ranges available to the SIFT and DT are 80 to 600 K and 30 to 400 K respectively. This represents an overall temperature range of 30 to 600 K, i.e. a factor of 20 change in temperature! These techniques have previously been used separately to study the temperature dependences of association reactions notably of CH_3^+ ions with several molecules (5) and of N_2^+ and O_2^+ ions in their parent gases (6). Now, in collaboration, we have undertaken to study several reactions over the uniquely wide temperature range available with these apparatuses and have initially studied the He stabilized reactions (7-10):



The aim of our experiments was to provide high quality experimental data which could be used as a critical test of recent theories of association reactions (11-14).

EXPERIMENTAL

The SIFT technique has been described in detail previously (2,3). N_2^+ and O_2^+ ions were created in a low pressure electron impact source (electron energy just above the threshold for ionization) mass filtered and injected into the He carrier gas (pressure ~ 0.5 Torr). It is known that $\sim 40\%$ of the N_2^+ ions produced in such sources are in the first vibrational level of the ground electronic state (15,16). Fortunately this vibrational excitation is efficiently quenched in collisions with N_2 (15,16) which must be added in relatively large concentrations (even at the lowest temperatures) to study the relatively slow reaction (1). Similar considerations apply to the reactant O_2^+ which is also expected to be in its ground vibronic state. In the SIFT experiments, reaction (1) was studied over the temperature range 80 to 520 K; however for reaction (2) the upper limit temperature was 200 K because of thermal decomposition of O_4^+ at higher temperatures.

The DT used in these studies has recently been described in detail (4). Again the ions were created in a low pressure electron impact source, mass selected and then injected into the He buffered drift tube (pressure ~ 0.03 -6 Torr). As in the SIFT experiments, any vibrational excitation in the reactant ions is quenched in collision with N_2 or O_2 . The ions then drift and react with the added N_2 or O_2 in a weak electric field before detection. Thus the energy gain in the electric field was insignificantly small (this was confirmed by experimental checks at different ratios of the electric field to gas number density, E/N). Reaction (1) was studied over the temperature range 45 to 400 K and reaction (2) from 48 to 200 K.

Very recently, the SIFT apparatus has been converted to a variable-temperature selected-ion flow drift tube (VT-SIFDT). With this apparatus, ionic reactions can be investigated at variable temperatures and variable E/N . Initially, as an extension to the present studies, we have used it to investigate the effect of E/N on the rate coefficient for reaction (1) and on the mechanism of collisional stabilization in the $N_2^+ + N_2 + N_2$ reaction (see below).

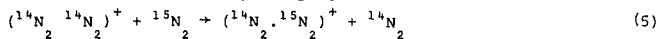
RESULTS

The combined data from the two experiments indicates that above 100 K the temperature dependences of k_1 and k_2 are

$$k_1 = 1.8 \times 10^{-29} \left[\frac{300}{T} \right]^{2.3 \pm 0.2} \text{ cm}^6 \text{ s}^{-1} \quad (3)$$

$$\text{and } k_2 = 5.5 \times 10^{-31} \left[\frac{300}{T} \right]^{2.7 \pm 0.3} \text{ cm}^6 \text{ s}^{-1} \quad (4)$$

The 300 K values for both k_1 and k_2 are in good agreement with previous values (7-10). Power law dependences are predicted by the recent theories by Bates (11,12) and by Herbst (13,14) which, more specifically, predict that $k \propto T^{-(\frac{1}{2} + \delta)}$ where $\frac{1}{2}$ is the number of degrees of freedom in the separate reactants ($\frac{1}{2} = 4$ in the reactions studied here) and δ describes the temperature dependence of the stabilization efficiency of the association reaction. Thus, for He collisional stabilization in reactions (1) and (2), δ is about 0.3 and 0.7 respectively. These values clearly differ from the δ values of about -0.3 and about 1.0 previously obtained in our DT for the N_2^+ and O_2^+ association reactions stabilized by N_2 and O_2 collisions respectively. These δ values are within the range indicated by Herbst's work (17) except for the negative value for which the theory makes no allowance. We have suggested (18) that the differences in the δ value for N_2 and He stabilization of $(\text{N}_4^+)^*$ excited complexes indicate very different stabilization mechanisms, with the stabilization by N_2 involving the switching of an N_2 molecule out of the $(\text{N}_4^+)^*$ complex rather than a super-elastic collision which presumably is the only process occurring in He collisions. Such a switching mechanism has been investigated at 80 K and 300K in the SIFT by studying the reaction



No reaction was observed with ground state N_4^+ ions. However when the energy of the interaction was increased in the VT-SIFDT, the reaction was observed to proceed at an increasing rate with increasing E/N reaching ~50% of the collisional rate coefficient at an E/N of ~70 Td. Under these conditions, the internal energy imparted to the N_4^+ ion in collisions with He carrier gas atoms is much less than the excitation energy of the N_4^+ intermediate complex when formed from N_2^+ and N_2 and thus reaction (3) is a viable stabilization mechanism. The efficiency of this switching reaction could also explain why the absolute value of the rate coefficient for the N_2 stabilized association reaction is several times greater than that for the He stabilized reaction. Interestingly, the overall rate coefficient for the N_2 stabilized reaction is almost independent of E/N (below 50 Td.) (19). However we have recently shown using the VT-SIFDT that k_1 changes dramatically with E/N!

In the DT study of reaction (2) below 100K, $\text{O}_2^+ \cdot \text{He}$ ions were observed as a significant component of the product ion spectrum. This suggests that, in addition to reaction (2), O_4^+ is produced by the "chaperon mechanism" viz:



The effect of these reactions on the determination of k_2 has been accounted for (20) by studying equilibrium in reaction (6). Below ~100K, the DT data apparently indicates a departure from the power law dependence of both k_1 and k_2 although this has been brought into question by some recent experiments conducted at temperatures as low as 20K (B.Rowe,priv.comm.).

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STABLE ISOTOPE RATIO ANALYSIS OF METALS USING FAB MASS SPECTROMETRY

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As many as 20 different metals perform functions necessary for life, growth and reproduction. It would therefore be desirable to have a method of following the absorption, distribution and excretion of metals in biological systems. Naturally occurring stable isotopes exist for many of these metals and may, in principle, be used as tracers. Although the relative abundance of stable isotopes of metals can be measured by several methods, there are very few reports of such tracer studies. The scarcity of results is related to the fact that, to be clinically useful, the method must be capable of measuring the isotopic abundance for many samples with high precision.

The present results show that FAB mass spectrometry may be an attractive alternative. Ions are desorbed from the surface of the sample by a beam of high energy xenon atoms and are analyzed by high resolution mass spectrometry. The relative abundance of isotopes may, therefore, be measured for metals which are present only as minor constituents of complex substances such as plasma and urine. Because of the low mass range and because the metals are mass deficient, a resolution of 5,000 is usually sufficient.

The detection of calcium in plasma is illustrated in Fig. 1 which is a plot of the ^{40}Ca ion signal as a function of time. Sample preparation for these measurements consists of placing 5 μL of plasma on the probe tip and drying in a stream of nitrogen. For a resolution of 5,000 and an FAB gun power of 7 watts, the ^{40}Ca signal remained greater than 10^5 ions/sec for 20 minutes. Since calcium is present in plasma at a level of only 100 ppm, these results show that extensive sample purification and preparation is generally not necessary.

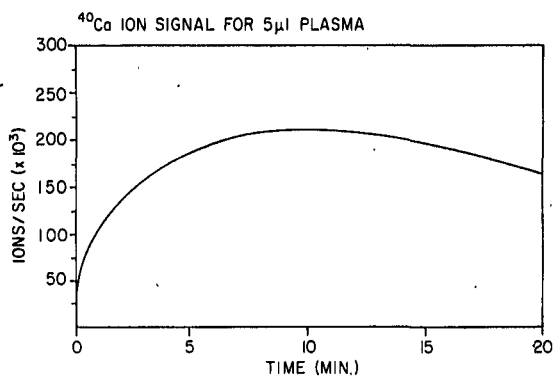
Sensitivity measurements were made on 1 μg quantities of pure samples of other metals to demonstrate the general utility of the method. These measurements were made using a resolution of 5,000 and are summarized in Table I which shows that more than 10^{10} ions/ μg are detected for the metals studied. These results suggest that FAB mass spectrometry can be used to accurately measure the isotopic abundance of metals which are present in complex biological samples.

TABLE I

The sensitivity of FAB mass spectrometry for different metal cations.

isotope	cou/ μ g	ions/ μ g
^{56}Fe	3.3×10^{-9}	2.0×10^{10}
^{64}Zn	5.3×10^{-9}	7.3×10^{10}
^{40}Ca	1.3×10^{-8}	8.1×10^{10}
^{24}Mg	6.6×10^{-8}	4.1×10^{11}

Figure 1



USE OF DOUBLE STABLE ISOTOPE TECHNIQUES IN MEASURING THE KINETICS OF BILE ACID TURNOVER; Fennessey, P.V., Everson, G., Kern, F. and Showalter, R.; University of Colorado School of Medicine, Mass Spectrometry Research Resource, Denver, CO 80262.

The state of the art method for the analysis of bile acid kinetics involves the use of a mechanical probe placed in the duodenum to collect bile near the opening of the common bile duct (sphincter of Oddi). Although stable isotopes and mass spectrometry are often used in the analysis of patients with suspected problems in bile acid circulation, the collection method is complex and risky. A number of laboratories are presently working on methodologies that involve simpler sampling media such as plasma (1-4).

Because of the analytical separation steps involved in bile acid analysis and the physiological constraints on the amount of isotopically labeled bile acid one could give to a patient, this analysis of isotope enrichment in plasma was a difficult task. A combination of improved instrument sensitivity, capillary chromatography and a dry injector system have alleviated the problems of sensitivity. Another problem that we encountered during the course of our analysis was a result of the constraint placed on this research by the limited availability of isotopically enriched bile acids. We have only been able to find compounds with a single ^{13}C enrichment for our studies. Further, the fact that these compounds fragment giving intense ions at a mass representing losses of the derivatized hydroxyl groups we found ourselves measuring enrichment of fragment ion clusters one mass unit apart. The di and tri hydroxy acetate derivatives gave fragment ions separated by two mass units. We began this project measuring m/z 368, 369, 370 and 371 and found a variable contribution to the small isotopically enriched cholic acid peak m/z 369 from the loss of an additional proton from the relatively large chenodeoxycholic fragment peak at m/z 370. This problem could be avoided by using the TMS derivatives which give a moderately intensive peak at m/z 458 for cholic acid.

We have carried out studies using artificial samples, using human volunteers and using patients with and without gall bladders. In these studies we have given $^2\text{H}_2$ enriched cholic acid or chenodeoxy cholic acid followed in a day by the corresponding $^{13}\text{C}_1$ isotope. In these studies we have taken multiple samples from plasma and bile to establish the relationship of all of these measurements of bile acid kinetics. This study has led to the following conclusions:

- 1) Bile acid concentration in plasma reflects the overall pool if the sample is taken after the gall bladder has been stimulated to contract and mixing has occurred.
- 2) Double isotope analysis works in patients but gives a C.V. of 25% for the pool size and fractional turnover rates calculated from pairs of data points.

An analysis of the errors leading to the large C.V. in the pool size and fractional turnover rate are in progress and preliminary data indicate that a large portion of the error is due to crosstalk in the peaks we are monitoring. Studies with $^2\text{H}_2$ -cholic acid substituted for $^2\text{H}_2$ -cholic acid are now being carried out to evaluate this hypothesis.

FRACTIONAL TURNOVER RATES (day $^{-1}$)

Patient Number	1*	2*	3	4
Multipoint analysis	.90	.28	.32	.17
Double isotope (sera)	.83	.25	.30	.22
Double isotope (bile)	.78	.22	.31	.34

* 1 & 2 - no gall bladder

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MEASUREMENT OF ENERGY EXPENDITURE BY DOUBLY LABELED
WATER IN HUMANS: SOURCES OF ISOTOPIC VARIATION.

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The doubly labeled water method is a noninvasive method for measuring energy expenditure in free-living subjects. The method was developed by Lifson et al (1) following an earlier collaborative study with Nier in which it was shown that the oxygen atoms in expired CO_2 were in isotopic equilibrium with body water (2). Because of this isotopic exchange, the kinetics of water elimination and respiration are interrelated. After a loading dose of $^2\text{H}_2^{18}\text{O}$, the ^{18}O is eliminated as H_2O and CO_2 ; while the ^2H is eliminated only as H_2O . Thus, the difference between the two isotopic elimination rates is a measure of CO_2 production, and hence energy expenditure.

Over the last three decades, the unique capabilities of the doubly labeled water method have been used to measure energy expenditure in small animals, but the method has been used in humans only recently (3). The time lag between the first use in animals and the first use in humans was probably due to cost. If the typical dose used in the earlier small animal studies was scaled up for use in humans, the cost of isotope alone would exceed \$4,000 per adult subject. Use of precise analytical methods based on differential isotope ratio mass spectrometry, however, has allowed us to use smaller, more economical doses.

Because of the cost, it is essential to use the minimum dose consistent with precise results. The minimum loading dose depends on the precision with which the isotopic enrichments in physiologic fluids can be measured. This precision may be influenced both by analytical error and biological variation. The aim of this study was to measure those variations and to determine the optimum isotopic loading dose.

Methods

Morning urine samples were collected from 17 subjects between the ages of 8 and 34 y/o. The subjects were living in metropolitan Chicago, Ill., and eating either a self-selected or parent-selected diet. A single urine sample was acquired from 12 subjects, and five urine samples were acquired at weekly intervals from the five remaining subjects. All samples were analysed in duplicate.

The $^{18}\text{O}/^{16}\text{O}$ isotope ratio analysis was performed as previously described (4). Briefly, 1.5 ml of urine was equilibrated with 1 ml STP of CO_2 at $25.0 \pm 0.1^\circ\text{C}$ for 48 hr. The CO_2 was purified under vacuum by cryogenic distillation and the isotope ratio was measured on a Nuclide 3-60 differential isotope ratio mass spectrometer.

The $^2\text{H}/^1\text{H}$ isotope ratio analysis was performed in a manner slightly different from that previously described (4). A 2ul aliquot was vacuum distilled, then reduced to H_2 over uranium at 700°C . The $^2\text{H}/^1\text{H}$ ratio was measured on a Nuclide 3-60 differential isotope ratio mass spectrometer. The mass spectrometer had a triple inlet and the analyses was performed by comparison against two standards in combination with electrical H_3^+ compensation (5).

Results

The standard deviations for the analytical error, week-to-week intraindividual variation, and interindividual variations are summarized in Table 1. The results are given in both atom % excess (6) and "del per mil" (6) (7). The analytical errors are associated

Table 1. The ^{18}O and ^2H isotopic variation in the analysis of human urine

SOURCE	^{18}O - STANDARD DEVIATION		^2H - STANDARD DEVIATION	
	ATOM % EXCESS	‰	ATOM % EXCESS	‰
ANALYSIS	3.1×10^{-5}	0.15	1.7×10^{-5}	1.1
INTRAINDIVIDUAL (7D)	4.0×10^{-5}	0.19	2.6×10^{-5}	1.7
INTERINDIVIDUAL	1.8×10^{-4}	0.86	9.2×10^{-5}	5.9

mainly with sample preparation as the instrumental measurement errors are 1.5×10^{-5} and 0.8×10^{-5} atom % excess for the ^{18}O and ^2H analyses respectively. The intraindividual were about 50% larger than the analytical errors, and the interindividual variations were about 6 times larger than the analytical errors.

The biological variations in the ^{18}O and ^2H isotopic abundances from the 37 urine samples were highly correlated ($r=0.83$). The slope of the regression of $\delta^2\text{H}$ onto $\delta^{18}\text{O}$ was 6.3 ($\text{SD}=0.7$). The correlation of the two isotope abundances was expected because the abundances of these two isotopes is highly correlated in natural water samples due to isotope fractionation during evaporation and condensation (8). The slope was more characteristic of evaporation under equilibrium control (theoretical slope=8) than kinetic control (theoretical slope=2.5) (8).

Because of the covariance of the biological variations and because a baseline sample of fluid is collected from each subject before giving the loading dose, the analytical error is the limiting factor in determining the dose. Based on these analytical errors, we predict that the optimum loading doses for the doubly labeled water method are between 0.25 and 0.3 g/kg total body water for H_2^{18}O (100 APE); and 0.10 and 0.12 g/kg total body water for $^2\text{H}_2\text{O}$ (100 APE).

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TOB 4 | APPLICATIONS OF STABLE ISOTOPES TO THE
STUDY OF PUTRESCINE METABOLISM, Patrick S. Callery, Linda A.
Geelhaar and M.S. Balachandran Nayar, University of Maryland,
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A major pathway for the metabolism of putrescine involves deamination with diamine oxidase to form Δ^1 -pyrroline which is oxidized further to γ -aminobutyric acid and 2-pyrrolidinone. At least three reasonable mechanisms can be proposed for the deamination reaction. In order to provide information on the mechanism, the deamination of putrescine catalyzed by diamine oxidase was carried out in deuterium oxide and deuterated buffers. Enamine and α,β -unsaturated intermediates were excluded based on the observation that deuterium was not incorporated into Δ^1 -pyrroline during its enzymatic formation in deuterium oxide. When the reaction mixture was buffered with phosphate, isolated Δ^1 -pyrroline contained two deuterium atoms at C-3, indicating that a phosphate-promoted, non-enzymatic isotope exchange had occurred. Using 5,5-dimethyl- Δ^1 -pyrroline as a model compound, the nature of the non-enzymatic deuterium exchange was studied and a bifunctional catalysis mechanism proposed. The results suggest that the choice of buffer could alter the conclusions drawn from enzyme mechanism studies involving imine-enamine tautomerization.

TOB 5 | ISOTOPIAL DETERMINATION OF AMINO ACID-
UREA INTERACTIONS IN EXERCISE IN HUMANS
Wolfe, R.R. (1), Wolfe, M.H. (2), Nadel, E.R. (3), Shaw,
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We recently reported that in light exercise (30% $\dot{V}O_2$ max) the oxidation of 1- ^{13}C -leucine was significantly increased, but the rate of urea production was unchanged (J. Appl. Physiol 52:458-466, 1982). We have therefore tested three possible explanations for this apparent incongruity. 1) We infused $NaH^{13}CO_3$ throughout rest and exercise and found that although altered bicarbonate kinetics in exercise resulted in greater recovery of $^{13}CO_2$, the difference between rest and recovery was small in comparison to the increase in the rate of $^{13}CO_2$ excretion during exercise when (1- ^{13}C)-leucine was infused. 2) We infused ^{15}N -leucine and isolated plasma urea N to determine directly the rate of incorporation of the ^{15}N . During exercise there was no increase in the rate of ^{15}N incorporation. 3) We tested our previous assumption that 1- ^{13}C -leucine metabolism in exercise was representative to the metabolism of other essential amino acids by infusing 1- ^{13}C - and α - ^{15}N -lysine throughout rest and exercise. We found that the rate of breakdown of lysine during exercise was not increased in a manner comparable to that of leucine.

Thus, these data confirm our original findings that leucine decarboxylation is enhanced in light exercise, but urea production is unchanged. It appears that in exercise leucine metabolism is not representative of the metabolism of all essential amino acids, and a model of whole body protein synthesis and catabolism based on that assumption is not valid. Our results support the concept that protein catabolism is not increased in light exercise.

THE ACCURATE DETERMINATION OF SERUM UREA AND CREATININE BY ISOTOPE DILUTION/MASS SPECTROMETRY

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INTRODUCTION. Serum urea and creatinine levels are frequently measured in clinical laboratories as tests for protein metabolism and renal function. As part of a program to develop highly accurate (definitive) methods for serum analytes, we have developed isotope dilution mass spectrometric (ID/MS) methods for urea and creatinine in serum. Our previous work on definitive methods for serum cholesterol (1), glucose (2), and uric acid (3) has shown that ID/MS is a technique capable of meeting the rigorous accuracy and precision requirements of a definitive method. These methods are used to certify analyte concentrations in serum pools which can then be used as an accuracy base for reference and field methods.

METHODS. Urea. To a known weight of serum is added a known weight of urea- ^{18}O such that the ratio of unlabeled to labeled falls in the range of 0.8-1.0. After an overnight equilibration, the serum is freeze-dried. Urea is extracted from the matrix with methanol, the methanol evaporated, and the urea sublimed. The sublimate is sealed in a vial with diketene and acetic acid and heated for 46-48 hours at 82-84° C to convert urea to 6-methyluracil in high yield. After excess reagents are evaporated, the derivative is silylated with bis(trimethylsilyl)acetamide (BSA). This derivative, 6-methyl-2,4-bis[[trimethylsilyl]oxy]pyrimidine, is left in excess BSA for analysis. Two sets of calibration standards, consisting of known mixtures of pure urea (Standard Reference Material 912) and urea- ^{18}O are independently prepared and derivatized. These standards, ten in all, cover the range of expected ratios from the serum samples.

For the ID/MS measurements, introduction of samples is via a gas chromatograph equipped with a 15 meter, nonpolar silicone phase capillary column directly coupled to the mass spectrometer. The mass spectrometer system, previously described (1, 2), has been modified by addition of a microcomputer, which controls switching of the magnetic field, provides a triangular wave form signal for the beam deflection plates, and handles data acquisition and processing. Ion abundance ratio measurements are made primarily in the EI mode on the $(\text{M}-15)^+$ ions at m/z 255 and 257. As a test for measurement bias, some of the samples are remeasured using other ions; the $[\text{M}-2(\text{CH}_3)]^{++}$ ions at m/z 120 and 121, and under isobutane chemical ionization conditions, the $(\text{M}+1)^+$ ions at m/z 271 and 273.

To measure the samples with the highest possible precision, we bracket the samples with calibration standards according to the following scheme. A preliminary measurement of the ratio of the sample is made and the calibration standards which are closest in ratio, above and below, to the sample are chosen. One of these standards is injected followed at 5.5 minute intervals by the sample and the other standard. As the peaks elute after a retention time of 25 minutes, the ion abundance ratio of each is measured. The amount of unlabeled urea in the sample is found by linear interpolation of the sample ratio between the ratios of the standard. The order of injection is reversed on a second day with the mean of the two runs taken as the reported result.

Creatinine. To a weighed amount of serum is added a known weight of creatinine- $^{13}\text{C}_2$ such that the ratio of labeled/unlabeled creatinine is approximately 4:1. After an overnight equilibration, the serum is put on an weak acid resin column and the column washed with water. Any creatinine present is eluted while creatinine is retained. For derivatization, creatinine is heated for three days at 85-86° C to form the ethyl ester of N-(4,6-dimethyl-2-pyrimidinyl)-N-methylglycine. Excess reagents are evaporated under a stream of nitrogen and the derivative is immediately taken up in distilled heptane for analysis.

For the ID/MS measurements, the $(\text{M}-\text{COOEt})^+$ ions at m/z 150 and 152 are used. Confirmatory measurements are made using the $(\text{M}+1)^+$ ions formed under isobutane CI conditions. Instrumentation, sample introduction, and the bracketing scheme are similar to that described for urea.

RESULTS. Urea. Our ID/MS method has been used to find urea levels in both lyophilized and frozen serum pools. The results, shown in Table 1, demonstrate the excellent precision attainable with this method. The oxygen- 18 label was chosen after earlier work (4) with urea- ^{13}C , $^{15}\text{N}_2$ gave results with high imprecision and measured ion abundance ratios which depended upon derivatization conditions. A primary isotope effect in the derivatization due to nitrogen-15 was believed to be the cause.

Table 1
ID/MS Results for Urea in Serum Pools

Serum	Number of Samples	Mean	Relative Standard Deviation (percent)
Lyophilized Human Serum (SRM 909)	18	7.244 mg/g dry serum	0.17
Frozen Pools A	3	.2393 mg/g liq. serum	0.18
B	3	.3564	0.17
C	3	.8259	0.16
D	3	.8828	0.18
E	3	1.5251	0.25

As a test for measurement bias, some samples from each pool were remeasured using other pairs of ions. The results, shown in Table 2, demonstrate the absence of systematic bias for measurements made using three different pairs of ions. Because of the high precision and the absence of any evidence of systematic bias with this method, we conclude that it satisfies the requirements for a definitive method.

Table 2
Comparison of Results Using Three Different Pairs of Ions

Serum	Number of Samples	Means			Range of Means (percent)
		255/257	120/121	271/273	
SRM 909	6	7.242	7.239	7.240	0.05
A	1	0.2393	0.2394	0.2392	0.10
B	1	0.3568	0.3571	0.3565	0.15
C	1	0.8250	0.8256	0.8257	0.08
D	1	0.8829	0.8829	0.8823	0.07
E	1	1.5254	1.5265	1.5294	0.27

Creatinine. Our first choice for a labeled analog, creatinine-d₃, proved to be unsatisfactory. As with urea, large imprecision and a dependence of measured ratios on derivatization conditions were observed. Preliminary results with creatinine-¹³C₂ appear to be promising. Much work remains to be done before this method can be considered "definitive."

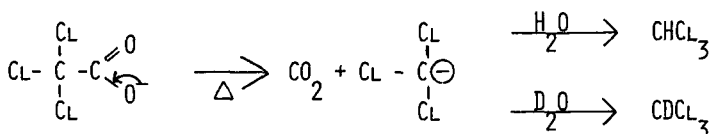
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USE OF D₂O TO DIFFERENTIATE BETWEEN PLASMA CHLOROFORM AND TRICHLORO-
ACETIC ACID; CARL D. PFAFFENBERGER, THOMAS V. BRIGGLE AND ANITA J.
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OUR LABORATORY HAS DEVELOPED A GC/MS METHOD BY WHICH HUMAN BLOOD PLASMA
MAY BE ANALYZED FOR CHLOROFORM/TRICHLOROACETIC ACID CONTENT. BY A PREVIOUSLY
USED GC METHOD (1) TRICHLOROACETIC ACID (TCA) WAS QUANTITATIVELY CONVERTED
INTO CHLOROFORM. ASSESSMENT OF BODY BURDEN LEVELS OF CIRCULATING CHLOROFORM
WAS THUS OBFUSCATED WHEN SIMULTANEOUS EXPOSURE TO TRICHLOROETHYLENE AND/OR
TETRACHLOROETHYLENE HAD OCCURRED, BECAUSE THESE VOLATILE CHLORINATED ORGANIC
SOLVENTS ARE METABOLIZED BY HEPATIC TISSUE TO TCA.

TO DIFFERENTIATE BETWEEN CIRCULATING CHLOROFORM AND CIRCULATING TRI-
CHLOROACETIC ACID IN THE BLOOD OF OCCUPATIONALLY EXPOSED WORKERS, PLASMA
WAS THERMALLY DECOMPOSED IN THE PRESENCE OF 80% D₂O. UNDER EXPERIMENTAL
CONDITIONS, THE REACTIVE INTERMEDIATE, THE TRICHLOROMETHYLCARBANION DERIVED
FROM TCA, ABSTRACTS DEUTERIUM FROM D₂O AND FORMS CDCL₃ WHICH IS EASILY DIS-
TINGUISHED FROM CHCL₃ BY MASS SPECTROMETRY. THE PERTINENT REACTIONS INCLUDE:



THE RELATIVE RATIOS OF THE POSITIVE FRAGMENTS OCCURRING AT $m/z = 83$ AND
 $m/z = 84$ ALLOW DIFFERENTIATION OF THE CIRCULATING CHLOROFORM FROM THE CIR-
CULATING TRICHLOROACETIC ACID. SPECIFIC EXAMPLES FROM BOTH THE GENERAL
POPULATION AND EXPOSED WORKERS ARE REPORTED IN THE ACCOMPANYING TABLE.
CALCULATIONS ARE MADE USING THE FORMULAS:

$$\% \text{ SHIFTED} = (I_{c84}/I_{c_{TOT}}) \times 100 + (6\% \times 1 - (I_{c84}/I_{c_{TOT}})) \quad (2)$$

$$\text{WHERE } I_{c_{TOT}} = I_{c84} + I_{c83} \text{ (ONLY)}$$

- (1) A.J. PEOPLES, C.D. PFAFFENBERGER, T.M. SHAFIK AND H.F. ENOS.
DETERMINATION OF VOLATILE PURGEABLE HALOGENATED HYDROCARBONS IN HUMAN
ADIPOSE TISSUE AND BLOOD SERUM. BULL. ENVIRONM. CONTAM. TOXICOL., 23
(1979), 244-250.
- (2) THE 6% CORRECTION FACTOR WILL BE EXPLAINED DURING THE FORMAL PRESEN-
TATION.

RANGES OF TCA CONCENTRATION

STUDY	RUN NO.	CONC. CHCL ₃	%SHIFTED	%UNSHIFTED
PALM BEACH	100	28 PPB	60.5	39.5
	400	26 PPB	78.9	21.1
PHASE III	1158	15 PPB	<u>34.7</u>	65.3
	1166	18 PPB	82.7	17.2
AIRCRAFT WORKERS	39	25 PPB	76.1	23.9
	49	1000 PPB	<u>89.9</u>	10.1

OBSERVED SHIFTS: FOR A RANGE OF TOTAL CHLOROFORM
VALUES OF 15 TO 1000 PPB, THE %
SHIFT HAS RANGED FROM 35 TO 90%.

THE FINANCIAL SUPPORT OF THE U.S. ENVIRONMENTAL PROTECTION AGENCY
THROUGH COOPERATIVE AGREEMENT CR-807714-02 IS GRATEFULLY ACKNOWLEDGED.

A NEW METHOD FOR THE DETERMINATION OF OXYGEN-18 IN ORGANIC MATERIALS

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Introduction This report describes the use of the Schutze-Unterzaucher procedure (1) for the determination of the abundance of oxygen-18 in organic substances. Organic oxygen is converted to carbon dioxide in three consecutive reactions i) pyrolysis of the sample, ii) reduction of the oxygen-containing pyrolysis products to carbon monoxide using nickel-coated carbon, and iii) oxidation of the carbon monoxide to carbon dioxide using iodine pentoxide.

Ideally, any sample preparation prior to the mass spectrometric measurement should leave the sample oxygen pool unaffected, so that the oxygen isotopic composition of the carbon dioxide is identical to that of the sample. In practice, an oxygen blank and a memory effect interfere with the isotopic analysis. Here, it is shown that a simple mathematical model can describe the isotopic aspects of this procedure, and that the isotopic information contained in the sample can be accurately and precisely reconstructed.

Model The oxygen inputs to the carbon dioxide are shown in figure 1. The pyrolytic decomposition of organic material yields a mixture of small molecules. Depending on the composition and structure of the intact sample, this may include CO, CO₂, H₂O, NO, COS, H₂, N₂, and CH₄. The oxygen-containing pyrolysis products react with the carbon surface in the reduction reactor to yield carbon monoxide. Some oxygen remains bound to the surface as chemisorbed CO which is freely exchangeable with CO in the gas phase. The result is a permanent sorbed-oxygen pool which acts as a buffer, charged with oxygen from previous samples (memory) and with oxygen from the quartz tube (a blank, derived from a reaction between carbon and silica).

As an organic sample is processed, all oxygen in the system is rapidly equilibrated between the chemisorbed CO and gas phase carbon monoxide. The abundance of oxygen-18 in the system is thus a weighted average of the isotopic abundances in the various inputs:

$$n_T^{18}F_T = n_S^{18}F_S + n_Q^{18}F_Q + \sum_j n_j^{18}F_j \quad (1)$$

$$n_T = n_S + n_Q + \sum_j n_j \quad (2)$$

where $^{18}F_i$ is the fractional abundance of oxygen-18 and n_i is the amount of oxygen ($\mu\text{mole O}$) in the i^{th} oxygen pool; the subscripts designate the oxygen pools [total (T), sample (S), quartz (Q), and previous samples (j)]. The numerical subscripts (j) define an index sequence of previous samples (2). The sample being analyzed is treated as the zeroth sample (for convenience, it is designated as S), so the index starts with the sample of most immediate precedence.

Assuming that no isotopic fractionation accompanies the partitioning of oxygen in the reduction reactor, the carbon monoxide oxygen pool is representative of all oxygen in the system. A relationship which is used to correct for the isotopic contributions of the blank and memory effect is then derived from equations (1) and (2):

$$^{18}F_S = ^{18}F_{CO} + (n_Q/n_S) \cdot (^{18}F_{CO} - ^{18}F_Q) + \sum_j (n_j/n_S) \cdot (^{18}F_{CO} - ^{18}F_j) \quad (3)$$

where the subscript (CO) designates the carbon dioxide oxygen pool.

Oxidation of carbon monoxide by iodine pentoxide merely doubles the amount of oxygen in the gaseous oxygen pool:

$$^{18}F_{CO} = 2 \cdot ^{18}F_{CO_2} - ^{18}F_I \quad (4)$$

where the subscripts designate the oxygen pools [carbon dioxide (CO₂) and iodine-pentoxide derived (I)].

Experimental Section A Carlo Erba Model 1106 Elemental Analyzer has been modified so that the effluent carbon monoxide can be oxidized to carbon dioxide using iodine pentoxide and the CO₂ can be collected, purified, and packaged for mass spectrometric analysis. The

reduction reactor was lined with nickel foil to minimize the reaction between carbon and silica which results in the blank. The carrier gas was doped with a mixture of n-heptane and carbon tetrachloride. The HCl produced by the pyrolysis of these compounds minimizes the sorbed oxygen pool by competition for sorption sites. Equations (3) and (4) were used to calculate the abundance of oxygen-18 in the sample from that measured in the carbon dioxide.

Results and Discussion The relationship expected to prevail between isotopic composition, sample size, and the history (i.e., memory) of the reactor are summarized in Fig. 2, which depicts a hypothetical series of replicate analyses (A, B, C, ..., Z) of a sample with a true isotopic composition represented by δF_2 . The initial sorbed oxygen pool in this case is assumed to be depleted in oxygen-18 relative to the samples so that the first result (the one on curve A) falls below the correct value, the precise position along the curve being established by the size of the sample (a large sample would give more nearly accurate results than a small one). The results of subsequent analyses of this same material do not fall on curve A because the isotopic composition of the sorbed oxygen pool has been changed. The memory of sample A defines curve B and, as shown, if the second sample analyzed is large, the third curve (C) is displaced more strongly upward. The memory will eventually be dominated by oxygen from the sample being analyzed, and curve Z represents the result expected if the blank is slightly depleted in oxygen-18 relative to the sample.

A curve (filled symbols) analogous to Z in the preceding example is shown in Fig. 3, which summarizes results of a series of analyses of benzoic acid of varying size. The sorbed oxygen pool had been "conditioned" to neutralize memory effects by repetitive processing of samples of this material just prior to this series of analyses. Corrections based on equations (3) and (4) have yielded the open symbols, which all are in excellent agreement with the known isotopic composition of this material.

Figure 4 summarizes similar corrections for a series of analyses interrupted by two samples very highly enriched in oxygen-18. It can be observed that the corrections are quite satisfactory even in this extreme case.

The method has also been applied satisfactorily to analyses of the isotopic composition of oxygen in standard sucrose samples and in standard water samples.

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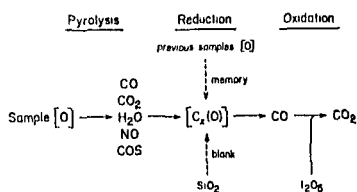


Figure 1

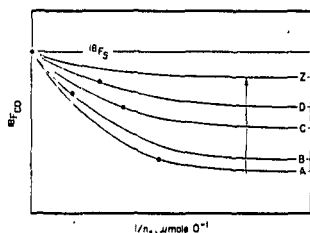


Figure 2

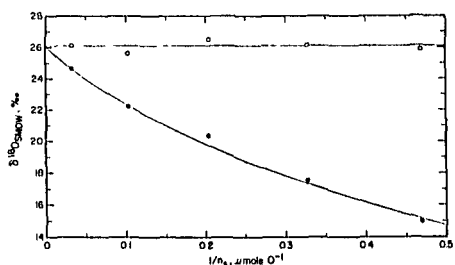


Figure 3

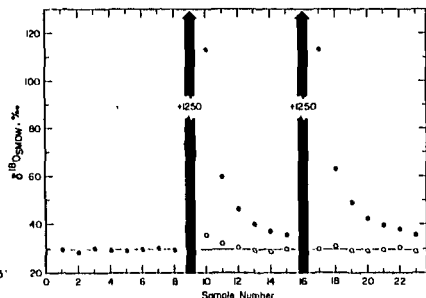


Figure 4

ISOTOPE CLUSTER CHROMATOGRAPHY: AN UPDATE

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The availability of computerized GCMS systems has led to data collection rates that far exceed the rate at which the data can be interpreted. In an effort to limit the amount of data which must be interpreted from a large data set, we have been developing computer software that will sort through the data set, seeking spectral features as desired by the analyst (i.e., selective data reduction). Isotope cluster chromatography (1) is a technique by which the computer searches for the unusual isotope patterns of chlorine and/or bromine atoms. The method can be extended to act as a chlorine-selective detector (2) or to search for isotope patterns from stable isotope labeled species (3).

While chlorine and bromine are the most familiar examples, they are by no means the only elements with distinctive naturally-occurring stable isotope distributions. Selenium has six stable isotopes spread over a range of nine u as shown in Figure 1.

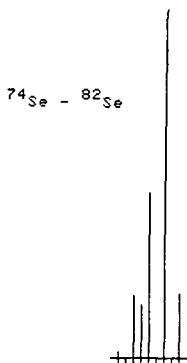


Figure 1. The natural isotope distribution of selenium.

The mass spectrum of any compound containing selenium should exhibit this pattern. The isotope cluster searching routine described above has been modified to sort through a data set of hundreds or thousands of mass spectra and locate those displaying a pattern of ions most like that of selenium. A score is assigned to each mass spectrum based on the likelihood that it contains a selenium cluster pattern and on the abundance of ions in that pattern. The searching routine has been evaluated using the EPA/NIH Mass Spectral Data Base containing spectra of over 31,000 compounds, including 146 selenium compounds. Depending on the search parameters used, 82-95% of the selenium compounds were assigned non-zero scores, with an average relative score of about 19,000. In contrast, only 2-9% of the non-selenium compounds were assigned non-zero scores, and their average relative score was substantially lower.

If an actual GCMS data set is searched, a plot of the relative score vs. scan number results in a selenium selective chromatogram as illustrated in Figure 2. In this experiment, a mixture of amino acids and the seleno-analog of methionine were trimethylsilylated and chromatographed. Figure 2a shows that two regions of the chromatogram are quite likely to contain selenium isotope patterns. Scan 511 is the spectrum of the TMS-Se-Met; scan 407 is an unidentified reaction side product; but both spectra do indeed exhibit the selenium isotope cluster.

** SELENIUM CHROMATOGRAM **

FRH 7300 SCAN 100 TO 620

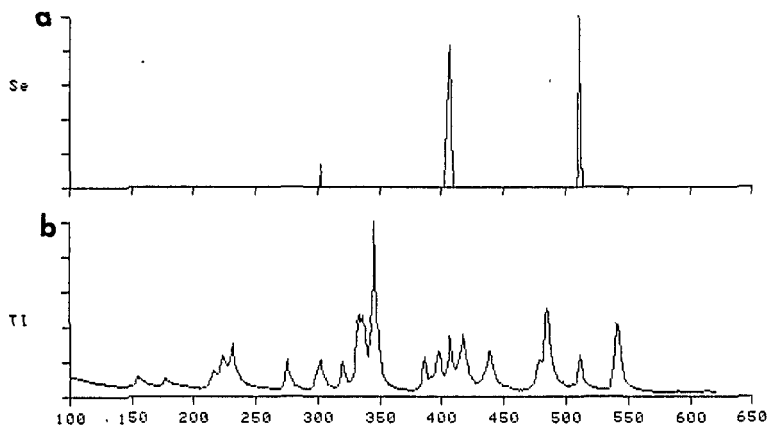


Figure 2. a) Selenium selective chromatogram and
b) total ion plot for a mixture of TMS-amino acids.

We feel this technique has great potential in the study of selenium metabolism and biochemistry. Selenium, an essential nutrient and a toxin, is found in a number of proteins including at least one enzyme active site (4).

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MICRODEGRADATION OF 1-¹³C-ORGANIC ACIDS INTO ¹³CO₂ FOR ISOTOPE RATIO ANALYSIS.
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Introduction

In clinical studies with stable isotopes the requirements for atomic mass resolution often exceed the specifications of quadrupole and magnetic sector instruments necessitating the use of the ratio mass spectrometer (RMS). Unfortunately, the RMS cannot measure isotopic ratios in most analytes other than breath unless the analytes are isolated and then degraded by combustion or by chemical reaction into low mass labeled end-products. This study surveys the microscale reactions applicable to C-organic acids of general clinical interest: fatty acids and their 2-amino-, 2-hydroxy-, and 2-keto-analogs. The objectives were a) to identify a repertoire of nonenzymatic manipulations affording high yields of ¹³CO₂ or CO; b) to define the sensitivity, accuracy and repeatability of C/¹²C ratios obtained by each microdegradation protocol on the SIRA 9 (VG Isogas) ratio spectrometer; and c) to compare these findings to the values obtained by microcombustion.

Materials and Methods

The following decarboxylation reactions were examined: 1-¹³C-palmitic acid with lead tetraacetate^{1,2} (PbOAc) or mercuric acid/bromine (HgO/Br); 1-¹³C-leucine with ninhydrin (NIN)^{3,5}, Chloramine-T (C-T), silver picolinate (AgPc) or PbOAc; 1-¹³C-2-ketoisocaproic acid and 1-¹³C-lactic acid with calcium hypochlorite (CaOCl)⁸, potassium superoxide (KO₂), tetrabutylammonium periodate (TbIO₄)¹⁰ or PbOAc. The action of these reagents on N-acetyl-leucine and its propylester were also examined, as well as the decarbonylating activity (RCOOH to CO) of rhodium chloride triphenyl phosphine¹¹ (Rh/tPP) on all acid analyte classes. We did not explore the Schmidt reaction¹, however, since a secondary objective was to maximize the yield of recoverable alkyl aldehydes or acids as co-products after decarboxylation.

Reaction stoichiometries for initial studies followed the specifications of Greenstein and Winitz⁴ for the microdegradation of amino acids: In general; 12.5 μ moles of substrate deposited by lyophilization into a 10 ml glass pressure vessel were treated with 625 μ moles in 2 ml of solvent and heated to 80°C, or to other temperatures as specified in the literature, for periods varying between 10 and 60 minutes. The solvents were for NIN, C-T, PbOAc: aqueous H₃PO₄ pH 2.5; AgPc: H₂O; CaOCl: H₂O plus 2% acetic acid; TbIO₄: dioxane; PbOAc (for non-water soluble substrates): pyridine/benzene 1:5 plus catalytic amounts of copper acetate; KO₂: dimethyl sulfoxide plus 1.5 x 10⁻⁴ of Crown ether; HgO/Br: carbon tetrachloride; Rh/tPP: no solvent, reagent added as solid.

The substrates were spiked with 1-¹³C-labeled analogues so as to afford ¹³C APE values (against standard) ranging from 0 to 100 x 10⁻³. In several experiments, 10,000 dpm of the corresponding ¹⁴C analog were also added in order to facilitate the determination of decarboxylation yields.

Upon completion of the decarboxylation reaction and addition of conc. H₃PO₄ where applicable to the basic reaction media, the reaction vessel was connected to an isopropanol/liq. N₂ cold-finger trap followed by a liq. N₂ cold-finger trap. The latter also served as the sample tube for RMS. Combustion experiments were carried out in quartz break-seal tubes by the classical method of DesMarais and Hayes¹³. Trapped ¹⁴CO₂ recoveries were measured by scintillation counting in Aquasol.

Results

Among the reagents explored C-T, HgO/Br, PbOAc and KO₂ were excluded from further study either because they afforded less than 90% recovery of theoretical CO₂ or because the CO₂ was contaminated with volatile colored by-products. Rh/tPP also was dropped from further evaluation because of the difficulties associated with concentration of CO samples for RMS.

Representative results with NIN on 1-¹³C-leucine are shown in Table 1.

TABLE 1: ¹³C-APE STUDY OF LEUCINE (+ 1 SIGMA)

SAMPLE (N=5)	THEORETICAL	COMBUSTION	NINHYDRIN
	12.5 x 10 ⁻⁶ M	(X6) 12.5 x 10 ⁻⁶ M	12.5 x 10 ⁻⁶ M
¹³ C	0	0 ± 0.25	0 ± 0.21
	10	11.4 ± 0.29	9.6 ± 0.37
	25	25.1 ± 0.43	27 ± 0.51
	50	53.9 ± 0.85	56 ± 1.1
	100	104.4 ± 1.9	98.9 ± 2.2

THEORETICAL vs COMBUSTION $y = 1.045x + 0.285$ (R = 0.999)

THEORETICAL vs NINHYDRIN (I) $y = 0.999x + 1.327$ (R = 0.997)

THEORETICAL vs NINHYDRIN (II) $y = 1.077x - 1.399$ (R = 0.998)

Recovery (average = 94%) of ¹³CO₂ by selected decarboxylation (y) showed a linear correlation with theoretical values (x) paralleling the results attained by microcombustion. Note should be made also of the regression equation labeled "Theoretical vs ninhydrin (II)". This was obtained by reaction of 1.25 μ moles of substrate with 62.5 μ moles of reagent (0.2 ml of total reaction solvent) in an otherwise identical experiment.

Additional successful correlations, in which y = observed, x = expected results over the 0 to 100 x 10⁻³ APE range as in Table 1, were:

- 1-¹³C-leucine + AgPC: $y = 0.963x + 0.653$, R = 0.992;
CO₂ average yield = 93%
- 1-¹³C-Na-2-ketoisocaproate + CaOCl: $y = 0.991x - 1.264$, R = 0.999;
CO₂ average yield = 96%
- 1-¹³C-Na-lactic acid + CaOCl: $y = 0.945x + 1.814$, R = 0.990;
CO₂ average yield = 97%
- 1-¹³C-palmitic acid + PbOAC: $y = 1.094x - 2.586$, R = 0.990;
CO₂ average yield = 90%

These results suggest that selected decarboxylation of 1-¹³C-organic acids affords linear recovery of labeled CO₂. Although the technique is laborious in contrast to automated combustion and despite the requirement for several micromoles of substrate, it may prove useful in the analysis of labeled plasma analytes. Work continues to validate this approach on analyte concentrations in the 1 to 2 μmole range, which are the ones actually found per dl of plasma and other fluids of biological interest.

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TOB 11 | THE EFFECT OF PHYSIOLOGICAL STATE
ON BACKGROUND ^{13}C ENRICHMENT IN EXPIRED AIR

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The natural enrichment of ^{13}C in energy substrates varies, and this variation must be taken into account when stable isotopic tracers are used in metabolic studies. We present data here to show that any change in the metabolic state can significantly alter the background enrichment of expired CO_2 . We show that not only can the introduction of natural energy sources alter the background enrichment, but also changes in substrate oxidation induced by different physiological states, such as exercise, can cause significant changes in CO_2 enrichment. Perturbations of this sort must be accounted for in studies in which oxidation is measured by means of a ^{13}C tracer.

TOC 1 | DISCRIMINATION OF HYDROCARBON ION
STRUCTURES ON THE BASIS OF THEIR REACTIVITY; P.
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Washington, D. C. 20234

In systems where a mixture of isomeric hydrocarbon ions are present, the abundances can be sorted out through (i) determinations of the specific reactivities of the isomers with selected reactants, or (ii) observation of differences in reaction channels. A major advantage of the study of ion structures through the examination of bimolecular reactivity is that information can be obtained about isomerization processes over a wide range of densities, from the pressures used in ICR experiments ($\sim 10^{-6}$ torr) to the liquid phase. In condensed phases, where mass analysis is not feasible, other experimental techniques such as absorption spectroscopy or neutral product analysis may be used. The major drawback of the approach is that isomerization of ions may be induced by "unreactive" collisions, so that one does not sample the "original" ion population. Several factors which control such collision-induced isomerizations in hydrocarbon systems will be discussed.

Systems will be highlighted in which ions of a particular structure or structures can be prepared through bimolecular reactions or through the unimolecular decomposition of molecular radical cations. In the latter case, selectivity can be achieved by controlling the internal energy content of the molecular ion, which can be formed through charge transfer reactions.

TOC 5 | MECHANISTIC STUDIES BY MEANS OF
ISOTOPIC LABELLING: RADICAL LIKE REACTIONS OF SOME
IONIZED CARBONIC ACIDS

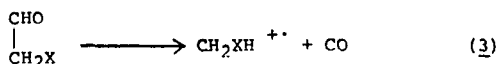
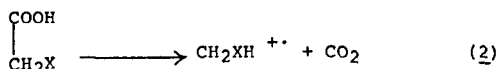
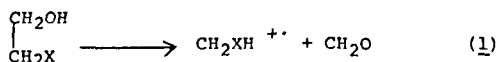
Helmut Schwarz and Thomas Weiske, Department of
Organic Chemistry, Technical University,
D-1000 Berlin 12, W.-Germany

In several recent review articles it has been shown convincingly that the unimolecular decay of gaseous cation radicals does not proceed in one step, even if the radical to be eliminated is already present as an intact structural group. To the contrary multi-step reaction paths are often energetically much more favourable than direct bond cleavages. For many systems it has also been shown that in this kind of pseudo-step decay processes, "hidden" hydrogen transfers play a particular role. Here, a hydrogen is transferred intramolecularly to a suitable ionized acceptor, and the newly-formed radical centre induces the actual bond cleavage, forming a double bond. With this concept, a variety of reactions can be readily explained which would otherwise be incomprehensible and regarded as anomalies. The palette of examples includes dissociations of C-H, C-C, C-O, C-S, C-N and C-halogen bonds, and the detailed study of C-C-cleavages in some ionized carbonic acids and their methyl esters seems to be of particular importance for mechanistic investigations. It will be shown in this contribution that in particular the gas phase chemistry of the cation radicals of the latter is in very close correspondence to the chemistry of free alkyl radicals.

Novel Gas Phase Ions, their Generation, Fragmentation Characteristics and Thermochemistry.

by John L. Holmes, Chemistry Department, University of Ottawa, Ottawa, K1N 9B4, Ontario, Canada.

In the past two years a remarkable number of novel ions of unconventional structure have been identified.¹⁻⁷ Part of the interest in these species arises from the success of *ab initio* calculations⁸⁻¹⁰ in predicting the stability of ions hitherto not observed by experiment. This report focuses attention on two types of new ion (a) those radical cations produced by dissociative ionisation which have collectively been described as radical-ion dipole complexes⁴ and (b) ions generated by charge reversal experiments. A third series of new ions, the oxy-carbenes, are described later this week by Dr. P.C. Burgers (paper ROC7). (a) The general method for the formation of these ions involves a dissociative ionisation in which an appropriate rearrangement is followed by the elimination of a small stable molecule, such as CH₂O, CO₂ and CO, e.g.



More than twenty of these ions have been identified to date and they include [CH₂XH]^{+\cdot}, where X = F, Cl, Br, I, OH, NH₂ and SH¹¹, [CH₂CH₂OH]^{+\cdot}, [CH₂CH₂NH₂]^{+\cdot}, [CH₃CHCH₂OH]^{+\cdot} and [CH₂CHOH.OH]^{+\cdot}. They have been generated via appropriate dissociative ionisations of the general type shown above and characterised by means of their collisional activation (CA) mass spectra, charge stripping (CS) mass spectra, metastable ion characteristics and thermochemical measurements. In each case the new ions all showed marked behavioural differences from their counterparts of conventional structure. Most striking were

- (i) the presence of intense doubly charged ions in CS mass spectra, normally absent in the conventional ions' CS spectra;
- (ii) fragmentation pathways completely absent in the conventional ions' unimolecular and collisional spectra, e.g. the isomers [CH₃OGH₃]^{+\cdot} and [CH₃CH₂OH]^{+\cdot} display no H₂O loss whereas for [CH₂CH₂OH₂]^{+\cdot} it is the dominant fragmentation process.
- (iii) Ionic heats of formation and dissociation energies were generally in excellent agreement with *ab initio* calculations, some of these are shown in the Figure, the energy diagram for the [CH₃F]^{+\cdot}, [CH₅N]^{+\cdot} and [CH₄O]^{+\cdot} systems.

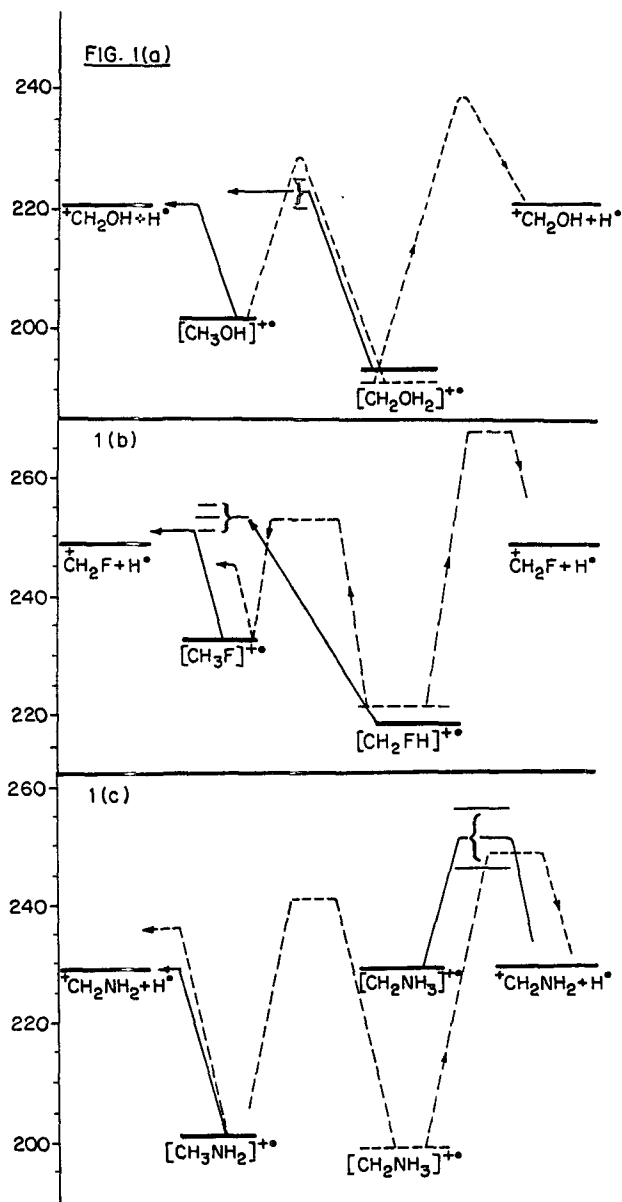
The full lines show measured threshold energies and ΔH_f° values for the ions while broken lines are for *ab initio* calculations by Radom et al.^{8,10}

(b) Charge reversal, in which a high velocity negative ion is collisionally converted by the loss of two electrons into a positive ion, is a vertical process and the product cation has initially the same geometry as its anion precursor. Such experiments permit the production of cations which are otherwise inaccessible (by any

dissociative ionisation). Thus for example we have recently studied the behaviour of HCOO^+ , CH_3CC^+ , $\text{CH}_2=\text{CHC}^+$ produced by charge reversal of the corresponding anions. The use of double collisional activation experiments has permitted a kinetic study of the rearrangement of these cations, to COOH^+ and CH_2CCH^+ respectively.

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ION STRUCTURES AND REACTION MECHANISMS FROM COLLISIONALLY ACTIVATED DISSOCIATIONS; F. W. McLafferty, C. Wesdemiotis, F. Turecek, I. J. Amster, M. T. Cheng, M. M. Mangano, and L. Lam, Chemistry Dept., Cornell Univ., Ithaca, NY 14853

Mass spectra formed by collisionally activated dissociation (CAD) are now commonly used for gaseous ion studies. An accompanying paper by Danis et al., describes their use for structures of neutral products of such reactions. This new technique, which we call "Neutralization-Reionization Mass Spectrometry (NRMS)," also can give more definitive ion structure information in special cases. For example, if butyl ions are neutralized with magnesium vapor and reionized by collision, the resulting CAD spectra from the *n*-, *iso*-, and *sec*-butyl ions are different. Even more striking, however, is that *tert*-butyl ions show no CAD spectrum (<1%), as the ionization energy of these ions (6.7 eV) is substantially lower than that of magnesium (7.6 eV). In keeping with theoretical predictions, CAD spectra of neutral species, negative ions, and positive ions of the same structure give very similar CAD positive ion spectra, except for the CAD products formed by low energy reactions from positive ions.

In a number of cases doubly-charged CAD product ions have provided definitive information on ion structures; collision gases other than He, such as N₂ and O₂, can give substantially higher yields of such ions. Recent applications include studies of four stable C₂H₃O⁺ isomers reaffirming the nonergodic metastable decomposition of the enolic isomer, isomers of C₇H₁₂N⁺, a reinvestigation of C₇H₇⁺ isomers, and ion-molecule reactions paralleling ionic solution reactions.

Instruments employing high (2-25 keV) ion accelerating energy are commonly used for such ion structure studies. They have a number of advantages over instruments such as the tandem quadrupole and ion cyclotron resonance which generally operate with ion accelerating voltages <100 eV. Discussion of these included the following points.

High velocity ions: (a) Quantitative abundances are independent of isomer internal energy. (b) CAD energies are high enough for A⁺ + A⁺⁺, A⁻ + A⁺, and A[•] + A⁺. (c) CAD efficiencies are 0.1% + >10% (with post acceleration). (d) Ion separation at high resolution is possible. (e) The energy deposited is proportional to the number of collisions.

Low velocity: (a) Spectra give qualitative structure information. (b) Spectra vary substantially with ion kinetic energy. (c) CAD efficiencies are 10% - 100%. (d) Ion separation at unit mass resolution is possible.

Do Electrophilic and Cycloaddition Reactions
Occur For Radical Cations?

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The reactivity of radical cations is an important issue in mechanistic chemistry in the gas-phase and solution. Major advances have occurred in recent years principally due to the application of tandem mass spectrometry (MS/MS) and collisionally activated decomposition (CAD) spectroscopy to this problem. One strategy we have adopted is to form reactants in selected energy states using charge exchange reactions at high pressure of the charge exchange reagent gas. The high pressure also serves to stabilize the ions. The ions are then selected for study by CAD spectroscopy. This strategy has been applied to electrocyclic reactions, specifically the ring-opening reactions of substituted cyclobutene radical cations.

Electrocyclic Reactions: A few years ago we reported that the cyclobutene radical cation undergoes facile ring opening to form the 1,3-butadiene. We have now turned to substituted cyclobutenes in order to assess the effect of substitution on the electrocyclic reaction.

The first system we studied was the methyl substituted cyclobutene radical cations. The 1-methyl and the 3-methyl cyclobutenes will form isoprene (eq. 1) and 1,3-pentadiene radical cations (eq. 2) if ring-opening occurs. Ionization of the corresponding neutrals by both low and high energy electron ionization produced two sets of C_5H_8 radical

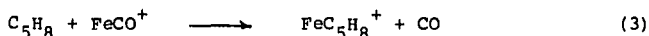


cations which gave identical CAD spectra. Thus, the electrocyclic reactions are essentially complete even at low ionizing energy. However, collisional ionization spectra of the isomers were unique. A notable feature of the spectra is that the cyclobutene ions gave almost no M^{2+} , whereas the doubly charged dienes were more stable in accord with the ability of the acyclic isomers to accommodate better two positive charges.

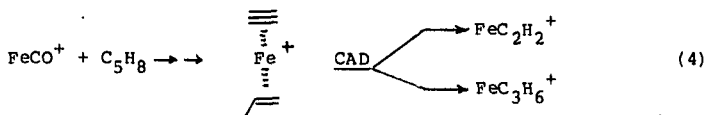
The seemingly contradictory results can be rationalized if one assumes that charge stripping samples ions that are near threshold in energy. Presumably near-threshold ions have retained structures corresponding to their neutral precursors. Addition of small quanta of

internal energy causes the ions to undergo electrocyclic reactions.

We next posed the question whether gas-phase metal ions would complex with the neutral methyl-cyclobutenes and cause ring opening. This point was investigated by conducting reactions of FeCO^+ (from ionized iron pentacarbonyl) with the four C_5H_8 isomers (see eq. 3) in a high pressure CI source. The product FeC_5H_8^+ was then selected using MS-I of a tandem



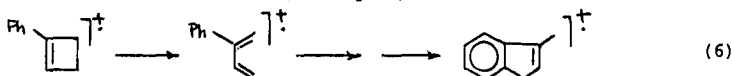
instrument, collisionally activated, and CAD spectra taken. All the $\text{Fe}(\text{C}_5\text{H}_8)^+$ ions gave unique CAD spectra. We interpret this to mean that Fe^+ inserts in various C-C bonds of the neutral C_5H_8 's to give, in part, ions with distinctive structures. However, all of FeC_5H_8^+ adducts gave common fragments in approximately the same relative ratios in their CAD spectra. These common fragments were FeC_2H_2^+ and FeC_3H_6^+ and are best understood to arise from decomposition of ring-opened adducts (see eq. 4). Thus, the reaction with FeCO^+ causes a fraction of the



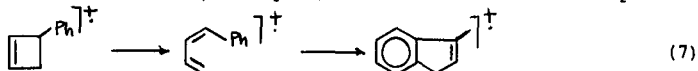
methyl-cyclobutene molecules to undergo ring opening followed by insertion in a C-C bond (see eq. 5).



The effect of phenyl substitution was investigated next. Both 1-phenylcyclobutene and 2-phenyl-1,3-butadiene were ionized with ever increasing internal energy deposition by using various charge exchange reagents (NO , toluene, CS_2) and by low and high energy electron ionization. The CAD spectra were taken as a function of internal energy. Both spectra were observed to change; the CAD spectrum of 1-phenylcyclobutene radical cation changed to resemble that of 2-phenyl-1,3-butadiene ion, and ultimately both spectra became nearly identical to that of 1-methylindene. These changes are not ascribed to internal energy effects; rather they can be explained in terms of sequential isomerization reactions (see eq. 6).



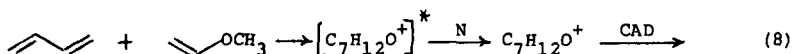
The CAD spectrum of 3-phenylcyclobutene ionized even at low electron energy is nearly identical to that of 1-methylindene which indicates an extremely facile electrocyclic ring opening to form a chemically activated 1-phenyl-1,3-butadiene ion which then rapidly isomerizes to the 1-methylindene radical cation (see eq. 7). Consistent with this picture



is the fact that the CAD spectra of 3-phenylcyclobutene, 1-phenyl-1,3-butadiene, and 1-methylindene radical cations give nearly identical CAD spectra when ionized at higher energies using CS_2 charge exchange (see paper by C. Dass in this volume for additional details).

Thermochemical measurements are in accord with this mechanistic picture. We find that the heats-of-formation of the ions decrease in the following order: 1-phenylcyclobutene and 3-phenylcyclobutene > 3-phenyl-1,3-butadiene and 1-phenyl-1,3-butadiene > 1-methylindene.

Cycloaddition Reactions: The question we now address is whether cycloadditions of gas-phase radical ions and olefin or diene neutral molecules occur. The experimental strategy is the same as for the study of the FeC_5H_8^+ adducts except the actual intermediate was studied by CAD spectroscopy. For example, we find that we can collisionally stabilize the $[\text{C}_7\text{H}_{12}\text{O}]^+$ (see eq. 8). The CAD spectrum of the stabilized adduct is

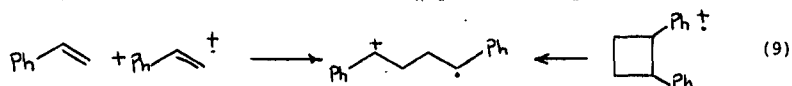


nearly identical to those acyclic $\text{C}_7\text{H}_{12}\text{O}$ radical cations formed from neutral acyclic $\text{C}_7\text{H}_{12}\text{O}$ molecules and considerably different than that of 4-methoxycyclohexene, the [4+2] carbon atom cycloaddition product proposed by Nibbering and Jennings. We believe both interpretations fit into a general mechanism of ionic cycloadditions which will be presented below.

Similar results were obtained for the reaction of the 1,3-butadiene radical cation and neutral 1,3-butadiene. The $\text{C}_8\text{H}_{12}^+$ intermediate or adduct was rapidly collisionally stabilized in relatively high pressure CS_2 (pressure of approximately 0.5 torr). The CAD spectrum of this adduct was almost identical to various acyclic C_8H_{12} reference ions. However, as the pressure of the bath gas (CS_2) was decreased, the CAD spectrum of the adduct smoothly changed and ultimately, at a pressure of less than 0.1 torr, became identical to that of the 4-vinylcyclohexene radical cation. Thus, we see that under conditions that are collision free or which

involve slow collision rates, the adduct is formed as a cycloadduct. However, increasing the rate of collisional stabilization allows us to trap an acyclic isomer. Therefore, gas-phase ionic cycloadditions are two step processes: the first encounter of the ion and neutral leads to the formation of an acyclic intermediate which, in the absence of stabilizing collisions, goes on to cyclize.

In an attempt to study the nature of the initially formed acyclic adduct, we investigated the nature of the intermediate formed in the reaction of styrene ion and neutral styrene. The CAD spectrum of the $C_{16}H_{16}^+$ intermediate is identical to those of cis- and trans-1,2-diphenylcyclobutanes and the radical cation formed in the ionic dehydration of $C_6H_5CH(OH)CH_2CH_2CH_2C_6H_5$. The spectrum is totally unlike those of any other $C_{16}H_{16}$ radical cations including 1- and 2-phenyltetralins, and various diphenylbutenes. This commonality is best explained by initial formation of an ion-dipole (see eq. 9). In the case



of the styrene intermediate, the ion-dipole is sufficiently stable to be observed whereas for the butadiene reactions, the intermediate either cyclizes or isomerizes to a more stable acyclic radical cation. We believe this mechanism to be general for reactions of olefin or diene radical cation and neutral olefins or dienes. Future work is directed at gathering additional evidence to support this picture. Details of these studies are found in a paper by G. Groenewold and E. Chess in this volume and will be published in J. Am. Chem. Soc.

REMARKS IN MEMORY OF J. L. FRANKLIN by F. H. Field, Rockefeller University, New York, NY 10021.

Our symposium today is dedicated to the memory of Joe Franklin, who died on August 25, 1982. It is appropriate that we do this as a recognition of the contribution that he has made over the years to mass spectrometry and as a manifestation of the affection and high regard in which he was held by all who knew him.

He was born in Natchez, Mississippi, in 1906, but his family moved to Texas relatively early in his life, and as a result he obtained his higher education at the University of Texas, taking the B.S. in 1929, the M.S. in 1930, and Ph.D. in physical chemistry in 1934. In 1930 and 1931 he was a fellow at MIT. After receiving his Ph.D. he joined the Humble Oil and Refining Company at Baytown, Texas, and except for a two year sojourn in Washington with the National Bureau of Standards, he worked in Baytown and Houston all his life. In the period from 1934 through 1963 he worked through the ranks in the Technical and Research Divisions of Humble, occupying positions of Assistant Section Head, Section Head, Assistant Division Head, and Research Associate. In 1964 he became the Robert A. Welch Professor of Chemistry in the Rice University. He served a tour of duty as Chairman of the Chemistry Department, and he became Emeritus Professor at the age of 70 in 1976. He continued research until 1981 when poor health required him to close his laboratory.

I shall not take the time to mention here in detail the large number of scientific organizations of which he was a member or officer and the numbers of committees or other groups on which he served. Of particular pertinence to this meeting, however, is the fact that he served as chairman of ASTM Committee E-14 on Mass Spectrometry in 1968-69, and he was instrumental in forming the American Society for Mass Spectrometry, serving as the first president in the year 1969-70. Awards and recognition of his services included the Southwest Regional Award in 1962 and the Southeast Texas Section Award in 1970, both from the American Chemical Society. From the American Institute of Chemical Engineers, he received the South Texas Section Publication Award in 1949 and the Distinguished Service Award in 1962. In 1973 he was chosen as a distinguished Alumnus of the University of Texas.

The range of his scientific interests and the large number of areas in which he worked were both remarkably large. His first professional activities with Humble involved chemical engineering and petroleum refining technology. As time went on he became increasingly interested in the application of basic concepts to provide a deeper understanding of this technology, and from about 1950 he devoted himself to fundamental investigations of nature with primary emphasis on gaseous ionic chemistry. He was the author and co-author of approximately 140 papers, co-author or editor of four books, and he was the holder of a number of patents. He worked in about 20 different areas of mass spectrometry, including: appearance and ionization of potential measurements and the energies of gaseous ions; ion-molecule reactions; ion kinetic energy studies; energies of ions and radicals; negative ion mass spectrometry and energetics; and so on.

It is perhaps a bit pretentious to attempt to select the most significant of his many scientific contributions, but I shall nonetheless express my preferences. One of his earlier contributions was the introduction of the Franklin group equivalent method of estimating the energies of molecules, radicals, and ions, a method which has been and continues to be of much value to scientific workers concerned with energetics and thermodynamic quantities. He was one of the first people to recognize the importance of gas phase data about ions to condensed phase phenomena such as alkylation, catalytic cracking and solution chemistry, and, more than that, to recognize that gas phase data could be obtained from mass spectrometric measurements. He was one of the earliest workers in the field of ion-molecule reactions, and papers on this topic issued from his laboratory for the rest of his life.

From early days also he deplored the relative scarcity of data on the mass spectrometry of negative ions, and when he moved to Rice in 1964, he mounted and maintained a major research effort to provide such data. At about the same time he and his co-workers discovered that a more or less fixed relationship exists between ion kinetic energy and total excitation energy in electron impact induced fragmentation processes. This discovery was used in determining more accurately the heats of formation of gaseous ions from appearance potentials.

Throughout his professional career he strongly emphasized the need for basic research, i.e., the need to understand at a fundamental level the phenomena and technology with which one is concerned. Coupled to this was an insistence on excellence in research, and he applied the highest standards of quality to the work which he did. He imparted these concepts and attitudes to the organizations and people with which he was associated.

In the non-scientific part of his life, Joe was also catholic in his interests. He was devoted to Mildred, his wife of 50 years, and to his three children. He was widely read and knowledgeable about music and art. He loved haute cuisine dining, and going on a company trip with him was usually a gastronomic experience. He was, in short, a gentleman of taste, culture and accomplishment.

I have referred to the many contributions to science that Joe Franklin made. These have been significant and valuable, but I am of the opinion that his greatest contribution was the scientific stimulation, guidance, and inspiration that he provided for many people. In part the influence he had on people was the result of his broad scientific interests and enthusiasm, his high standards, and his impressive intellectual capacity. These qualities made him widely respected. But at least as important as these qualities were the warmth and friendliness of his personality which manifested itself as an interest in and a concern for the people with whom he came in contact; and for those qualities he was widely loved.

Because of the contributions he himself made and the contributions made by others whom he has influenced, the disciplines of mass spectrometry and ionic chemistry and physics have been significantly advanced. Because of his friendship and example, many of us, and certainly myself, have greatly profited. With his death we have suffered the loss of a valued colleague and a dear friend, and we cannot but be sad. However, I think that it is proper to adopt a larger point of view: Life is inexorably finite; Joe's allotment was long, productive and happy. We should be sad because of his death, but we should be happy that he lived and made the contribution that he did. I suggest that we dedicate this symposium to him in this latter spirit, that it should be a celebration of the things that were dear to Joe: the pleasure of intellectual endeavour, the excitement of discovery, the warmth of collegial fellowship, and the joy of the scientific life.

TOD 1 | THE Cf-252 FISSION FRAGMENT IONIZATION
MASS SPECTROMETRY OF CHLOROPHYLL a. F.H. Field and B.T.
Chait, Rockefeller University, New York, N.Y. 10021

We report on the Cf-252 fission fragment ionization positive and negative spectra of chlorophyll a. Time-of-flight mass analysis was used. An important part of the study involved the use of retarding potentials both in the initial ion accelerating region and at the end of the flight tube. These retarding potentials sharpened the spectra and permitted the study of the metastable decomposition reactions occurring. Networks of these were worked out for both positive and negative ions. Some rate constants for the reactions were determined. We suggest that fission fragment induced fragmentation processes involve many of the concepts involved in the quasi-equilibrium theory of mass spectra, e.g., the formation of reactant ions with a wide range of energies, which results in a network of sequential and competing unimolecular reactions with variable and wide-ranging rate constants.

TOD 3 | RATES AND MECHANISMS OF ION-MOLECULE
REACTIONS John I. Brauman, Department of Chemistry,
Stanford University, Stanford, Ca.

Many ion-molecule reactions, especially those of large organic systems at thermal energies, appear to proceed through statistical complexes. Some of these reactions are inefficient and their slowness can be attributed to potential barriers. The barrier heights can, in turn, be corrected for exothermicity and then related to intrinsic molecular properties.

Other dynamic effects can, in principle, affect the rates. Some of these will be discussed as well.

Some Aspects of Intermediate Complex Formation
in Thermal Ion-Molecule Interactions

Eldon E. Ferguson

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I appreciate the opportunity to share in this tribute to Joe Franklin, whose loss to this community is deeply felt. Joe was a particularly important person in my own career because I received my introduction to the subject of ion-molecule chemistry from him, a story I previously related [1] at the session of this Conference honoring Joe in 1975. 25 years ago when I was a physics professor at the University of Texas, Joe visited the campus and gave a few of us, including my first two graduate students, Fred Fehsenfeld and Art Schmeltz, an informal lecture on his research at Baytown. That time was the first I heard of ion-molecule reactions and was influential in my decision to start a program in ion-molecule chemistry in Boulder a few years later. In subsequent years, it was always a pleasure to discuss new results with Joe and I certainly miss that. His enthusiasm for science was an inspiration and his friendship was a warm pleasure.

Some years ago we started measuring temperature dependences of ion-molecule reaction rate constants in our Flowing Afterglow system in Boulder and we found the generality that slow ion-molecule reactions (i.e. $k \ll k_c$) almost always have rate constants which decrease with increasing temperature near 300K. We attributed this to the role of intermediate complexes, the idea being that the lifetime of the complex would decrease with increasing temperature and that the probability of reaction increases with complex lifetime. One also finds that three-body ion-neutral association rate constants decrease with increasing temperature and this is also attributed to the decreasing lifetime of the complex with increasing temperature.

When we extended our energy range with the Flow-Drift Tube, we found that these slow reaction rate constants generally decreased with kinetic energy to some minimum value at an energy of typically a few tenths of an eV and then increased. We interpreted this as implying an intermediate complex mechanism at low energies and a direct reaction mechanism at high energies. The general location of the rate constant minimum was comparable to the expected electrostatic bond energy, which seems at least plausible. The complex lifetime depends strongly on the ion-neutral interaction well-depth, as well as on the complexity and details of the molecular structure. Lifetimes as low as $\sim 10^{-12}$ s can be estimated from three-body association rate constants for weakly bound clusters such as $O_2^+ \cdot N_2$ ($D = 0.225$ eV) and as long as 2.5×10^{-10} s for more strongly bound clusters

such as $O_2^+ \cdot H_2O$ ($D \sim 1\text{eV}$). Lifetimes of 10^{-12}s are of the same order as rotational lifetimes of the thermal energy complexes. The concept of complex implies an exchange of energy between relative kinetic energy of the ion and neutral reactant and the internal energy of the reactants. Recently, Bates [2] and Herbst [3] have extended RRKM theory to the special case of ion-neutral three-body association in order to better understand the temperature dependence for extrapolation to interstellar cloud conditions near 20K.

Many charge-transfer reactions have a decrease in rate constant followed by an increase with increasing energy, suggesting that slow charge-transfer reactions also go via intermediate complexes in the same way as ion-atom interchange ("chemical") reactions do. There appear to be no observations of thermal energy charge-transfer rate constants exceeding the collision rate constant, i.e. no rate constant evidence for long range electron jumps occurring outside the orbiting impact parameter radius. Recent measurements of the charge-transfer between the ions CO_2^+ , N_2O^+ , NO_2^+ , and H_2O^+ with NO show this characteristic behavior with kinetic energy [4].

The ideal test of mechanism would be a crossed-beam study of the reaction kinematics encompassing the energy region of the rate constant minimum to see if the angular product distribution makes a transition from spherically symmetric to asymmetric at this energy. Unfortunately, beam experiments tend to avoid charge-transfer reactions, to avoid slow reactions, and to not reach the low energies where the rate constant minimum occurs. Accordingly, no such test has yet been carried out.

Beam experiments have shown that some reactions do occur via persistent long-lived complex formation, even up to energies of several eV, the endothermic $C^+ + D_2 \rightarrow CD_2^+ + D$ reaction being one example [5, 6]. In this case, a deep energy well exists because of the chemical stability of CD_2^+ . A large three-body association rate constant implies a deep well and hence complex formation. As an example, the anomalously large association rate constant for $Si^+ + O_2 + M \rightarrow SiO_2^+ + M$, which is much larger than for Fe^+ , Mg^+ , Ca^+ , Na^+ , or K^+ association with O_2 , reflects a deep (4.5eV) accessible well because of the stability of SiO_2^+ [7]. The accessibility is a result of the only slight endothermicity of $Si^+ + O_2 \rightarrow SiO^+ + O - 0.35\text{eV}$, which allows the Si^+ to enter the deep well between the O atoms with the collision energy available at close impact. One can predict that if the $Si^+ + O_2 \rightarrow SiO^+ + O$ reaction were carried out in a beam experiment, the long lived intermediate would be evident in the angular product distribution up to relatively high energies, just as for $C^+ + D_2$.

It is found [4] that the linear ions CO_2^+ , N_2O^+ , and NO_2^+ have strongly enhanced charge-transfer rate constants with NO at near thermal energies

when the ions are vibrationally excited in the bending mode. It has been suggested [4] that the vibrational angular momentum of the degenerate bending vibrations may couple with the orbital angular momentum of the ion-neutral collision complex, allowing an efficient transfer of collision energy into internal vibrational energy, thereby increasing the lifetime of the complex and, hence, the reaction rate constant. The magnitude of such coupling remains to be calculated, however. The nonlinear ion H_2O^+ also shows some vibrational enhancement of its charge-transfer rate constant with NO so there must be other mechanisms operating in addition to the Angular Momentum Coupling mechanism, if indeed it is operative.

A very interesting role of intermediate complexes has been demonstrated recently for vibrational relaxation of molecular ions in collisions with neutrals [8, 9]. The vibrational relaxation of O_2^+ ($v=1$) and O_2^+ ($v=2$) ions have been measured for a number of neutrals, He, Ne, Ar, Kr, H_2 , D_2 , CO, CO_2 , SF_6 , CH_4 and H_2O . A strong correlation between quenching rate constant and O_2 - neutral bond energy is evident. When the bond energy is not available, the neutral polarizability gives a qualitative measure of the bond strength for non-polar neutrals. It is proposed that the $V \rightarrow T$ transfer is in effect a $V \rightarrow V$ transfer in the complex, from the O_2^+ bond to the weak O_2 - neutral bond, which then dissociates. The mechanism is very similar to the well studied vibrational predissociation of weakly bound neutral van der Waals molecules for which a wealth of experimental and theoretical information is rapidly becoming available. Some vibrational relaxation studies of N_2^+ ($v=1$) [10, 11] and also NO^+ ($v=1$) [12] have been carried out at Innsbruck. The same general correlation of k_q with neutral polarizability is exhibited by the extensive NO^+ ($v=1$) relaxation data. The quenching rate constants of $\text{O}_2^+(v)$ by CO_2 and Kr and $\text{NO}^+(v)$ by CO_2 , CH_4 , and N_2 were found to decrease with increasing kinetic energy, consistent with an intermediate complex model.

If one assumes that the vibrational quenching efficiency (k_q/k_c) is equal to the ratio of the vibrational predissociation rate constant k_{vp} to the complex unimolecular decay rate k_u , we can compute $k_{vp} = (k_q k_u / k_c)$. The value of k_u can be obtained from three-body association rate constants k^3 of the ion and neutral where they are known, $k_u = k_c k_s / k^3$, where k_s is the third-body stabilization rate constant. The following values of k_{vp} in units of 10^9 s^{-1} are obtained; for $\text{NO}^+(v=1)$: $\text{N}_2(1.7)$, $\text{CO}_2(1.8)$, $\text{CH}_4(v \geq 1)$, for $\text{O}_2^+(v=1)$: $\text{N}_2(1.4)$, $\text{CO}_2(2.9)$ and $\text{H}_2\text{O}(2.0)$. Except for $\text{NO}^+ - \text{N}_2$, $k_{vp} = 2 \pm 1 \times 10^9 \text{ s}^{-1}$ fits the data well whereas the values of k_q vary by a factor of 50. The value $k_{vp} = 2 \times 10^9 \text{ s}^{-1}$ is rather close to the

value $k_{vp} = 5 \times 10^9 \text{ s}^{-1}$ estimated for the vibrational predissociation of $(\text{HF})_2$ dimer [13] and $k_{vp} \sim 3 \times 10^9 \text{ s}^{-1} - 2 \times 10^8 \text{ s}^{-1}$ calculated for $\text{Ar} \cdot \text{I}_2$ ($v=1$) [14]. The anomaly in $\text{NO}^+ - \text{N}_2$ is attributed to a resonant V - V transfer, $\text{NO}^+(v=1) + \text{N}_2(v=0) \rightarrow \text{NO}^+(v=0) + \text{N}_2(v=1) + 14 \text{ cm}^{-1}$, an almost exact resonance. We consider the accumulated evidence very strong for an intermediate complex role in molecular ion vibrational relaxation and predict generally efficient vibrational relaxation by neutrals of polarizability $> 1\text{\AA}^3$ with an increasing efficiency for lower frequency vibrations.

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CHEMICAL IONIZATION MASS SPECTROMETRY:
ANALYTICAL APPLICATIONS OF ION/MOLECULE REACTIONS

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Chemical ionization mass spectrometry, CIMS, is an analytical application of ion/molecule reactions. In order to understand, to explain, and perhaps to predict chemical ionization mass spectra and sensitivities for different compounds, one must understand the fundamental processes.

The reaction rate constant is the fundamental molecular parameter in CIMS; it is the analog of the ionization cross section, σ , in electron ionization mass spectrometry. One can easily show (with CH_4 as the reagent gas, for example) that the total sample ionization or sensitivity depends on the sample concentration, (M); ionic reaction time, t ; ionic concentration, (CH_5^+) and (C_2H_5^+); and the rate constants for the ionic reactions, k_1 :

$$\Sigma I_i^+ = \{k_{17}(\text{CH}_5^+) + k_{29}(\text{C}_2\text{H}_5^+)\} (M) t \quad (1)$$

Experimental data on rate constants of ion/molecule reactions indicate that many proton and charge transfer reactions are fast if they are a few kcal/mol exothermic and very slow if they are endothermic.^{1,2} One may use data on rate constants to predict and to explain selective ionization with different reactant ions. Rate constants are generally not available for reactions with complex molecules; so one must reason by analogy and the estimates are generally qualitative.

For example, one can compare the reactions of s-butyl ions from n-butane with those of t-butyl ions from isobutane. From the few data that are available for reactions of $\text{s-C}_4\text{H}_9^+$ and analogies with reactions of $\text{s-C}_3\text{H}_7^+$, one would expect s-butyl ions to react rapidly by hydride transfer to alkenes and somewhat more rapidly by proton transfer to c-hexanone. The t-butyl ions should give virtually no ionization with n-alkanes and very slow hydride transfer with tertiary alkanes, but very fast proton transfer to c-hexanone.³ Fig. 1 shows a flame

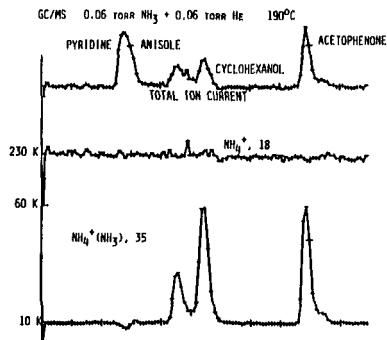
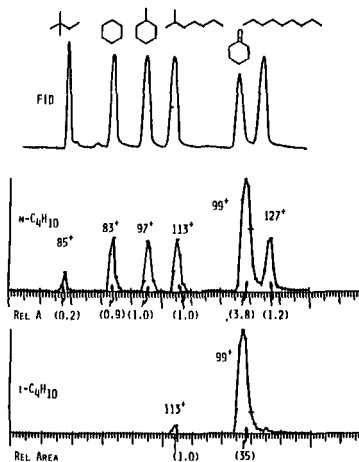
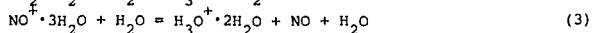
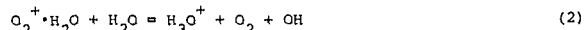


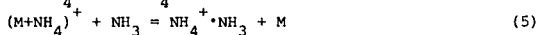
Fig. 2. Sample Catalyzed Solvation of Ammonium Ions

Fig. 1. Comparison of $\text{s-C}_4\text{H}_9^+$ and $\text{t-C}_4\text{H}_9^+$ as CI Reagent Ions

ionization detector trace for an equimolar mixture of hydrocarbons and c-hexanone. Some selectivity is indicated for $\text{s-C}_4\text{H}_9^+$ since the total area of $(\text{M}+\text{H})^+$ from c-hexanone is 3.8 times the area for $(\text{M}-\text{H})^+$ from 2-methylheptane. Little variation is noted among the sensitivities of the C_6 - C_{10} hydrocarbons. The predicted high selectivity for t-butyl ions is readily apparent in this figure since there are no detectable ions from c-hexane or n-nonane and the total area for $(\text{M}+\text{H})^+$ from c-hexanone is 35 times the area for $(\text{M}-\text{H})^+$ from 2-methylheptane. Switching reactions, such as the following, have been reported as important processes in determining the composition of atmospheric ions:⁴



Somewhat similar reactions may explain observations in NH_3 CIMS. The relative sensitivities as $(\text{M}+\text{NH}_4)^+$ ions for polar, but not strongly basic, compounds decrease with increasing ammonia pressure. That is, abundant $(\text{M}+\text{NH}_4)^+$ ions are observed for cyclohexanol and abundant $(\text{M}+\text{H})^+$ ions are observed for pyridine at low pressures of NH_3 , 0.05 torr; and at 0.5 torr, abundant $(\text{M}+\text{H})^+$ ions are observed for pyridine and virtually no ions are observed for cyclohexanol. A mixture of 1% NH_3 in CH_4 at 0.5 torr gives abundant $(\text{M}+\text{NH}_4)^+$ ions for cyclohexanol and abundant $(\text{M}+\text{H})^+$ ions for pyridine. In addition, Fig. 2 shows the increase in ion current at $m/z = 35$, $\text{NH}_4^+ \cdot \text{NH}_3$, in GC/CIMS experiments at 0.05 torr NH_3 when polar, but non-basic compounds elute. The ion current at $m/z = 35$ decreases as the more basic pyridine elutes. We attribute these observations to the following reactions:



Since there is conservation of charge in CIMS, for every sample ion produced, a reactant ion is removed. Reactant ion monitoring, RIM, is the technique of observing changes (if any) in the reactant ion current during the course of a GC/CIMS experiment. The decreases in reactant ion current (or sensitivities for the different compounds) are related to sample concentration, ionic reaction time, and ionic reaction rate constant. For C_2H_5^+ and CH_5^+ , it is observed that the relative sensitivities per gram for many compounds are essentially independent of molecular weight and molecular structure. One may obtain 'semiquantitative' analyses on complex mixtures by RIM without knowing the chemical nature of all of the components of the mixture. Moderately accurate values of sensitivities for complex molecules can be obtained from the relative rate constants calculated from theoretical models.⁵

Selective detection in Reactant Ion Monitoring is achieved with low energy ions that react with molecules possessing a certain property. From compilations of proton affinities (like those of Franklin⁶ or of Rosenstock⁷), it is known that methanol is a stronger base than alkanes or cycloalkanes, but that it is a weaker base than most alkenes. Consequently, CH_3OH_2^+ is expected to, and does, transfer a proton to and identify alkenes in GC/CIMS experiments with mixtures of saturated and unsaturated aliphatic hydrocarbons. From compilations of ionization potentials (like those of Franklin⁸ or of Rosenstock⁹), one could select C_2H_5^+ or $\text{C}_2\text{H}_5\text{Cl}^+$ as selective reactant ions to identify aromatic hydrocarbons in fossil fuel mixtures. Fig. 3 shows a RIM trace for a gasoline sample using C_2H_5^+ from benzene as the reactant ion. The FID trace shows all of the components and the RIM trace shows only the unsaturated compounds. The spectra obtained at the various minima in the RIM trace for C_2H_5^+ contained only M^+ ions as indicated. To a good approximation, the mole % composition of the unsaturated compounds and the area % from the RIM traces are the same. Other selective reactant ions have been examined by RIM: $\text{CH}_3\text{ClCH}_3^+$ and $\text{CH}_3\text{BrCH}_3^+$ are selective methylating reagents. Trimethylsilyl ion, $(\text{CH}_3)_3\text{Si}^+$, from tetramethylsilane reacts with many hydrocarbon derivatives to give $(\text{M}+73)^+$ adduct ions; it even forms adduct ions with butyl chloride. A typical RIM trace for $m/z = 73$, trimethylsilyl ions, is shown in Fig. 4. $(\text{M}+73)^+$ ions are formed for the indicated compounds. Additional selectivity can be obtained by complexing the trimethylsilyl ion with other species, acetone, in this example. Although bromobenzene, nitrobenzene, and dodecyl thiol react with trimethylsilyl ion, they do not displace acetone from the solvated ion at $m/z = 131$.

Fig. 5 shows another use of RIM, the determination of proton affinities of complex molecules. The compound in question is introduced into a chemical ionization source in the presence of a protonating reagent and the ion current of the $(\text{M}+\text{H})^+$ ion of the compound is monitored as a series of bases are eluted from a gas chromatograph into the source of the mass spectrometer. If the compound in question is less basic than the compound eluting through the source, then proton transfer will occur and the ion current for $\phi_3\text{PH}^+$, for example, will decrease. If no reaction of $\phi_3\text{PH}^+$ occurs, we assume that the proton transfer reaction does not occur because it is endothermic. One can then bracket the proton affinity of the compound in question by choosing reference bases of known and closely spaced proton affinities. For the example of Fig. 5, the proton affinity of triphenylphosphine is less than the proton affinity of triethylamine and greater than the proton affinity of di-isopropylamine.

One of the major advantages of CIMS is the simplification of the spectra and one goal of research in CIMS has been to find reagent ions which give only one ion per compound. Fragment ions are necessary, however, for structural characterization. Increased fragmentation can be obtained in CIMS by high pressure collisional activation, HPCA. The source for HPCA is a long path, high pressure or chemical ionization source which is separated into two regions by a wire mesh grid. In the first region the conventional CI reactions occur at a low field strength. A high field strength can be applied in region 2 to accelerate the ions and allow them to undergo dissociative collisions. If low fields are present in both regions, one obtains CI spectra, as shown in Fig. 6 for ethylbenzene and p-xylene. When a low field is maintained in region 1 and a high field is applied to region 2, the CI ions of the sample $(\text{M}+\text{H})^+$,

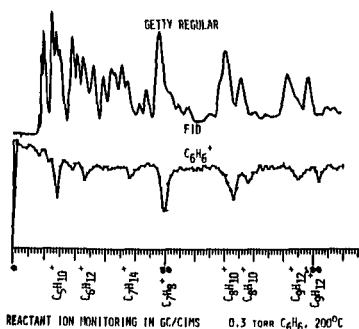


Fig. 3. FID and $C_6H_6^+$ RIM Traces for Gasoline Sample

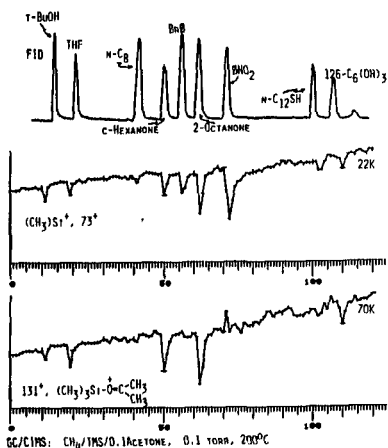


Fig. 4. FID, 73^+ and 131^+ RIM Traces in GC/CIMS with TMS

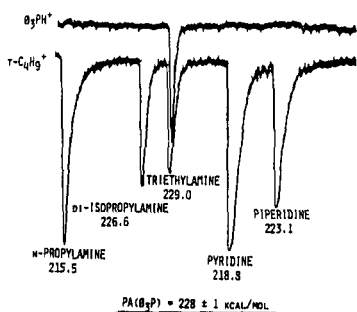


Fig. 5. Determination of Proton Affinities by RIM

$(M+C_2H_5)^+$, and $(M+C_3H_7)^+$ in this case) acquire significant kinetic energy from the field and convert sufficient kinetic energy into internal energy that significant fractions of these ions are decomposed. The CH_3 CI spectra of ethylbenzene and p-xylene are very similar, but the HPCA spectra are easily distinguished, and the decomposition products are easily explained in terms of the differences in structures of the two species. One may rapidly alternate the fields in region 2 so that alternating CI and HPCA spectra may be obtained across a peak in a GC/CIMS experiment. Under CI conditions, one obtains the simplified spectrum and molecular weight information; under HPCA conditions, one obtains extensive fragmentation and structural information.

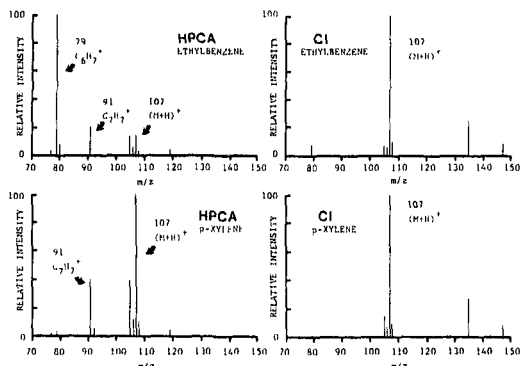


Fig. 6. CH_3 CI and HPCA Spectra of Ethylbenzene and p-xylene

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TOD 4 } CHEMICAL IONIZATION MASS SPECTROMETRY:
ANALYTICAL APPLICATIONS OF ION/MOLECULE REACTIONS; Burnaby
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Chemical ionization mass spectrometry is a direct result of early fundamental studies of ion/molecule reactions at increasingly higher pressures. The sensitivities of a CI reactant ion with different compounds are directly related to the rate constants of the ion/molecule reactions. Reasonable agreement is observed between experimental relative sensitivities from Reactant Ion Monitoring studies and the values calculated from ADO theory.

Selective reactivity or non-reactivity with classes of compounds can be achieved by varying the acid strength of the reactant ions. Similar selectivities can be achieved with this endothermic/exothermic criterion for charge exchange reactions. Reactant ion monitoring and CI spectra can also determine the proton affinities and other thermochemical data for complex molecules.

Exchange reactions, switching or solvent displacement reactions, and clustering or adduct formation reactions are also observed as CI processes. "Self CI" or sample ion/sample molecule reactions have been used in GC/CIMS and FT-MS analyses. High pressure collisional activation can be used for analytical characterization and ion/molecule reaction studies.

FAST ATOM BOMBARDMENT SPECTRA OF GOLD COMPOUNDS:
EXPECT THE UNEXPECTED!

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Our initial interest in the FAB spectra of gold compounds began about two years ago when we were trying to solve a structure elucidation problem where FD results produced ambiguous answers. It was thought that FAB spectra might clarify the situation and provide a solution to the problem. Hence, a FAB spectrum of Ridaura (1) was obtained from glycerol. Surprisingly, this spectrum showed a strong peak at m/z 977 which was indicative neither of the molecular weight of 1 (M.W. 678) nor of a glycerol adduct thereof. Subsequent analysis of this peak by triple quad MS¹ and analysis of the isotopic cluster composition demonstrated that m/z 977 corresponded to an ionic species (i.e. a cluster) of the formula $[C_{18}H_{45}Au_3P_3S]^+$. After obtaining this unexpected but interesting FAB result, it seemed worth while to look at the FAB spectra of other gold compounds to see if they would produce similar spectra. The following paragraphs summarize the results obtained for the FAB spectra of compounds 1, 2 and 3 in both glycerol and thioglycerol and compare these results with the FD spectra of compounds 1, 2 and 3.

In the FAB spectrum of 1 from glycerol, in addition to the m/z 977 peak sample related peaks are observed at m/z 315 $[AuPET_3]^+$, m/z 433 $[Au(PET_3)_2]^+$ and m/z 679 $[MH]^+$. However, the FAB spectrum of 1 from thioglycerol shows a slightly different spectrum. In addition to the peaks observed in the glycerol FAB spectrum there is a strong peak at m/z 993 which apparently is an $AuPET_3$ adduct of 1. In contrast, the FD spectrum of this

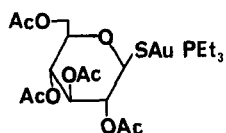
compound shows strong peaks at m/z 678 $[M]^+$, m/z 331 $[$  $]$

and m/z 118 $[PET_3]^+$. The FD spectrum also shows a small peak at m/z 993, but none at m/z 977.

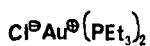
The FAB spectrum of 2 from glycerol shows peaks at m/z 433 and m/z 315 with no sign of a peak at m/z 977. However, when this compound is dissolved in thioglycerol, the FAB spectrum shows a strong peak at m/z 977. Furthermore, when the FAB spectrum of 2 is obtained from glycerol after thioglycerol has been used in the instrument, it shows an m/z 977 peak. Apparently, the gold is reacting with sulfur from residual thioglycerol which is not removed even with careful cleaning in the source and/or inlet system to produce the m/z 977 peak. The FD spectrum of 2 shows the base peak at m/z 350 (1 Cl) $[ClAuPET_3]^+$ and a small peak at m/z 433, neither of which is unexpected.

The FAB spectrum of 3 from glycerol shows the presence of a strong m/z 977 peak in addition to the anticipated peaks at m/z 315 and m/z 433, even when it has been determined that the instrument is free of residual sulfur contamination. The FD spectrum of 3 shows the anticipated molecular ion at m/z 350 (1 Cl), but no evidence of contaminants in the sample. Therefore, we may speculate that the m/z 977 peak observed in the glycerol spectrum of 3 may be caused by trace residual contamination of the sample by a sulfur containing impurity, or possibly that 3 may be reactive enough to extract trace amounts of sulfur from the environment. Efforts are currently being made to resolve this issue.

From the results obtained in this limited study, a number of conclusions about the FAB spectra of gold compounds can be drawn. In the first place, gold apparently is a good scavenger for sulfur and appropriate precautions must be taken when running samples to be sure the source and sample stage are free of sulfur contamination. Secondly, thioglycerol is not a good dispersing medium for obtaining FAB spectra of gold compounds. Thirdly, gold tends to form clusters under FAB conditions (e.g. m/z 977 and m/z 993). Finally, FAB spectra obtained on gold compounds may be confusing and FD should still be considered a useful tool for confirming molecular weights of some compounds (e.g. 1 and 3).



1



2



3

Note: All FAB spectra were obtained from a VG-ZAB-1F mass spectrometer equipped with a FAB source, a high field magnet and a 2035 data system. We wish to thank Mr. Walter Johnson for providing the FD data for compounds 1, 2 and 3.

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MASS SPECTRAL INVESTIGATION OF BIOLOGICALLY-ACTIVE TRANSITION METAL COMPLEXES

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Desorption ionization methods such as secondary ion mass spectrometry (SIMS) and fast atom bombardment (FAB) which employ keV particle sputtering from a condensed phase are applicable to a wide variety of biologically important transition metal complexes. The charge state of the complex is particularly important to successful analysis. Neutral complexes such as $\text{Pt}(\text{NH}_3)_2(\text{Cl})_2$ do not readily undergo cationization (1) during solid or solution sputtering. Loss of Cl^- generates the $\text{Pt}(\text{NH}_3)_2(\text{Cl})^+$ ion observed in static SIMS (2), while substitution by DMSO in a DMSO/glycerol matrix yields the $\text{Pt}(\text{NH}_3)_2(\text{Cl})(\text{DMSO})^+$ ion seen in FAB.

Preformed cationic complexes such as Tc and Fe radiopharmaceuticals are also particularly sensitive yielding representative spectra from both solids and solutions. Generating long-lived spectra which allow high resolution and/or ms/ms analysis of secondary ions requires a solution matrix.

The ability to obtain spectra of specific mass-selected secondary ions is invaluable in deconvolving complex mixtures of radiopharmaceuticals. For most cationic complexes, metastable dissociations observed in MIKES are sufficient to characterize the sputtered cation, however, additional structural information may be obtained by collisional-activation. The lability of certain complexes to oxidation, substitution, and rearrangement makes ion structural analysis a necessity for complete characterization.

A series of Tc and Fe radiopharmaceuticals have been characterized (3) using this approach. The resulting MIKE spectra provide a data base for ordering the relative ion intensities of ligand losses from a Tc or Fe metal center (Tables I, II, and III). Representative MIKE spectra are shown in Figures 1 and 2. Metastable loss of the equatorial chelating ligand is favored when the alkyl (-R) substituent is bulky, indicating steric repulsion is the major driving force for dissociation. Ring size, unsaturation, chelate planarity, and Tc-Y stability show less pronounced effects upon dissociation. This is contrasted with the effects of differing metal center, viz., Tc vs. Fe, where ligand losses are reversed.

Comparisons are made with bond lengths obtained by X-Ray Diffraction (4) and from infrared analysis. Projections are made to distinguish oxidation states and ligand stabilities from the mass spectral data.

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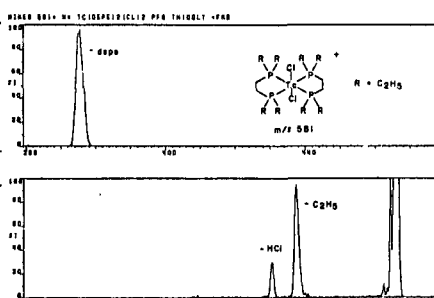
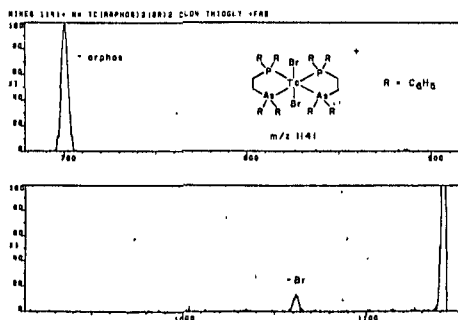


Figure 1

Figure 2

TABLE I
RELATIVE INTENSITIES OF LIGAND LOSSES

COMPOUND	Y	R	-X or -HX/-LIGAND
$\text{Tc}(\text{dmpe})_2(\text{Cl})_2^+$	P	CH_3	5.9
$\text{Tc}(\text{depe})_2(\text{Cl})_2^+$	P	C_2H_5	0.31
$\text{Tc}(\text{dbpe})_2(\text{Cl})_2^+$	P	C_4H_9	0.071
$\text{Tc}(\text{arphos})_2(\text{Cl})_2^+$	P/As	C_6H_5	0.18
$\text{Tc}(\text{dpe})_2(\text{Cl})_2^+$	P	C_6H_5	0.41
$\text{Tc}(\text{dpe})_2(\text{Br})_2$	P	C_6H_5	0.09
$\text{Tc}(\text{dae})_2(\text{Br})_2^+$	As	C_6H_5	0.12
$\text{Fe}(\text{dmpe})_2(\text{Cl})_2^+$	P	CH_3	0.00

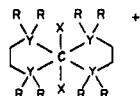


TABLE II
RELATIVE INTENSITIES OF LIGAND LOSSES

COMPOUND	Y	R	-X or -HX/-LIGAND
$\text{Tc}(\text{diphos})_2(\text{Cl})_2^+$	P	CH_3	large (50)
$\text{Tc}(\text{diars})_2(\text{Cl})_2^+$	As	CH_3	large (50)
$\text{Tc}(\text{diars})_2(\text{I})_2^+$	As	CH_3	large (50)
$\text{Tc}(\text{mppb})_2(\text{Cl})_2^+$	P	$\text{CH}_3/\text{C}_6\text{H}_5$	2.9
$\text{Tc}(\text{dppb})_2(\text{Cl})_2^+$	P	C_6H_5	0.88

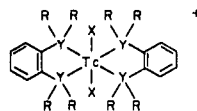


TABLE III

RELATIVE INTENSITIES OF LIGAND LOSSES

COMPOUND	Y	R	-X or -HX/-LIGAND
$\text{Tc}(\text{dpe})_2(\text{Cl})_2^+$	P	C_6H_5	0.41
$\text{Tc}(\text{dppe})_2(\text{Cl})_2^+$	P	C_6H_5	0.44
$\text{Tc}(\text{dppp})_2(\text{Cl})_2^+$	P	C_6H_5	0.25



ANALYSIS OF PLATELET ACTIVATING FACTOR (AGEPC) BY FAB-MS

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Acetyl glyceryl ether phosphocholine (AGEPC) has been described (1,2) as the lipid platelet activating factor (PAF) which was released by rabbit basophils upon IgE dependent antigen stimulation (3), and as the antihypertensive polar renomedullary lipid (APRL) (4). The importance of this molecule as a mediator of inflammatory responses, as well as its antihypertensive properties, is becoming apparent (5), and its potent effects have been demonstrated by various bioassay methods at concentrations on the picomolar range. These bioassay methods are not specific for AGEPC and confidence in the quantitative data from such methods is limited. We have sought to develop a specific and sensitive method of analysis using the physicochemical properties of this unique molecular species as revealed by fast atom bombardment mass spectrometry (FAB-MS).

AGEPC (alkyl = hexadecyl) yields an intense FAB-mass spectrum (Figure 1) characterized by an abundant protonated molecular ion at m/z 524. The most abundant ion, however, occurs at m/z 184 (not shown in Fig. 1) from the phosphorylcholine residue I. There is also an abundant ion from the fatty ether moiety at m/z 224 (II). The AGEPC subjected to FAB-MS in Fig. 1 was dissolved in saline, hence the appearance of the alkali attachment ions for sodium and potassium.

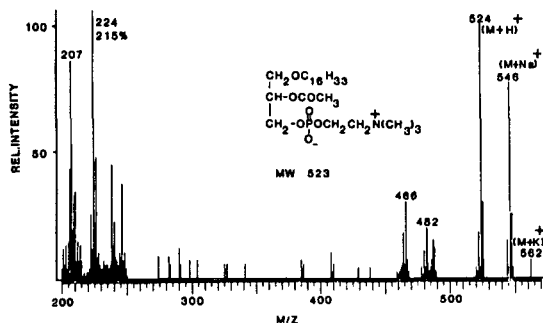
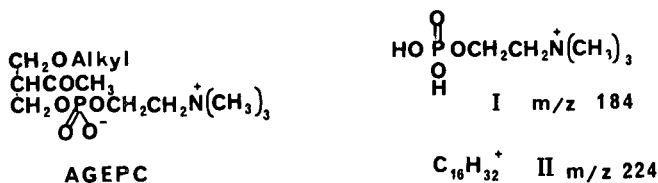


Figure 1. FAB-Mass spectrum of AGEPC.

Development of a quantitative mass spectral procedure for AGEPC requires consideration of an appropriate internal standard. Chemical analogues are not useful as internal standards because of uncertainties related to ionization efficiencies, differential matrix effects and variable recoveries in isolation procedures. Also, there is an ionization suppression effect observed as the concentration of the measured compound increases (6) past some maximum value. For these reasons we felt that stable isotope internal standards would be required and D3-AGEPC was synthesized from lyso-AGEPC and D6-acetic anhydride. This D3-AGEPC gave a protonated molecular ion at m/z 527. Figure 2 shows the mass spectrometric results (analog recording) of mixing 0.5, 1, 5, and 10 μ g of AGEPC with a constant amount (5 μ g) of D3-AGEPC. In general the absolute signal for D3-AGEPC decrease as the AGEPC increased as expected for the saturation effect. When the ratios of m/z 524/527 were measured and plotted relative to the amount of AGEPC, a straight line was obtained (Figure 3). The data had a linear correlation coefficient of 0.99. The lower limit for quantitation of AGEPC by this method was 10 nanograms (S/N 2) using 60 ng D3-AGEPC. These results suggest that FAR-MS can be used as a sensitive, quantitative analytical technique when stable isotope internal standards are employed.

ACKNOWLEDGEMENT. This work was supported by grants from the National Institutes of Health HL 25785 and RR 01152.

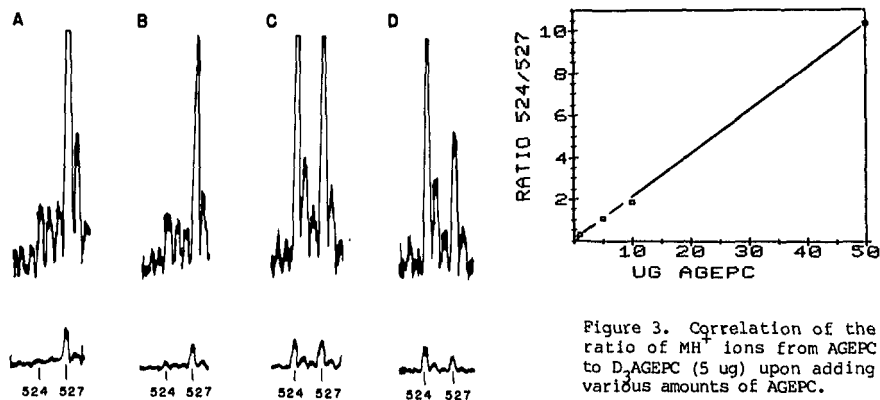


Figure 3. Correlation of the ratio of MH^+ ions from AGEPC to D₃AGEPC (5 μ g) upon adding various amounts of AGEPC.

Figure 2. UV-recorder trace of MH^+ ions from D₃-AGEPC (m/z 527, 5 μ g) added to target after mixing with (A) 0.5 μ g, (B) 1 μ g, (C) 5 μ g and (D) 10 μ g, native AGEPC (m/z 524).

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SUMMARY

The potential of soft ionization procedures, field desorption (FD) and fast atom bombardment (FAB) mass spectrometries, for the structural analysis of underivatized glycosphingolipids was examined with gangliosides and related glycosphingolipids. Asialo-gangliosides were analyzed by FD-MS with a carbon emitter. Molecular ion with sodium ion, $(M+Na)^+$, were detected as the base peak. Fragment ions were not detected at the emitter current producing the $(M+Na)^+$ base peak. In contrast with neutral glycosphingolipids, the analysis of gangliosides by FD-MS and FAB-MS was very difficult probably due to the presence of sialic acid. However, we could detect the molecular ion species of gangliosides by use of a FAB-MS matrix of triethanolamine (TEA) with a few drops of 1,1,3,3-tetramethylurea (TMU). With this new matrix, the molecular ion species of gangliosides, more intense than those obtained with a conventional glycerol matrix, were detected by positive ion FAB-MS. Fragment ion species for determining the carbohydrate sequence were still less informative. Negative ion FAB-MS spectra of gangliosides and asialo-gangliosides gave high intensity molecular ions species $(M-H)^-$, and fragment ions cleaved at glycosidic linkages sequentially from the non-reducing end with or without ceramide portion. These fragment ions were found to be extremely useful for the structural analysis of glycosphingolipids.

INTRODUCTION

Possible participation of glycosphingolipids in many biological processes have been clarified and the structural elucidation of glycosphingolipids increasingly becomes an important subject.^{1,2)} Mass spectrometric procedures in the field of glycosphingolipid chemistry, together with chemical and enzymatic methods, and high resolution nuclear magnetic resonance spectroscopy, considerably facilitated the molecular weight determination, the linkage, branching and sequence determination of carbohydrate moiety, and the conformation of molecular species including ceramide. Recent progress in ionization procedures and improvement of instrumentation have further expanded the applicable region of glycosphingolipids analysis by mass spectrometry. Particularly, the introduction of soft ionization procedures allows the ionization of underivatized glycosphingolipids directly from solid phase by FD-MS³⁾ and from liquid matrices by fast atom bombardment mass spectrometry.⁴⁾ The potential and capability of these newly developed procedures on glycosphingolipid chemistry are not yet known in detail. In this report, we describe the soft ionization mass spectrometries useful for the structural elucidation of underivatized glycosphingolipids.

MATERIALS AND METHODS

Materials; Gangliosides were extracted from bovine brain and purified by DEAE-Sephadex A-25 and Iatrobeds column chromatography. The purity of isolated gangliosides (GM1, GD1a, GD1b, GT1a and GT1b) was confirmed by thin layer chromatography. Asialo-gangliosides (CMH, CDH, GA2 and GAl) were prepared from ganglioside by formic acid-treatment and purified by Iatrobeds column chromatography.

Methods; FD-MS: Asialo-gangliosides solutions in chloroform-methanol (1:1, v/v) were loaded on a carbon emitter with a microsyringe and then analyzed a JMS-DX300 gas chromatography mass spectrometer (JEOL Ltd., Tokyo) and a JMA-3500 data system (JEOL Ltd., Tokyo).

POS / NEG -FAB-MS: Sample solutions (chloroform-methanol) were mixed with TEA and a few drops of TMU on a stainless steel sample holder. The analysis was performed by detecting positive/negative ions with a JMS-HX100 ultra high resolution mass spectrometer with a FAB-ion source (JEOL Ltd., Tokyo) and a JMA-3500 data system (JEOL Ltd., Tokyo).

RESULTS AND DISCUSSION

Purified glycosphingolipids contained stearic acid and two kinds of sphingosine (C-18 and C-20). FD-MS: FD mass spectra of asialo-GM2 (GA2) are shown in Fig.1. Molecular ion species $(m/z)1143$ and $m/z1115$ are detected as base peak ion at low emitter current (15-17mA) and fragment ions are not observed (Fig. 1-A). When the emitter current is raised to 18-20mA, the fragment ions, $(CDH+Na)^+$, become the most intense ion in the spectrum (Fig. 1-B). At 21-22mA emitter current (Fig. 1-C), the intensity of fragment ions further increases, reflecting the importance of emitter current in the formation of fragment and molecular ion species. Fig. 2 shows the FD and FD-linked scan mass spectra of N-acetylpsycosine. At the optimum emitter current producing the molecular ion species as the base peak the daughter ion linked scan method performed on the molecular ion species was useful in obtaining fragment ion information (Fig. 2-A,B). POS -FAB-MS: The molecular ion species of GM1 ganglioside were not detected by POS -FAB-MS using a glycerol matrix (Fig. 3-A). With the TEA

and TMU, molecular ion species were clearly detected,⁴⁾ suggesting that the selection of matrix to cleave the hydrogen bond is effective in the ionization of ganglioside. Fragment ions were not detected and the POS.-FAB MS was a rather less informative method to determine the carbohydrate structure (Fig. 3-B). NEG -FAB-MS: Negative ion FAB-MS spectrum of GM1 ganglioside is shown in Fig. 4. In contrast with the POS -FAB-MS spectrum (Fig. 3-B), molecular ion species and fragment ions, which were sequentially cleaved at glycosidic linkage from the non-reducing terminal, are detected.

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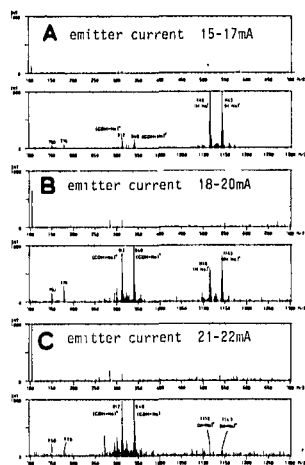


Fig. 1. FD Mass Spectra of GA2

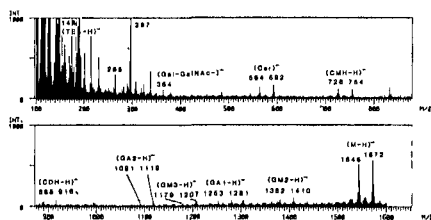


Fig. 4. Negative Ion FAB Mass Spectrum of GM1

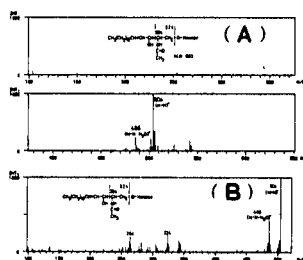


Fig. 2. FD Mass Spectra of N-Acetyl Psychosine
(A) Normal FD
(B) Collision activated Linked scan
(B/E=const.)

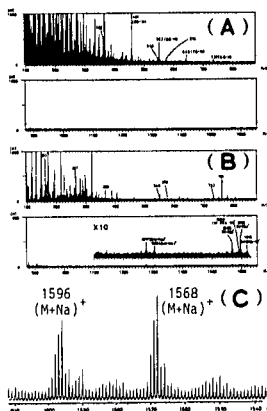


Fig. 3. Positive Ion FAB Mass Spectra of GM1
from Bovine Brain
(A) Glycerol matrix
(B) Triethanolamine + Tetramethylurea matrix
(C) Molecular Ion Regions with (B) matrix

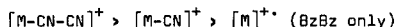
MATRIX EFFECTS IN FAB ANALYSIS OF COBALAMINES

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The fast atom bombardment mass spectra (FAB/MS) of corrin cobalt (III) complexes (e.g. Vitamin B12, cobyrinic acid derivatives) obtained from glycerol matrices frequently do not provide molecular mass information in the positive ion mode [1]. Neither $[M]^{++}$ nor $[M+H]^+$ are obtained for cobyrinic acid derivatives in contrast to the usual results under these conditions [2]. This is apparently due to a very easy loss of the axial ligands, which is also known to occur under EI and FO conditions (see references cited in [1]).

In general, our measurements obtained on cobyrinic acid derivatives confirm the results reported by Schwarz [1]. The peak group of highest abundance in the upper mass range normally represents the corrin system after loss of the axial ligands L1 and L2 [1]. Additional peak groups above this mass number arise from undetermined chemical reactions between sample and matrix or sample fragments [3], but are certainly not simple matrix/molecule adducts. Thus no information on the chemical nature of the axial ligands of cobyrinic acid derivatives can be obtained (Fig. 1). Replacing glycerol by other matrix compounds improves the situation markedly. Promising results were obtained with the ethyl and butyl esters of citric acid (TEC, TBC), benzoic acid benzylester (BzBz) and 2-nitro-phenyloctylether (NPOE).

The spectra of heptamethyl-Co α ,Co β -dicyano-cobyrate obtained with the ester matrices provide information on either one (TEC, TBC) or both (BzBz) axial ligands. Additional peak clusters are either of low abundance or arise from easily interpretable attachment of matrix constituents ($[M-L1-L2 + 73/101/121]^+$ for TEC/TBC/BzBz). The spectra from the citrate ester matrices do not provide molecular mass information, but an ion for $[M-L1]^+$ of significant intensity (m/z 1062, ~20 %) is present. In benzoic acid benzylester a $[M]^{++}$ ion (m/z 1088, ~20 %) and the $[M-L1]^+$ ion (~60 %) is observed. In all three matrices the relative intensities follow the same pattern:



The spectra obtained with NPOE are dominated by the $[M-CN]^+$ ion, the second and third intense signals being due either to $[M]^{++}$ or $[M-CN-CN]^+$. It appears that the slightly oxidizing matrix can prevent the well known metal valence change Co(III) $\rightarrow$ Co(II) to a large extent. The abundance of these two ions is directly proportional to the time the sample was subjected to atom (or ion) bombardment. The highest intensity of the $[M]^{++}$ ion is detected immediately after introduction of the sample into the source (120 - 150 % of $[M-CN-CN]^+$) and decreases to 20 - 30 % after a few minutes.

A similar compound, heptamethyl-Co-cyano-Co-aquoperchlorato-cobyrate, was subjected to the same experiments. The most abundant peak group in the spectra determined in ester matrices arises from the corrin molecule $[M-L1-L2]^+$ but, in contrast to the dicyano complex, no signals for either $[M-L1]^+$, $[M-L2]^+$ or $[M]^{++}$ are present. In NPOE the most abundant signal arises from $[M-L1-L2]^+$ (m/z 1036) with $[M-H_2OClO_4]^+$ (m/z 1062) ranking second (~80 %). No evidence for the aquocyano complex (m/z 1080) is found, but signals for the perchlorato complex (m/z 1161; loss of CN=1135) are present at low abundance but clearly above the chemical background. A time dependence of the $[M-H_2OClO_4]^+$ ion similar to the dependence of the $[M]^{++}$ ion of the dicyano complex is found.

Heptamethyl-Co α ,Co β -dicyano-5,6-dioxo-5,6-ssecocobyrate and heptamethyl-Co α ,Co β -dicyano-14,15-dioxo-14,15-ssecocobyrate did not give rise to significant signals corresponding to the molecular mass (m/z 1120) or loss of one axial ligand (m/z 1094) in a glycerol matrix. These compounds however are reduced by glycerol under FAB conditions as indicated by the high abundance of the m/z 1070 ($[M-CN-CN+2H]^+$) peak. Less pronounced, but clearly discernible such reductions by glycerol are also evident in the $[M-CN-CN]^+$ cluster of the heptamethyl-dicyano-cobyrate discussed above (see m/z 1038 in Fig. 1). In spectra obtained with the NPOE matrix, the high mass and is dominated by peak clusters arising from $[M-CN]^+$ (m/z 1094, 100%), $[M-CN-CN]^+$ (m/z 1068, ~80%) and $[M]^{++}$ (m/z 1120, ~15%) with no significant reduction of the oxygenated corrin system being observed. The 5,6-dioxo and the 14,15-dioxo compounds can be differentiated by the medium abundant ions of m/z 771 and m/z 785 respectively, formed by loss of the methylpyrrolinylketone moiety each.

CONCLUSIONS

The FAB/MS analysis of corrin samples is profitably carried out in a weakly oxidizing matrix compatible with the experimental requirements for this technique. Such a matrix allows for the direct determination of the correct chemical composition of corrins (Fig. 2) and similar (e.g. porphyrin) complexes and prevents chemical degradation during the desorption and/or ionization process like reduction of functional groups or changes of metal valences. It constitutes thereby a more adequate system for the mass spectrometric analysis of these compounds than the conventional one with glycerol as matrix for sample admission.

ACKNOWLEDGEMENT

We thank Dr. B.Kräutler for providing the samples and his valuable discussions on the chemistry of the corrins.

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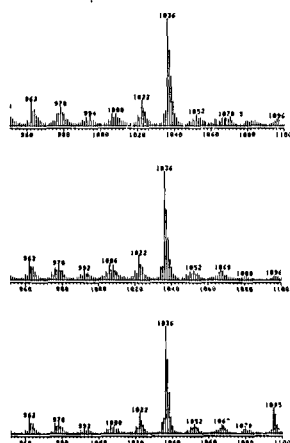


Fig. 1. Heptamethylcobyrate
Matrix : Glycerol
axial Ligands L1 and L2 :
Top : CN / CN
Middle : CN / H₂O.C₁₀₄
Bottom : CH₃ / H₂O.C₁₀₄

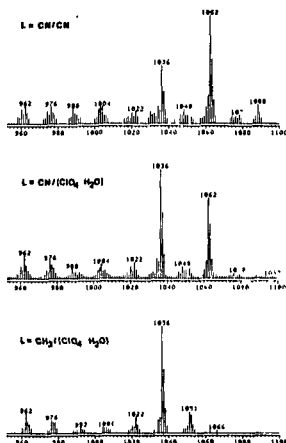


Fig. 2. Heptamethylcobyrate
Matrix : NPDE
axial Ligands L1 and L2 :
Top : CN / CN
Middle : CN / H₂O.C₁₀₄
Bottom : CH₃ / H₂O.C₁₀₄

CHARACTERIZATION OF CHEMICALLY MODIFIED CHLOROPHYLL MODELS BY ^{252}Cf -PDMS

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We are engaged in the synthesis, isolation, structural elucidation and determining photophysical properties of models for the photoreaction center in green plant photosynthesis. Our approach is to modify nature's own chlorophyll molecule chemically to produce simple well characterized compounds which mimic some of the photochemical and optical properties of the complex intact in vivo green plant photoreaction center.

Particle induced desorption is the method of choice for determining the molecular weight of these nonvolatile, thermally labile compounds. ^{252}Cf -Plasma Desorption Mass Spectrometry has been chosen to characterize these model systems. The mass spectrometer, designed by W. H. Ulbricht, is in principle similar to the instrument of Macfarlane.¹ However, there are some notable differences.² The ionization region acts in a reflection mode versus a transmission mode, the flight path length is 1.5 m, and the probe, maintained at +10kV, is a stainless steel plate.

The model compounds are in the 600-2000 amu molecular weight range. Small amounts of material (<1mg) are obtained after separation by HPLC. The samples are dissolved in THF, electrosprayed³ onto the sample plate and placed in the ionization region of the mass spectrometer. A typical compound is linked pheophorbide a. This compound is covalently linked at the C-10 positions on ring V by butylene glycol moiety. The compound has a molecular weight of 1300 amu and is confirmed in the PDMS spectrum. Table 1 lists some of the compounds synthesized and their observed molecular weights.

Table 1 ^{252}Cf -PDMS of Chlorophyll Pairs

Compound	Molecular Weight	
	Calculated	Observed
bis(methyl pheophorbide <u>a</u> ethylene glycol diether (C-10, C-10)	1299.50	1300.00
10-(butylene glycol pheophorbide <u>a</u>)-methyl pheophorbide <u>a</u> (C-7, C-10)	1268.6	1268.5
bis(10-hydroxybutoxy-methyl pheophorbide <u>a</u>) - 1,4- dichlorophthalate (C-10, C-10) (cis)	1587.4	1587.7
bis(methyl chlorophyllide <u>a</u>) ethylene glycol diether (C-10, C-10)	1344.1	1344.0

*Rockwell International, Rocky Flats, CO

A compound designed specifically to mimic the charge separation properties of the photoreaction center is based on tetraphenyl porphyrin (TPP). The synthesis links two TPP molecules together through the para-phenyl position. Figure 1 shows the ^{252}Cf -PD mass spectrum of one TLC fraction. The molecular ion at m/z 1152 corresponds to bis(TPP) and an unexpected ion at m/z 1804 corresponds to tris (TPP).

Our experience has shown that ^{252}Cf -PDMS is a rapid, simple, and reliable analytical method for characterizing these higher molecular weight compounds. Further synthetic work involving attachment of chlorophyll molecules to proteins is planned. The demonstrated high mass range of ^{252}Cf -PDMS makes it the method of choice for analysis of the chlorophyll-protein models.

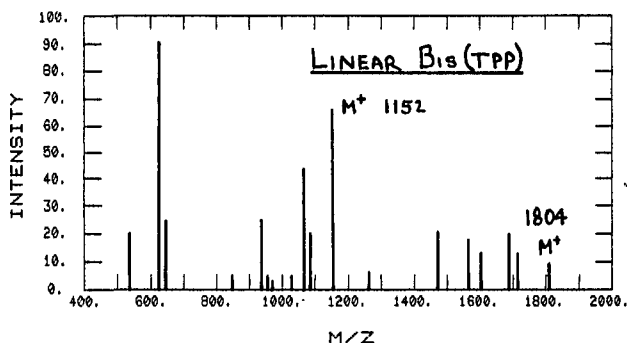


Figure 1

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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF ANIONIC
SURFACTANTS AND OTHER SULFONATE SALTS

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Sulfonate salts have been of commercial interest for many years, principally as surface-active agents such as detergents. Their analysis by mass spectrometer has, until FAB, required derivative preparation for volatility. Mass spectra of the salts can now be obtained easily. Some of the compounds examined recently are sodium and triethanolamine salts of alkylbenzene sulfonic acids, sulfated alcohols such as lauryl sulfate, an N-acyl taurate, and a sulfated oleate ester. All yielded spectra that were relatively easy to interpret, knowing in advance the structures involved. Several samples from a commercial collection of surfactants proved to be mislabeled.

In general, the spectra of sulfonates are relatively simple. The spectrum of the glycerol matrix was often completely absent or reduced to two or three recognizable peaks. In the FAB(+) spectrum of sodium dodecanesulfonate, the most intense peaks at masses higher than 150 consist of the monomer and clusters of sulfonate anions, each of which contains enough sodium ions to make the net charge plus one. For example, three anions are associated with four sodium ions to give an intense peak at m/z 839. In the FAB(-) spectrum of the same sample, clusters of anions are associated with the right number of cations to give species of charge minus one. There are no ions containing glycerol, but there is evidence for free sulfate. A peak at m/z 80 is due to a singly charged ion containing one sulfur and three oxygens. It is ubiquitous in negative ion spectra of sulfonates and can occur also as a component of clusters. A peak at m/z 352 is probably made up of it plus one dodecanesulfonate and a sodium cation.

The base peak of the FAB(+) spectrum of ammonium cyclohexanesulfonate is at m/z 182 and consists of one anion, one neutral ammonia, and two protons. It is arguable whether there might be an ammonium ion with a single proton, but the bookkeeping is simpler with the former arrangement and this is demonstrated by the set of three ionic clusters at m/z 346, 363, and 380. These contain two of the sulfonate moieties and three protons each and one, two, and three ammonia molecules, respectively. The same phenomenon is observable in the FAB(-) spectrum. At m/z 491, 508, and 525 are peaks that contain three sulfonate ions and two protons each with zero, one, and two ammonias.

The application of FAB to quantitative analysis is not easy. Spectra are clearly not additive relative to the composition of the condensed phase. Glycerol yields a strong spectrum in either positive or negative ion mode; but when a sulfonate salt is added, the sulfonate dominates the spectrum and the glycerol disappears almost completely. This nonadditive character bodes

ill for quantitation. However, if the components to be determined are chemically very similar, the situation is optimum. Such is the case for the molecular weight distribution of alkylbenzene sulfonates with a highly branched propylene oligomer chain.

"ALKANE 56" sulfonate consists of a spread of molecular weights in a homologous series, and the problem is to determine the distribution and average molecular weight. The peaks with more than one anion cannot be used because each is a mixture containing up to four different pairs of anions plus the three necessary sodium ions. The negative ion spectrum might be expected to be preferable because the species of interest are anions. Nevertheless, in practice the two appear to be equally useful. Molecular weight distributions and average molecular weights were calculated from the FAB(+ and -) spectra assuming equimolar sensitivities. Similar information was obtained from the starting hydrocarbon by a routine batch analysis and from a GC/MS run assuming equal sensitivities for the molecular ions formed by electron impact. The agreement among all four methods was astonishingly good.

The FAB(-) spectrum of the sodium salt of the sulfate ester of a polyoxyethylated octylphenol contains peaks due to one through seven ethylene oxides per molecule, each with its terminal sulfate group. Also readily visible are peaks due to dioctylphenol and its sulfated ethylene oxide adducts. Combination peaks containing all kinds of pairs were identified.

The addition of barium chloride to a glycerol solution of sodium dodecane-sulfonate almost completely suppressed the sulfonate spectrum just as the sulfonate suppressed the glycerol spectrum. The large number of peaks led to the writing of a computer program to test all possible combinations of candidate ions and neutrals against the observed masses and against the electric charge requirements. The program is called CMBNS and exists in both BASIC to run on an Apple II+ home computer and in FORTRAN to run on a large Timeshare computer. Listings are available on request.

Input data are the masses of species known or suspected to be present and limits and charges for each. A list of peaks seen in the spectrum is then typed in and the program responds with masses and the combinations that it found to fit and a list of the masses for which it could find no match. The program can then be rerun without reentering the list of observed peaks but with different constraints. Examples with a proton added or subtracted were presented. When the three lists were combined, it was seen that most of the peaks in the spectrum were given possible combinations.

FAB(+ and -) has proved to be a powerful and simple tool for the examination of anionic surfactants. Spectra are usually simple. A computer program is available to help with the interpretation in other cases.

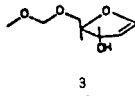
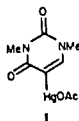
ANALYSIS OF REACTION MIXTURES CONTAINING ORGANOMETALLIC
INTERMEDIATES BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY

Henry T. Kalinoski,^{a,b} Uli Hacksell,^a Douglas F. Barofsky,^b Elisabeth
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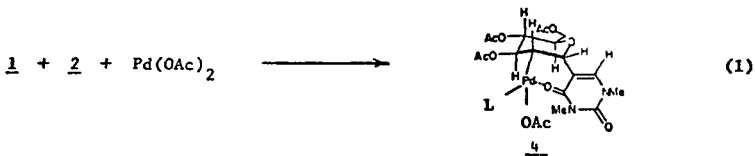
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Fast atom bombardment (FAB) mass spectrometry,¹ which utilizes a liquid matrix and efficiently samples preformed ions, has potential to provide information concerning details of condensed phase reactions.^{2,3} To assess this potential we have employed FAB mass spectrometry in conjunction with ongoing synthetic efforts in study of palladium(II)-mediated reactions of aryl or heteroarylmercuric acetates with chiral furanoid⁴ and pyranoid glycals⁵ (cyclic enol ethers). These particular reactions present attractive features for testing the basic hypothesis since the course of such reactions, which occur regio- and stereospecifically and lead to C-glycosides, is complex and postulated mechanisms^{4,5} involve a number of (usually) undetected metal containing complexes. Our initial results are encouraging; we have succeeded in identifying a number of metal containing species present in reaction mixtures which were added directly to a glycerol matrix and analyzed by FAB mass spectrometry and have obtained information relevant to a fuller understanding of these complex, multicomponent organo-metallic reaction systems.



For our initial experiments, we used a system well studied in our laboratory involving palladium(II) acetate mediated coupling of 1,3-dimethyl-2,4-(1H,3H)-pyrimidinedione-5-yl-mercuric acetate (**1**) with pyranoid glycal **2** (3,4,6-tri-O-acetyl-D-glucal) or furanoid glycal **3** (1,4-anhydro-2-deoxy-5-O-(methoxymethyl)-D-erythro-pent-1-enitol) in acetonitrile. In each instance, we have established that the reaction occurs regio- and stereospecifically and yields an adduct **4** (isolated after stabilization by ligand exchange and characterized) or **5** (not isolated but characterized by study of decomposition products).



The procedure used involves addition of reaction mixture aliquots to a glycerol droplet placed on the molybdenum surface of a probe-mounted FAB target. In a typical experiment, a 1 μ l aliquot of a reaction mixture (consisting of 0.05 mmoles each of **1**, palladium(II) acetate and **2** or **3** in 2 mL of acetonitrile) was added to a 3 μ l glycerol droplet on the FAB target, the target was introduced into the mass spectrometer ion source and bombarded with a beam of 5-7 Kev argon atoms at a incidence angle of 70° and the resulting positive ion mass

spectrum was recorded.

The results obtained in these experiments are summarized in Table 1. FAB mass spectra of the reaction mixture obtained by addition of equimolar quantities of 1, 2 and palladium(II) acetate in acetonitrile, exhibit prominent palladium-containing ions at m/z 517 and 245 (Table 1, experiment 1). The ion at m/z 517 arises by ionization of adduct 4 through loss of an ionic ligand (acetate) with concomitant loss of a neutral ligand (probably acetonitrile) and is characteristic of the intact palladium-bearing C-nucleoside. The ion at m/z 246 corresponds to a pyrimidinyl moiety bonded to palladium(II); i.e., the organopalladium species, formed by transmetalation from the corresponding organomercurial, which is the reactive intermediate in palladium-mediated carbon-carbon bond forming reactions of olefins. Results with furanoid glycal 3 were similar (experiment 2). The intact adduct 5 gave rise to an ion at m/z 446 by loss of acetate (with retention of a solvent-derived acetonitrile ligand). As in experiment 1, the pyrimidinylpalladium transmetalation product was present as evidenced by an ion at m/z 246.

These results are significant. Complex organopalladium adducts such as 4 and 5 which lack stabilizing ligands (e.g. R₃P) decompose readily and have not proved amenable to mass spectrometric analysis. More noteworthy is the direct observation of the pyrimidinyl-palladium transmetalation product. A large body of data provide compelling, but indirect, evidence for the intermediacy of such species in palladium mediated reactions; however to our knowledge direct evidence, as obtained in this study, has not been available previously.

In further studies to gain information concerning bonding interactions of the various reactant pairs and the progression of the overall reaction, experiments 3-7 were conducted which involved systematic omission of one reactant of the three component reaction mixtures. That is, the three possible binary reactant combinations were formulated and FAB mass spectra were recorded before and after addition of the third reactant. Surprisingly, acetonitrile solutions containing the pyrimidinylmercuric reagent 1 and palladium(II) acetate (experiment 3) did not give rise to the ion at m/z 245 indicative of transmetalation. Similarly, no evidence for interaction of organomercurial 1 with glycals was seen (experiments 6 and 7). Palladium(II) acetate forms complexes with the glycals (experiments 4 and 5). These results indicate that the possibility that transmetalation occurs following complexation of palladium and glycal must be considered.

TABLE 1
PALLADIUM- AND MERCURY-CONTAINING IONS OBSERVED IN FAB MASS SPECTRA OF REACTION MIXTURES^a

EXPERIMENT	REACTANTS PRESENT	METAL CONTAINING IONS OBSERVED (m/z) ^b			
		Pd-glycal complex	Hg ⁺² , ^d	R ₂ Pd ⁺	R ₂ Pd-glycal adduct
1	<u>1</u> + <u>2</u> + Pd(OAc) ₂		341	245	517 ^o
2	<u>1</u> + <u>2</u> + Pd(OAc) ₂			245	446 ^f
3	<u>1</u> + Pd(OAc) ₂		341		
4	<u>2</u> + Pd(OAc) ₂	469 ^g			
5	<u>3</u> + Pd(OAc) ₂	425 ^h			
6	<u>1</u> + <u>2</u>		341		
7	<u>1</u> + <u>3</u>		341		

^aReaction mixtures contained equimolar portions of the reactants indicated in acetonitrile as solvent. ^bsee reference

13 for method used to assign m/z values for multi-isotopic metal-containing ions. ^cR=1,3-dimethyl-2,4-(1H,3H)-pyrimidin-5-yl. ^dThis ion is accompanied by an ion at m/z 481 corresponding to [R₂HgH]⁺. ^e[Adduct 4-LOAc]⁺. ^f[Adduct

5-OAc]⁺. ^g[Pd⁺²(glycal 2)(glycerol)-H]⁺. ^h[Pd⁺²(glycal 3)-H]⁺.

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QUANTITATIVE ANALYSIS OF PAINT ADDITIVES BY FABMS

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INTRODUCTION

FABMS has become a widely used soft ionization technique for thermally labile and/or nonvolatile compounds. Although many articles have been presented which demonstrate the usefulness of FABMS as a qualitative technique, reports concerning its utility as a quantitative technique have been very limited.¹ A recent interest in our laboratory to monitor the photostabilizer additive bis(2,2,6,6-tetramethyl-4-piperidinyl) sebecate (TPS, Figure 1.) in cured paint as a function of weathering time instigated the development of a quantitative FABMS technique for this compound. Quantitation using external and internal standard procedures were investigated.

EXPERIMENTAL

The FAB analyses were performed on a VG ZAB-2F equipped with a VG modified Ion Tech Ltd. B11 Saddle Field Ion Source and B50 power supply. Xenon was used as a bombarding gas with an energy of 8kV and at a power supply limiting current of 1mA. Attempts to analyze the TPS directly in cured polymer by FABMS were not successful because of a lack of sensitivity. However, it was found that the compound could be quantitatively extracted from the cured polymer with methylene chloride and then analyzed in a glycerol matrix. The analysis procedure involves adding 300 µg of glycerol diluted in methanol to the sample probe tip followed by a known amount (1-2 ul) of sample extract. These materials are mixed and the solvents allowed to evaporate prior to analysis. The resulting spectrum is given in Figure 1. The sample signal (M+1 plus M+2 ions) is integrated over the life of the signal during FAB desorption (3-5 minutes). Internal standard techniques were investigated using the compounds tri-cyclohexyl citrate and bis(2,2,6,6-tetramethyl-4-piperidinyl)-2,2,9,9-tetradeutero sebecate (D₄-TPS) as internal standards.

RESULTS AND DISCUSSION

Quantitation by external standardization initially looked promising in that a linear relationship between mass of TPS and response was obtained. However, day-to-day reproducibility proved to be highly dependent on the manner in which the sample was distributed on the probe tip, instrument stability, and changes in the sample matrix. The former two problems were greatly reduced by incorporating the internal standard tri-cyclohexyl citrate in the sample extract. However, the response of this compound proved to be highly dependent on the sample matrix and this dependency did not parallel that of TPS.

To overcome these problems the deuterated analog of TPS, D₄-TPS, was synthesized and incorporated as an internal standard. Quantitation based on this procedure gave excellent results. The precision and recovery of this technique for known amounts of TPS in a polymer-free matrix, an uncured polymer extract, and a cured polymer extract are given in Table 1. The lower recoveries of TPS obtained for the cured polymer samples are attributed to chemical reactions which occur in the curing process. The limit of detection for the technique is about one nanogram of TPS which gives a S/N ratio of approximately 2-5.

¹ Scheifers, S., R. Hollar, K. Busch, R. Cooks, Amer. Lab., 14(3),19(1982).

Table 1. Recovery and Precision of D₄-TPS Internal Standard Technique

	<u>% Recovery</u>	<u>% Rel. Std. Dev.</u>	<u>Amount (ng)</u>	<u>Number Samples</u>
Polymer-free Matrix	94	4.1	200	7
Uncured Polymer Extract	96	3.4	100-500	10
Cured Polymer Extract	80	2.4	100-200	8

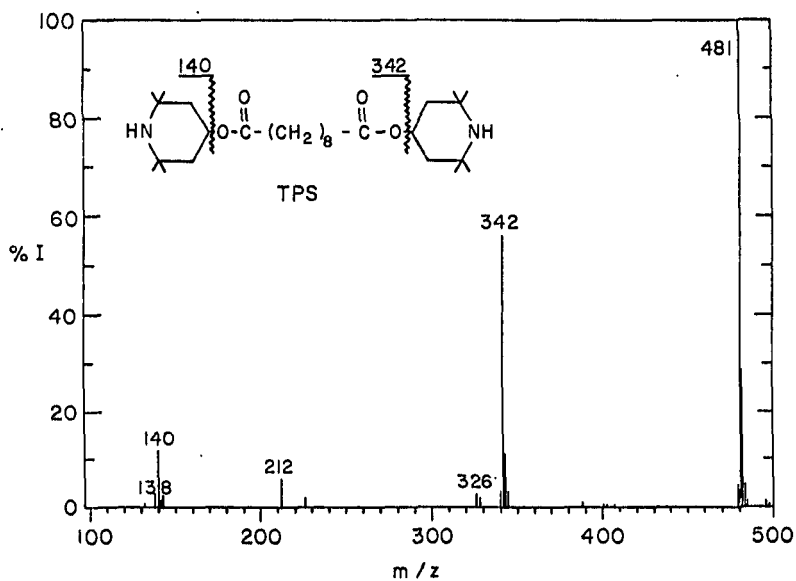


Figure 1

Field Desorption of Hydrocarbon Polymers

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Numerous synthetic polymers of low molecular weight ($\leq 10^4$ u) have been studied successfully in the past by field desorption (FD-MS). These include several aromatic systems such as polystyrene,¹⁻² phenolic resins,³⁻⁴ and aromatic amines.^{1,4-5} In all of these oligomeric systems the dominant FD-MS ions were $[M]^+$; none of these materials showed a tendency to form proton (or cation) attachment species. Aliphatic hydroxyl-terminated polymers, in contrast, do tend to form cation attachment ions in FD-MS analysis. In extensive studies of polyglycols,⁶⁻⁷ $[M+H]^+$ or $[M+Na]^+$ ions were dominant.

With this background of reports we were surprised by a recent article reporting the FD-MS analysis of butyl-terminated polybutadiene (PBD) that reported a dominance of $[M+H]^+$ ions for the various oligomers.¹⁰ It seemed unlikely to us that a pure nonpolar hydrocarbon polymer such as PBD would form ions by proton attachment, since there is no favorable site available for this to occur. The FD-MS behavior of PBD has been re-examined, and contrary to this earlier report,¹⁰ molecular ions $[M]^+$ do dominate in the FD mass spectrum of this polymer. Three other butyllithium catalyzed polymers have also been examined; polyisoprene (SI), polyethylene (PE), and polystyrene (PS). These polymers also yielded $[M]^+$ ions. In extensions of earlier work^{7,11} we show that accurate average molecular weight determinations can be made using FD-MS data for these polymers.

The low molecular weight polymer samples studied are listed in Table I. The polymers were analyzed by field desorption mass spectrometry using either a Varian MAT 731 mass spectrometer (Wiesbaden) or a Varian MAT 311A instrument (Brecksville). Standard high-temperature activated carbon emitters were used. A composite spectrum of polybutadiene sample CDS-B-1 (average of twenty-three scans) is shown in Fig. 1. The principal ions observed are $[M]^+$, and the spectrum is "clean," i.e., higher mass fragment ions are essentially absent. FD mass spectra for the other hydrocarbon polymers were similar in nature to PBD. For number average ($\bar{M}_n$) and weight average ($\bar{M}_w$) molecular weight determinations, several spectra were recorded (with repetitive scanning) as the field emitter was slowly heated with up to 40 mA heating current. Accumulated spectra were summed, and the average molecular weights were calculated as described previously.^{7,11} The results are listed in Table I. Molecular weight averages derived from FD-MS data agreed well with values obtained by conventional techniques.

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TABLE I
Molecular Weight Parameters for Hydrocarbon Polymers

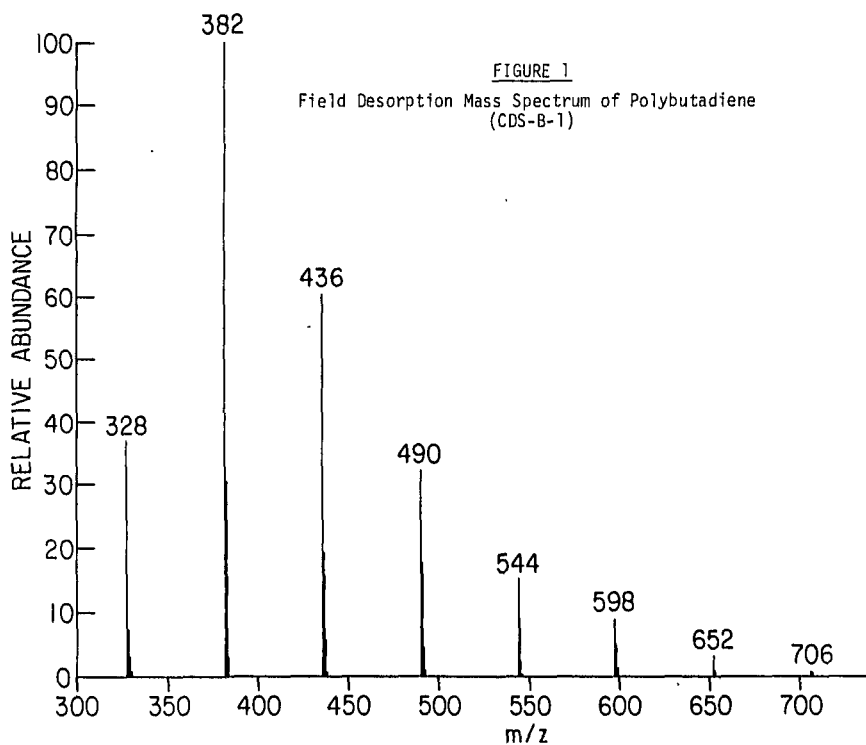
Polymer Type	Batch	Field Desorption		Manufacturers' Values	
		$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
Polybutadiene	CDS-B-1	430 ^a	1.03	420	1.10
	CDS-B-2	885 ^a	1.04	960	1.07
	CDS-B-3	2450 ^a	1.02	2350	1.13
Polyisoprene	940	931 ^b	1.08	940	1.10
		1070 ^a	1.12		
Polyethylene	700	644 ^a	1.05	640	1.10
	1000	1030 ^a	1.08	910	1.10
Polystyrene	61004	941 ^{b,c}	1.08	811	≤1.30
		961 ^a	1.11		
	12a	1690 ^{c,d}	1.15	1710	≤1.10
		1680 ^a	1.14		

^a Varian MAT 731.

^b Varian MAT 311A.

^c Repeated from Reference 11.

^d Kratos MS-50 (see Reference 11).



LIQUID IONIZATION MASS SPECTROMETRY OF PHOSPHOLIPIDS

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Recently field desorption (FD)¹⁾ and fast atom bombardment (FAB)^{2,3)} mass spectrometry were reported as powerful techniques for obtaining a molecular ion of a phospholipid. However, the interpretation of their spectra seems not easy due to a few and complexed fragment ions.

Liquid ionization mass spectrometry we have developed⁴⁾ was applied to analysis of phospholipids (synthetic compounds in this paper). A sample containing 1-10 µg of a lipid in 1-5 µl of methanol (or methanol-chloroform) solution was mixed well with 1 µl of a matrix (as used in FAB) and deposited on a needle tip (sample holder). A sample solution concentrated by warming it was also examined without matrix. Glycerol was found to be a moderately good matrix, but liquid paraffins which are good for nucleosides⁴⁾ were found useless, because liquid paraffins did not solve (nor suspend) the sample. Proton affinities of phospholipids examined must be larger than that of glycerol. A mass spectrum obtained from the concentrated solution was similar to that with glycerol.

As an example, mass spectra of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (di 16:0 PC) is shown in Fig.1. The MH^+ ion was observed as the base peak soon after the sample introduction (within 5 s) at an adequate heater current (1.25 A : around 150°C) and then fragment ions increased their abundances as shown in Fig.1a that is the summed mass spectrum of 4 scans (ca 40 s). If the heater current was slightly increased (1.30 A : around 180°C), the peaks at m/z 551 became dominant (Fig.1b) and stable. Since this peak is weak at lower temperature, the mass 551 ion may be produced from thermal decomposition products of the parent molecules. The similar change in mass spectrum with the temperature and measuring time were observed for all samples. The main fragment ions observed at m/z 551, 464 and 196 are likely to be produced by a bond cleavage at a, b, and c, respectively, as shown in Fig.1. The same types (a, b and c) of fragmentation were observed for other standard compounds and their mixtures as shown in Figs. 2-4. The MH^+ ions and a-type ions (e.g., m/z 551 for di 16:0 PC and m/z 607 for di 18:0 PC) give the information about the sum of two acyl groups. Each b-type fragment ion (e.g., m/z 464 for di 16:0 PC and m/z 490 for di 18:1 PC) includes one acyl group which is assumed to be attached to the position-2 from its mass number, although it has not yet been confirmed. All spectra in Figs. 2-4 show another type of fragment ions consisted of one acyl group ($RCOOCH_2$) plus 59u, i.e., m/z 300, 328 and 356 in Fig.2, and m/z 352, 354 and 356 in Fig.4 (Abcissa of Fig.4 is partly enlarged to show these ions). The fragment peak at m/z 196 is observed for all PCs examined so far, being characteristic of phosphatidylcholines. The same (c) type ion was observed at m/z 154 for di 16:0 PE, too. The ions at m/z 372 and 621 in Fig.3 and at m/z 617 (and 621) in Fig.4 are produced by the addition of H_2O to the ions of mass 354, 603 and 599, respectively. It is interesting to note that such water adduct ions are more abundant for the compounds (PC) containing more unsaturated acyl groups. The water adduct ions were less abundant at higher temperature.

Figures 2 and 4 indicate that the intensities of each MH^+ peaks and each b- and d-type ion peaks correspond to each relative abundances in the samples, although some calibration in the sensitivity of each peak is required. For the compounds containing saturated acyl groups (Fig.2), the ions produced from a larger molecule appear to be less abundant and a-type ion peaks may be useful for quantitation, too. The relative intensities of a-type ion peaks for unsaturated compounds (Fig.4) should be corrected by considering the abundance of their water adduct ions.

A phosphatidylethanolamine (di 16:0 PE) and its mixture with di-16:0 PC were examined. The PE also showed the a-, b- and c-types of fragmentation and other fragment ions. The MH^+ ion was not observed, but $MH^+ - H_2O$ ion appeared. The ions from PE appeared at lower temperature and the ions from PC appeared at higher temperature. Thus both compounds can be analysed without pre-separation of them.

By this method (LI) using a μg scale of sample, a mass spectrum corresponding well to the structure of phospholipid (PC) was obtained. The mass spectrum is somewhat different from FD, FAB and SIMS spectra. Quantitation of each component in a mixture appears to be possible, if a good quadrupole mass spectrometer with a good data processing system.

We wish to thank Prof. K. Saito of Kansai Medical School for kind supply of the samples.

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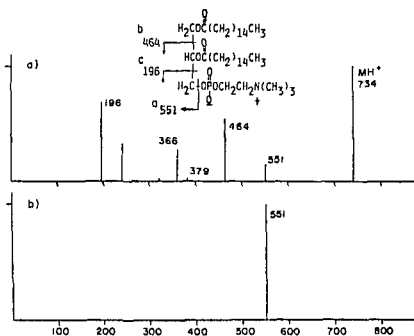


Fig.1 Mass spectra of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (di-16:0 PC)

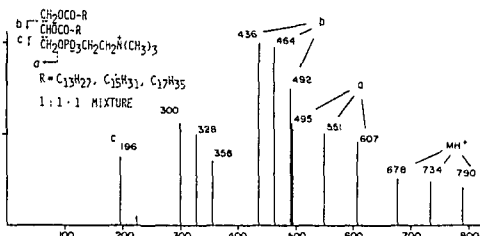


Fig.2 Mass spectrum of an equimolar mixture of di-14:0 PC, di-16:0 PC and di-18:0 PC

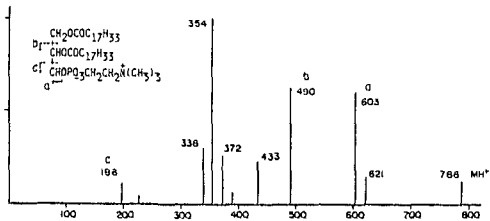


Fig.3 Mass spectrum of 1,2-dioleoyl-sn-glycero-3-phosphocholine

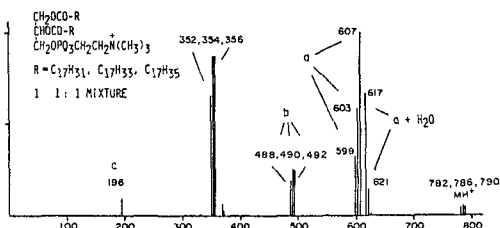


Fig.4 Mass spectrum of an equimolar mixture of di-18:2 PC, di-18:1 PC and di-18:0 PC

COLLISION ACTIVATED DISSOCIATION SPECTRA
OF IONS PRODUCED BY
PARTICLE-INDUCED DESORPTION

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The combination of a particle-induced desorption (PID) ion source with a tandem quadrupole mass spectrometer system is a powerful instrumental technique for the identification and/or structure confirmation of polar or thermally labile molecules. On the Finnigan TSQ® system, particle-induced desorption is accomplished using a specially designed ion volume which provides separate regions for generation of bombardment particles by a high voltage discharge and for desorption of ions from a typical FAB-type target. The bombardment particles are a mixture of atoms and ions; however, the spectra produced are typical of those produced by atom guns. The system is easily operated in both positive and negative ion modes.

With the system operating in a "normal" mode with a single stage of mass filtering, the spectra produced consist primarily of molecular protonated ions (positive ion mode) or molecular deprotonated ions (negative ion mode), along with cluster ions from the matrix used to mobilize the sample. In the MS/MS mode, these molecular protonated or deprotonated ions can be mass selected by the first quadrupole for fragmentation by collision activated dissociation in the collision chamber (second quadrupole). These CAD daughter ions can then be mass filtered by the third quadrupole, detected, and recorded for interpretation.

This PID-CAD combination has been used for a variety of sample types, including thermally labile compounds, such as underivatized oligopeptides and catalysts, and polar compounds, such as phosphonic acids, carboxylic acids, and compounds containing both acidic and basic functionality. The examples discussed include a variety of compound types, for which the combination of molecular weight information from PID and fragmentation from CAD provide sufficient information to characterize the chemicals in question.

- 1) Following molecular weight determination in the PID mode using positive and negative ions, the structure of N-formyl iminodiacetic acid was elucidated by CAD of the protonated ion.
- 2) Using diethanolemine as a matrix to promote negative ion formation by PID, the molecular weight of a trisphosphonic acid was established. CAD of the molecular deprotonated ion gave structure confirmation for the compound.
- 3) PID was used to determine the molecular weight and partial sequence information for an underivatized tetrapeptide. Subsequent CAD analysis provided increased structural information. In most cases, CAD provides complete sequence confirmation.

In summary, the PID-CAD combination provides significant advantages to the mass spectrometrists. The combination gives both molecular weight and fragmentation information for the analyte. In many cases, the reduction in chemical noise (especially from matrix ion clusters) results in an increased signal to noise ratio for the CAD spectra. It also allows lower m/z fragments to be detected free from the large interference of bombardment gas ions. Finally, and perhaps most important, the combination greatly expands the range of chemical compounds which are amenable to mass spectrometric characterization.

Breakdown curves - graphs of relative abundances of fragment ions as a function of internal energy of parent ions - are the most fundamental representation of mass spectra. Until the advent of the photoionization-photoelectron coincident technique, breakdown curves were obtained either by QET calculations or via charge-exchange mass spectrometry. Information similar to breakdown curves can be obtained from a collision-induced dissociation experiment by determining the relative abundances of fragment ions scattered at an angle to the main beam path. The experimental results, when plotted as normalized ion intensity vs. the scattering angle in the laboratory frame of reference, resemble breakdown curves closely. From a comparison of ARMS spectra and breakdown curves, it has been concluded that the internal energy deposited during the ion-neutral collision is a function of the scattering angle. This is illustrated in Figure 1 which compares the relative abundances of $[C_7H_7]^+$ and $[C_7H_8]^+$ as determined in an angle resolved experiment to photodissociation MS/MS results.¹

Different interpretations have been proposed to explain the observed $\text{int} - \theta$ relationship. These include the impact parameter approach and the kinetic energy release associated with the fragmentation. According to the former, the internal energy deposited during the collision is inversely proportional to the square of the impact parameter, the distance of closest approach. This hypothesis was based on an extensive study of collision-induced dissociation mass spectra of methanol obtained at various angles using a number of target gases.² In the alternative rationalization, the perpendicular velocity component acquired by the fragment ion as a consequence of the kinetic energy released during fragmentation is thought to be the dominant source of scattering. One of the conclusions of this model is that polyatomic ions are not scattered through substantial angles.³ However, a study on polyatomic ions which survive the collision through substantial angles indicate that this is possible. Table 1 shows the ion signal at angles greater than 5° both in the absence and in the presence of collision gas. Enhancement in the ion signal at high angles as a result of collision is due to substantial scattering of polyatomics. That polyatomics are scattered inelastically is proven by the observed changes with the scattering angle in the relative abundances of 71^+ and 58^+ formed in a subsequent dissociative collision of 2-pentanone ions scattered at various angles in a non-dissociative collision.⁵

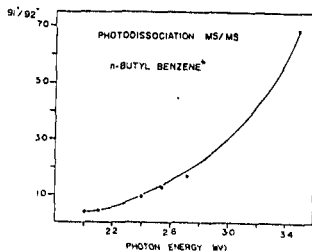
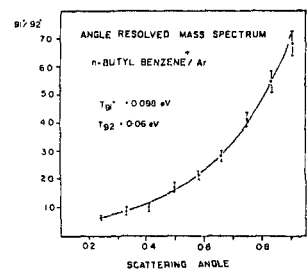


Figure 1

Table 1
NON-DISSOCIATIVE SCATTERING
OF
POLYATOMIC IONS
(Derived from NITROMETHANE)

ION	DEFLECTION POTENTIAL (Volts)	COUNTS/min without collision gas	COUNTS/min with collision gas
6 ⁺	500	33	7376
46 ⁺	500	358	35815
30 ⁺	720	29	7551
	820	0	3200
	920	0	1580
	1020	0	440
	1120	0	115

Neither of the models proposed explain the experimental results. For example, n-butylbenzene shows a linear dependence of energy deposited on the scattering angle in contrast to the square dependence predicted by the impact parameter approach. Similarly, the relative abundances of 91^+ and 92^+ generated from benzylmethyl ether and of 131^+ , 100^+ and 69^+ from perfluoropropylene⁶ cannot be rationalized in terms of kinetic energy release alone.

The experimental results shown in Table 1 were obtained using a new instrumental configuration for the ARMS experiment.⁴ The new instrument uses the first field-free-region of a reverse geometry instrument as the collision region. The primary ion beam is deflected through an angle prior to collision using the z-focusing plates. Target gas is introduced at the source slit via a collision needle. The fragment ions scattered along the optical axis of the instrument are detected by a B/E linked scan. Performance of this configuration is comparable to these previously used as illustrated in Figure 2.

Besides the fact that angle resolved mass spectrometry provides fundamental information such as breakdown curves and furthers our understanding of collision-induced dissociation, ARMS spectra can be useful in structural problems. Figures 3 and 4 show ARMS and ERMS spectra of isomeric $C_3H_4^+$ ions, respectively. Both procedures of varying the internal energy associated with a dissociating ion allow the isomers to be distinguished.

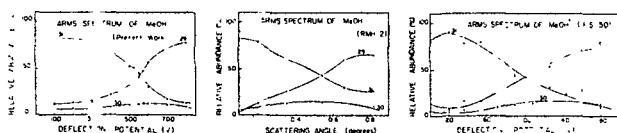


Figure 2

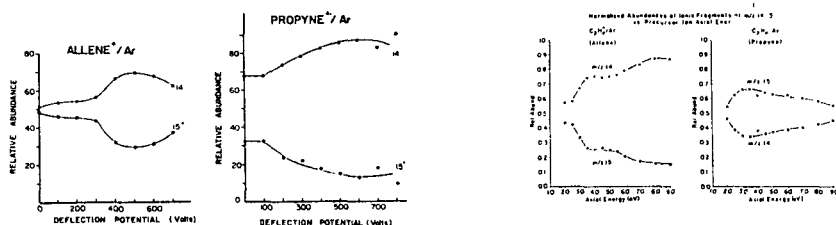


Figure 3

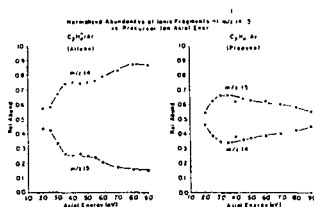


Figure 4

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The quantitation at low levels of components in mixtures which are not amenable to chromatographic separation

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Selected ion monitoring (SIM) combined with chromatography is a well established technique in mass spectrometry. In addition, high resolution can be used to add a further dimension of specificity to the detection of the desired compound. However, chromatography can be a time consuming technique, particularly if the sample has to go through complex purification and derivatisation procedures prior to analysis.

In this work, a dramatic saving in time in the quantitation of salicylic acid is demonstrated. Salicylic acid has been used as a preservative in food, and is used as a topical antiseptic and keratolytic agent. It is present at low levels in mixtures which would require several stages of preparation prior to chromatography/mass spectrometry.

The technique involves the analysis of charged products of ion decompositions occurring during the flight from the ion source to the detector of a standard MS25 mass spectrometer. Typically the sample, spiked with methyl salicylate as an internal standard, is subjected to one stage of solvent extraction. Quantitation is then performed on the extract by evaporation of the mixture from a solids probe into the ion source, which operates in the electron impact mode of ionization. Both methyl salicylate (m.w 152) and salicylic acid (m.w 138) have a common fragment ion at m/z 120. The intensities of ions undergoing decomposition to this fragment in the first field free region of the instrument (between the source and electrostatic analyser) are measured by varying the accelerating voltage using a DS55 data system controlled power supply at fixed electrostatic analyser and magnetic field strengths.

Analysis of hair treatment samples show incorporation of salicylic acid at concentrations of around 1 mg per ml. This technique provides the basis for very rapid quality control for this type of preparation.

STRUCTURE AND ISOMERIZATION OF ORGANOPHOSPHONATE IONS
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The gas-phase structure of the molecular and fragment ions of dimethylmethylphosphonate (DMMP) and a structural isomer, trimethylphosphite (TMP), have been investigated using collision-induced dissociation (CID) with mass-analyzed ion kinetic energy spectrometry (MIKES). Structures for the ions produced by electron-impact ionization of DMMP and TMP have been postulated from a comparison of their CID-MIKES spectra, and they are generally in agreement with earlier work on similar compounds [1].

The CID-MIKES spectrum of the molecular ion of DMMP did not exhibit a m/z 109 fragmentation corresponding to loss of a phosphorous-bound methyl radical. However, this is a significant fragment ion in the electron-impact spectrum of DMMP. This unexpected behavior could result from a keto-to-enol isomerization of the DMMP molecular ion after ionization. Earlier work on methylacetate [2] structural analogs suggests that the molecular ion of DMMP could exist in one of three isomeric forms with the enolic isomer probably being the most stable (Figure 1). In addition, the enolic isomer cannot readily lose the phosphorous methyl group to produce the m/z 109 fragment ion.

**POSSIBLE STRUCTURES FOR THE
DMMP MOLECULAR ION**

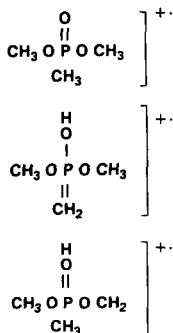


Figure 1

The keto-to-enol isomerization of the DMMP molecular ion was confirmed by comparison of the CID-MIKES spectrum of the DMMP molecular ion (m/z 124) and the m/z 124 radical cation produced from dimethylmethylthiomethylphosphonate (DHMTMP). The CID-MIKES spectra of the two m/z 124 ions were found to be qualitatively similar; however, the relative intensities of the m/z 93 and 94 fragment ions were different. In an effort to account for these differences, the CID-MIKES spectrum of the DMMP molecular ion generated by charge exchange with CS_2 , COS, CCl_4 , and Ar and by 70 eV electron impact ionization were compared with the CID-MIKES spectrum of the m/z 124 ion obtained from DMMTMP under electron impact. The relative intensities of the

m/z 93 and 94 fragment ions from the DMMTMP ion were intermediate between the m/z 93 and 94 fragment ion abundances obtained from the DMMP molecular ion produced the charge exchange and electron impact (Table 1). In addition, the ratio

Table 1
Intensity Variation of m/z 94 and 93 Fragment Ions

<u>DMMP</u>	<u>%I (EI)</u>		
Charge Exchange Reactants (Recommendation Energy eV) Recombination Energy eV	94	93	93/97
CS ₂ (10.06)	21.71	9.11	0.31
COS (11.18)	21.26	8.98	0.32
CCl ₄ (11.7)	22.89	9.57	0.31
Ar (15.76)	23.40	9.44	0.32
DMMTMP			
Electron Impact	28.72	6.28	0.23
DMMP			
Electron Impact	30.71	4.74	0.15

of the 93/99 fragment ion intensities follows the same trend. These data can be interpreted in the following manner. The DMMP molecular ion, produced with low internal energy by charge exchange, exists primarily in one isomeric structure -- presumably the enolic isomer. The m/z 124 ion of DMMTMP and the molecular ion of DMMP produced under electron impact conditions exist as a mixture of the enolic structure and at least one additional isomer.

Further work to characterize more fully the isomers in this system includes a study of the m/z 124 ion of DMMTMP as a function of internal energy, a comparison of the CID-MIKES spectra from other isomeric m/z 124 ions, and isotope labeling experiments.

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ELECTROCYCLIC RING OPENING OF SUBSTITUTED GAS-PHASE CYCLOBUTENE RADICAL CATION

Chhabil Dass and M. L. Gross

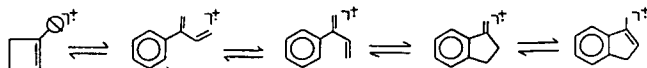
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The electrocyclic ring opening of cyclobutene and its derivatives to the corresponding 1,3-butadienes is a well-established phenomenon. The transformation takes place in a stereospecific conrotatory manner for the thermally allowed process (1). For cyclobutene, the experimental activation energy is 32.9 kcal/mole for the thermal process (2). Earlier studies of substituted neutral cyclobutenes have shown that the rate of the electrocyclic ring opening is enhanced by substitution at the 3- and 4-positions, whereas substitution at the double bond retards the rate of the corresponding reaction compared to the unsubstituted cyclobutene (3). In order to determine if the effect of substitution on the electrocyclic reactions of open shell systems is similar to that of their closed-shell counterparts, we have investigated the electrocyclic ring opening reactions of phenyl and methyl substituted gas-phase cyclobutene radical cations by mass spectrometry/mass spectrometry (MS/MS). The specific strategy we have chosen is to form stable ions, i.e., the ions that do not decompose or isomerize in the mass spectrometric time frame of $< 10^{-5}$ s, obtain their collisionally activated dissociation (CAD) and collisional ionization spectra using the high resolution capability of MS I of triple analyzer MS/MS instrument (4). These spectra are then compared with those of the corresponding ring-opened radical cations.



The first question we addressed was whether 1-phenylcyclobutene radical cation undergoes electrocyclic ring opening to 2-phenyl-1,3-butadiene ion. The CAD spectra of these isomeric ions formed at various ionizing energies are compiled in Table 1. The losses of CH_3 and C_2H_4 are characteristic features of the spectra of these ions. As the ionizing energy is increased, the loss of CH_3 increases, whereas the loss of C_2H_4 decreases. Therefore, the ratio of relative abundances of m/z 115/102 is a sensitive probe for distinguishing these isomeric structures. The loss of C_2H_4 is characteristic of the ring-closed isomer as is expected by the cyclo-inversion reaction. Based on these results we conclude that at energies close to the threshold for ionization, the 1-phenylcyclobutene ion is a stable structure. On increasing the ionizing energy, it not only undergoes electrocyclic ring opening to 2-phenyl-1,3-butadiene ion, but also rearranges to stable 3-methylindene ion (Scheme 1). The latter we found as the most stable ion among several other $\text{C}_{10}\text{H}_{10}$ isomers studied. This conclusion was reached based on thermochemical measurements and by the fact that the spectrum of 3-methylindene ion does not change with internal energy (Table 1). We have estimated that the activation energy for the electrocyclic ring opening of the 1-phenylcyclobutene radical cation is less than 14 kcal/mole.

SCHEME 1



We also studied the effect of phenyl substitution at the 3-position. The results of CAD studies are shown in Table 2. These results show that compared to the 1-phenylcyclobutene ion, the electrocyclic ring opening of 3-phenylcyclobutene ion is more facile. Even at low internal energies its spectrum shows the features of trans-1-phenyl-1,3-butadiene and 3-methylindene ions. The conversion of 3-phenylcyclobutene ion to ultimate structure occurs via Scheme 2.

SCHEME 2

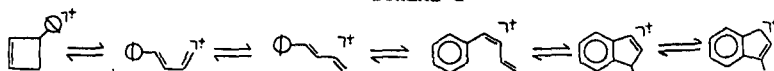


TABLE 1 - PARTIAL CAD SPECTRA

Ioniz	Compd	115	102	91	77	51	R
Low-eV		22	23	2.5	9	12	1.0
		27	14	2.5	11	13	2.0
		41	10	0.6	6	8	4.1
CE by		27	19	2.6	11	12	1.5
$[C_7H_8]^+$		31	15	3.1	11	12	2.0
CE by		29	18	2.7	10	11	1.6
$[NO]^+$		35	14	2.9	10	10	2.5
70 eV		34	17	2.2	8	10	2.0
		35	13	2.6	8	11	2.6
		43	10	0.7	6	8	4.3
CE by		39	12	1.4	8	9	3.3
$[CS_2]^+$		38	11	1.3	7	9	3.5
		41	10	0.7	7	8	4.1

R = m/z 115/102

TABLE 2 - PARTIAL CAD SPECTRA

Ioniz.	Compd.	115	102	91	77	51	R
Low-eV		39	12	1.2	8	8	3.3
		28	13	1.7	11	12	2.2
		31	13	1.8	12	11	2.4
70 eV		41	10	0.6	6	8	4.1
		43	11	0.8	7	8	4.0
		35	12	1.2	8	9	2.9
CE by		39	10	1.3	7	9	3.9
		43	10	0.7	6	8	4.3
		42	10	1.0	7	8	4.2
$[CS_2]^+$		39	10	0.9	7	9	3.9
		41	10	0.7	7	8	4.1

R = m/z 115/102

The results obtained in case of phenyl substituted cyclobutene ions led us to study the effect of other substituents. We have studied the electrocyclic reactions of 1-methyl and 3-methyl cyclobutene ions. The results of the CAD studies for the ions formed by low-energy electron ionization do not permit us to distinguish the ring-closed and the corresponding ring-opened isomers. However, the collisional ionization spectra of these isomeric ions are different (Table 3). The reason for this is that the CA method samples ions predominantly having energies close to the threshold for decomposition which may have already isomerized, whereas, the collisional ionization technique samples ions of very low internal energies. We, therefore, conclude that the ions formed at very low internal energies are of intact structures.

TABLE 3 - COLLISIONAL IONIZATION SPECTRA

Compound	34	33.5	33	32.5	32	31.5	31
	-	3	16	27	19	8	28
	8	10	22	23	12	6	21
	7	6	18	18	18	7	25
	37	12	15	13	9	3	11

The results of phenyl substituted cyclobutene radical cations have been communicated to the Journal of American Chemical Society.

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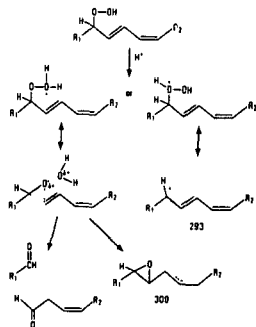
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Studies of Isomeric Fatty Acid Hydroperoxides by
Chemical Ionization via Direct Exposure Probe

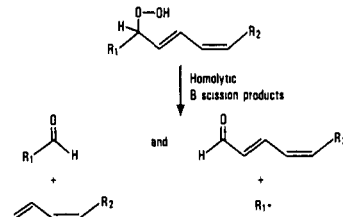
R. D. Plattner and H. W. Gardner
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Fatty acid hydroperoxides customarily have been reduced prior to MS analysis, because these compounds are thermally labile and thus unstable to separation and analysis by GC-MS. However, 9- and 13-hydroperoxides can be separated by HPLC and then analyzed intact by MS via the direct exposure probe (DEP). Figure 1 shows the isobutane CI spectra of methyl 9-hydroperoxylinoleate and methyl 13-hydroperoxylinoleate. The use of a DEP allows a few nanograms of the hydroperoxide to be volatilized rapidly into the CI source, yielding ions for analysis by MS/MS. The rapid heating of the DEP technique minimizes decomposition of the sample. The predominant ions observed in isobutane CI (0.13 torr) spectra of the isomeric hydroperoxylinoleates are at m/z 311 ($(MH-O)^+$, m/z 309 ($(MH-H_2O)^+$) and m/z 293 ($(MH-H_2O_2)^+$). A protonated molecule (MH^+) at m/z 327 and a proton abstracted molecule ($M-H$) at m/z 325 are also observed as small signals along with other minor unexplained adduct ions/reaction products at m/z values above the molecular weight of the hydroperoxides. Ions in the CI spectrum may arise from two pathways: (1) molecular protonation via CI leading to ions that can be compared with the acid-catalyzed reaction of the linoleate hydroperoxides in solution (scheme 1); or (2) oxy radical β -scission reaction derived from homolytic cleavage of the hydroperoxide (scheme 2). Certain fragment ions were indicative of an acid-type heterolysis; i.e., the 9-hydroperoxide isomer afforded a protonated C9 aldehyde ester (m/z 187) and a protonated C6 aldehyde (m/z 101), whereas the 13-hydroperoxide gave a protonated C6 aldehyde (m/z 101) and a C12:1 aldehyde ester (m/z 227). A homolytic scission mechanism of the 9-hydroperoxide isomer was indicated by the protonated fragments of a C8 ester (m/z 159), a C9 aldehyde ester (m/z 187, and a C10:2 aldehyde (m/z 153). Homolytic scission of the 13-hydroperoxide also was implied by the protonated fragments of C6 aldehyde (m/z 101) and C13:1 aldehyde ester (m/z 239).

The size of the m/z 311, 309, and 239 fragments is strongly dependent on the source temperature and rather insensitive to probe heating rate. The largest signals for m/z 311 and 309 are obtained at a source temperature of $70-100^\circ\text{C}$. MS/MS analyses of collisionally activated decomposition (CAD) daughters of m/z 309 and 311 are clearly different for the 9- and 13-isomers. The m/z 309 ion is proposed to be the epoxyallylic carbonium ion shown in scheme 1. Cleavage of the epoxide toward the saturated end of the molecule yields m/z 185 in the daughters of the 9-hydroperoxy isomer and m/z 99 in the 13-hydroperoxy isomer (figure 2). Similarly, the CAD daughter spectra of m/z 311 for both hydroperoxide isomers are different.



Scheme 1



Scheme 2

Ammonia CI of the hydroperoxides (table 1) (0.25 torr) shows an intense adduct ion $(M+NH_4)^+$ at m/z 344, and intense ions at m/z 328 $(M+NH_4-O)^+$ and m/z 326 $(M+NH_4-H_2O)^+$. An additional adduct ion is observed 16 amu above the base peak at m/z 360; this probably arises from reaction of the molecule with NH_3 from the CI gas. The intensity of this ion and the base peak are dependent on NH_3 pressure in the source. At ~ 0.1 torr m/z 328 is the base peak, with the m/z 344 adduct being about 20% as intense. At this pressure, m/z 360 is not observed. In the ammonia CI spectra, fragments corresponding to NH_4^+ adducts of C9 aldehyde ester (m/z 204) and C13:2 aldehyde ester (m/z 256) are observed in the 9- and 13-hydroperoxide isomers, respectively. Again, the CAD daughter spectra of m/z 344, 328, and 326 are different for both hydroperoxide isomers, making them discernible by the use of MS/MS.

Table 1

Ammonia CI Spectra of 9- and 13-Hydroperoxy Linoleate Isomers

Isomer	Spectra	
	Type	
9-Hydroperoxylinoleate	CI	360(7), 344(100), 328(35), 326(48), 310(30), 309(28), 204(40), 187(20), 170(30)
13-Hydroperoxylinoleate	CI	360(9), 344(100), 328(22), 326(40), 310(32), 309(41), 256(31), 226(26)

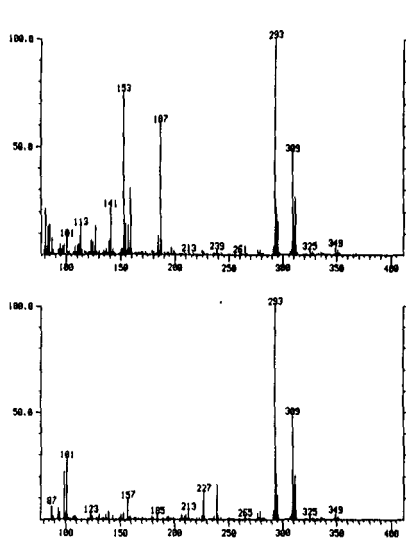


Fig. 1. CI-MS (isobutene) of 9- and 13-hydroperoxylinoleate.

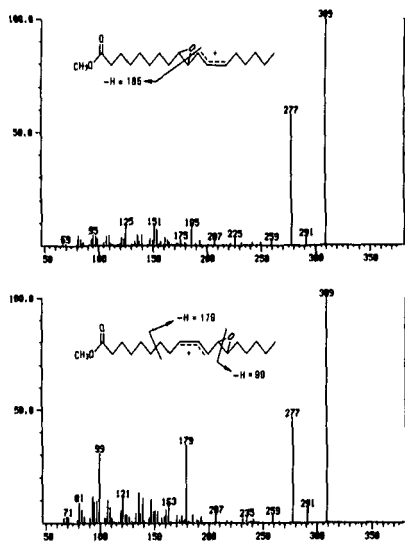


Fig. 2. CAD daughter spectra of m/z 309 from CI and MS of 9- and 13-hydroperoxylinoleate isomers.

**FEMTOGRAMS AND PARTS-PER-TRILLION:
ULTRATRACE ANALYSIS BY TANDEM MASS SPECTROMETRY**

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Tandem mass spectrometry (MS/MS) has been developed into a powerful technique for the trace analysis of compounds in complex mixtures. Detection limits in the femtogram range (sample concentration in the part-per-trillion range) have been achieved for real samples. The high selectivity of MS/MS often makes it possible to perform these analyses with minimal sample clean up and no preconcentration. Furthermore, the limited sample preparation and elimination of chromatographic separation makes analyses extremely rapid (up to 100 samples/hour).

As an example of the detection limits which can be achieved, consider the trace analysis of chlorinated compounds in human blood serum and urine. The goal of this work is the development of a rapid screening and quantitation procedure for hexachlorobenzene (at the 100 ppt level) and trichlorophenol (10 ppb). A short GC column (1 mm id x 50 cm) was chosen for sample introduction in order to allow more rapid analysis and prevent loss of these volatile compounds with the direct insertion probe. Methane was employed as the carrier gas, with negative ionization selected for maximum sensitivity. For HCB the only daughter ion from collision-induced dissociation of the ^{35}Cl molecular ion was monitored ($284^+ \rightarrow 35^+$); for TCP, the loss of HCl from the molecular ion ($196^+ \rightarrow 160^+$) was employed. Although 1 μL samples of serum and urine could be injected directly on column, better precision and extended column life were achieved by first extracting the samples with solvent (1:1) and injecting 1 μL of the extract.

Figure 1 shows the data for triplicate analysis of six serum extracts, six standards, and six urine extracts for TCP. A total of 60 injections were analyzed in 36 minutes. The reproducibility in peak area averaged $\pm 10\%$. The limits of detection for HCB and TCP evaluated at a signal-to-noise ratio of 5:1 are shown in Table 1.

FIGURE 1

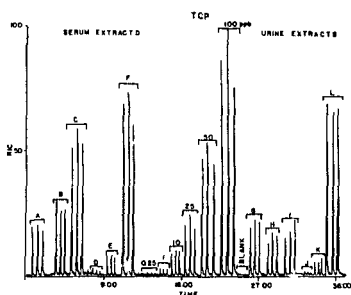


TABLE 1

MS/MS Limits of Detection			
conc ^a	mass ^b	moles ^c	molecules
Hexachlorobenzene			
50 ppt	50 fg	180 amol	1.1×10^8
Trichlorophenol			
250 ppt	250 fg	1.3 fmoI	7.7×10^8

^a concentration in 1 μL samples
^b mass in femtograms (10^{-15}g)
^c mol in femtomol and attomol (10^{-18}mol)

Another example of the ultimate detection limits achievable by MS/MS involves the quantitation of tryptolines, indoleamine-aldehyde condensation products, in brain tissue extracts. The compounds have been converted into their heptafluorobutyryl derivatives to allow their introduction through the short GC column and to enhance their negative CI sensitivity. Instrumental sensitivity and limits of detection for standards are shown in Table 2. The calibration curves shown in Figure 2 indicate linear response down to below 1 pg, with curvature at lower amounts presumably due to contamination and other problems inherent in preparing ppt-level standards. Unfortunately, the HFB derivatization of real tissue extracts leads to the formation of numerous other fluorine-containing compounds which co-elute with the tryptoline-HFB and interfere with the negative chemical ionization process. Research to eliminate this problem is continuing.

TABLE 2

TRYPTOLINE STANDARDS

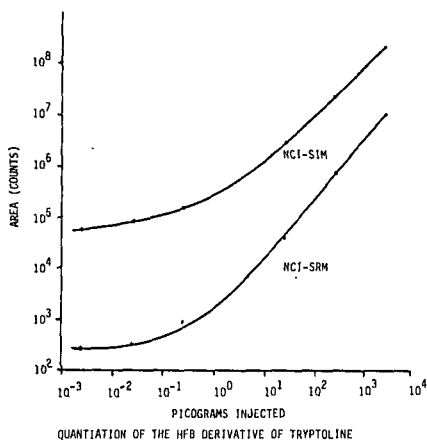
Sensitivity

Ion Source Efficiency (NCI)	10^{-3}
Transmission (Q1/Q2/Q3)	10^{-2}
CID Efficiency ($348^+ \rightarrow 179^+$)	10^{-1}
EM Gain (1800 V)	10^5
Data System Sensitivity (counts/ion)	2
Overall Sensitivity (counts/molecule)	2×10^{-6}

Limit of Detection

Blank	300 counts
3 x blank	900 counts
	= 4×10^8 molecules
	= 700 attomoles
	= 250 femtograms
	= 250 parts per trillion (in 1 μ l)

FIGURE 2



TRACE ANALYSIS IN AIR USING MS/MS AND CI WITH AIR AS THE REAGENT GAS

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Atmospheric Pressure Chemical Ionization (APCI) achieves its high sensitivity to trace gases by allowing ion-molecule reactions to proceed as far as they can go in a recombination-limited steady-state plasma.

Not generally appreciated is the fact that the ionization/recombination cycling in such a plasma can reduce and even remove a true trace constituent and replace it with false trace gases. Since the ionization potentials of positive ions exceed the electron affinities of negative ions with which they recombine, excess energy of several eV is available and the recombination can be dissociative, i.e., rather than returning an ionic species back into the undissociated neutral molecule it was before becoming ionized, neutral fragment molecules are formed.

Because the gas residence time in an APCI source is long due to the limited rate at which vacuum pumps can handle gas flow from the source, many recombination cycles occur. To prevent sample alteration by the source one can either (1) reduce the plasma density (at the expense of reducing signal) or (2) reduce the residence time, which calls for larger apertures and sub-atmospheric operating pressure.

Studies have been performed with air admitted into a source at a pressure of about 2 torr. The source was Extranuclear's Simulscan tandem CI/EI source. No reagent gas is used; only air is admitted. To introduce a known trace gas, a portion of the air passes over the headspace of a container holding a small amount of dimethoxy-methyl-phosphonate (DMMP), $(\text{CH}_3\text{O})_2\text{P}(\text{O})\text{CH}_3$. Enough is admitted to allow clear detection above the background mass peaks using EI. From knowing the ionization cross section for Ar and estimating the total cross section for producing both molecular and fragment ions of DMMP at $20 \text{ \AA}^2$, the amount of DMMP admitted can be estimated. Typically it is in the neighborhood of 1 ppm.

The air is then examined using Air-CI. A typical Air-CI spectrum is shown in Fig. 1. The primary ion is O_2^+ formed by charge transfer between N_2^+ and O_2 . NO^+ , formed by N^+ reacting with O_2 , is also prominent. Low number water clusters of H_3O^+ are seen as primary ions. DMMP $^+$ at 124 AMU is formed by charge (electron) transfer, probably with O_2^+ . Because excess energy is left in the reaction, fragment ions at 79 and 94 AMU appear. Protonated DMMPH $^+$ at 125 is also seen, formed presumably by proton transfer from the water cluster ions. Peaks at 154 and 156 AMU also appear.

Collision induced dissociation spectra are examined using Extranuclear's Model 6000A Triple Quadrupole MS/MS instrument. Fig. 2 shows CID daughter spectra of DMMP $^+$ and DMMPH $^+$. The collision gas was Ar and the collision energy was about 13 eV in this run. Most striking in this spectrum, where the DMMP level was about 1.2 ppm is the good signal-to-noise ratio. The much larger daughter peaks of DMMP $^+$ than DMMPH $^+$ is also striking.

Mass 97 reveals a single prominent daughter at 79, suggesting that it is a water cluster. Daughters of 156 were found to be 124, 97, 94 and 79 suggesting that 156 is 124 with an O_2 attached. A single small daughter of 154 was seen at 125 AMU which is presumably DMMPH $^+$.

Upon reducing the amount of DMMP by re-apportioning the amounts of the clean and the DMMP-doped air, and estimating the concentration by comparing the 124 peak height with that of O_2^+ in the Air-CI spectrum at the lower pressure and at the higher pressure where the amount of DMMP could be estimated from the EI spectrum, DMMP at the level of a few ppb have been detected in MS/MS. Unquestionably further improvement can be made. It seems probable that with modification of the source, sub-atmospheric pressure air chemical ionization can extend its sensitivity into the ppt range without encountering the severe clustering problems that attend APCI.

With respect to the recombination recycling problem, the appearance of a small peak at 116 AMU at the highest ionizing electron current (and therefore highest plasma density in the CI source) is believed to confirm that the phenomenon does occur even at the low

plasma densities here, and suggests that in most APCI sources where the plasma density is high the problem may be quite significant.

On the basis of these studies, which are continuing, it appears that Air-CI, with MS/MS for positive identification of the ions at high signal-to-noise ratios, is a very attractive method to detect and identify trace constituents in air in the ppb range. Unlike APCI where declustering of ions degrades mass resolution, Air-CI retains good resolution throughout the entire spectrum.

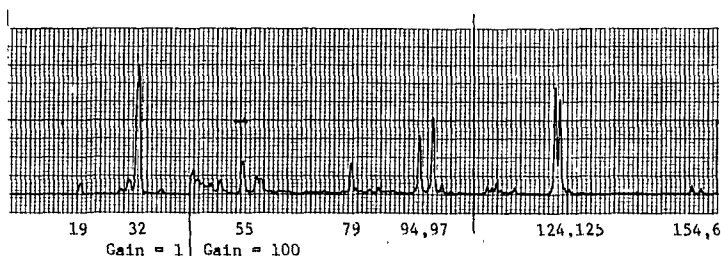


Fig. 1. Air-CI spectrum with 1.2 ppm of DMMP

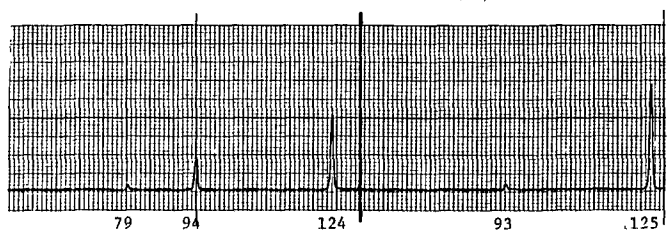


Fig. Daughter spectra of DMMP^+ and DMMPH^+

THE EFFECTS OF THE RADIOFREQUENCY ON COLLISION ENERGETICS AND TRANSMISSION IN QUADRUPOLE COLLISION CELLS, J.E. Fulford and B.I. Shushan, SCIE^x, 55 Glen Cameron Road, Unit 202, Thornhill, Ontario Canada L3T 1P2

INTRODUCTION

Collisionally induced dissociation (CID) using MS/MS is becoming widely used for the analysis of complex mixtures [1], the measurement of thermochemical properties [2] and for studies of the fundamentals of the CID process [3]. Instrumentation for low energy CID most frequently uses a collision cell which employs a two dimensional quadrupole field to efficiently contain the collision products for transmission into the final mass analyzer. Parent and daughter ions which traverse the quadrupole collision cell are influenced by the radiofrequency (rf) electric fields in the transverse dimensions, but remain unaffected in the axial direction [4]. The parent ion collision energy (axial) is, however, controlled by the potential difference between the ion source and the collision cell.

Due to the effect of rf fields in the collision cell, parent ions absorb energy thereby obtaining a velocity component in transverse directions which may influence the collision energetics. The extent of this influence is of concern when measuring kinetic and thermochemical parameters and in establishing standard conditions for the compilation of CID mass spectral libraries. Earlier work [8] established some theoretical expectations for transverse energy effects, however, no direct quantitative evidence of transverse ion energies has been reported.

The dissociation of n-butylbenzene parent ions has been well characterized by photoexcitation [5,6] and by high energy [6] and low energy [7] CID. The fragmentation of the $m/z=134$ parent ion by CID yields major daughters at $m/z=92$ and 91. The $m/z=91$ and 92 daughter ions account for >90% of the total daughter ion yield. Relative yields of $m/z=91$ and 92 are extremely sensitive to parent ion internal (vibrational) energy [5,6] and therefore are dependent on precursor ion translational energy and target thickness. Figure 1 demonstrates that there is a linear dependence of the $91^+/92^+$ intensity ratio over the range of collision energies used in this experiment. Furthermore the slopes of these plots increase significantly at higher target thickness reflecting increased translational to vibrational energy transfer. This chemical system has, in the present study, also been used to measure the contribution of the collision cell rf voltage toward the transverse kinetic energies of butylbenzene molecular ions. Butylbenzene is particularly suitable for this study since the mass dependent transmission efficiency of the collision cell can be neglected due to the proximity in the mass of the $m/z=91$ and 92 daughter ions.

EXPERIMENTAL

The n-butylbenzene was ionized in the atmospheric pressure ion source of a TAGA[®] 6000 triple quadrupole by charge transfer from benzene molecular ions. Axial ion energy was maintained at 3.4 ± 0.9 eV (15 ± 4 eV Lab) throughout and argon was the target gas. The rf voltage on Q2 was controlled as a constant ratio to Q3 rf voltage (constant daughter transmission) and this ratio was altered from 0.2 to 1.0 (180-900 volts) for the rf voltage experiments.

RESULTS

The change in 91/92 was linear (Figure 2) with rf voltage and, as in Figure 1, the slope is steeper at higher target thicknesses. (The curvature at the highest target thickness is presumably due to preferential scattering of the $m/z=91$ daughter ion (ie. the product with the lower translational energy [7]). The slopes of the straight line portions from Figure 2 can be used as a measure of the susceptibility of the system to rf voltage in the collision cell; accordingly this is plotted in Figure 3 as a function of target thickness. The effect of voltage at target thicknesses below 3.0×10^{14} cm⁻² is small compared with that at higher thickness, since the translational to vibrational energy transformation is likely to be more efficient with more collisions. This has implications for the selection of standard operating conditions, in that lower target thickness ($<3.0 \times 10^{14}$ cm⁻²) should be selected if collision cells of different manufacture are to yield comparable results; as well this implies that for increased fragmentation the method of choice would be to increase the axial ion energy rather than the target thickness.

The magnitude of the transverse energy contribution over the range of available Q2 rf voltage may be estimated using the measured 91/92 dependence on axial ion energy (Figure 1). At target thicknesses below 3.0×10^{14} cm⁻² ($2 - 3 \times 10^{14}$ is typical) the transverse energy component may vary by 0.5eV in the centre of mass frame (~ 2 eV Lab) between the

extremes of available rf voltage. In the general case the largest transverse velocity contribution would be observed when the daughter ion mass is closest to that of the parent ion (eg. H atom loss). Extrapolation of the present data to this limiting case results in a transverse energy component of up to 0.7eV (~ 3 eV Lab). In our laboratory, standard operating practice is to set the Q2 rf voltage to one half that of the Q3 value (constant q daughter) in order to optimize the transmission of parent and daughter ions alike. This procedure reduces the effect of the collision cell voltage on the transverse energy component to approximately 0.35eV in the centre of mass system or 1.5eV in the Lab frame. Thus under typical operating conditions the transverse energy contribution from the rf excitation appears to be insignificant when compared with the axial ion energies in general analytical use (>20 eV Lab). For measurements of onset energies, however, the contributions by the transverse energy component would be significant (especially if axial ion energy distributions can be narrowed to 1eV) and result in an underestimate of critical energies for CID onsets.

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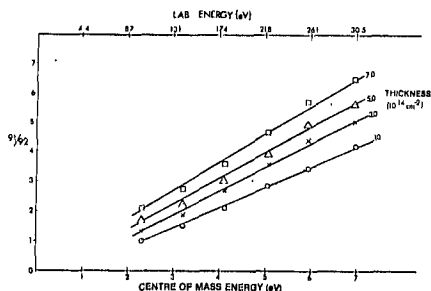


FIGURE 1. EFFECT OF C OF M AXIAL ENERGY ON 91/92 BUTYLBENZENE

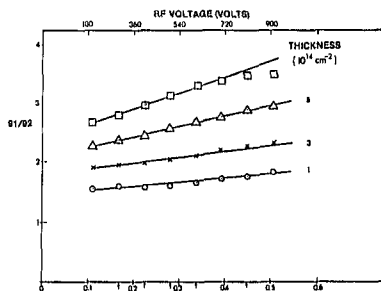
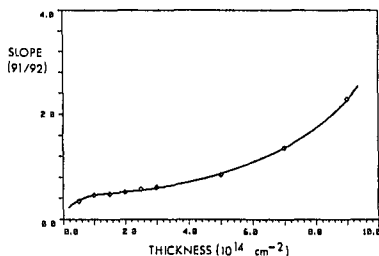


FIGURE 2. EFFECT OF COLLISION CELL VOLTAGE ON 91/92 BUTYLBENZENE



EFFECT OF TARGET THICKNESS ON SUSCEPTIBILITY TO COLLISION CELL VOLTAGE

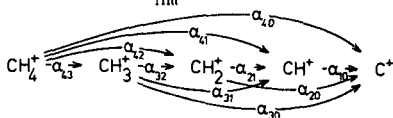
FIGURE 3.

TARGET PRESSURE DEPENDENCE OF MS/MS SPECTRA AND COLLISION ENERGY EFFECTS OVER A RANGE OF 10 - 6000 eV.

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Recent studies regarding collision energy effects (under single collision conditions) and target pressure effects (at a single ion kinetic energy) in MS/MS are described herein. The former experiments were designed primarily to follow energy deposition into the ion as a function of collision energy while the latter measurements were undertaken to test a simple model which describes the effect of target gas pressure on the MS/MS spectrum. All measurements were carried out using our tandem mass spectrometer which has been described in detail [1].

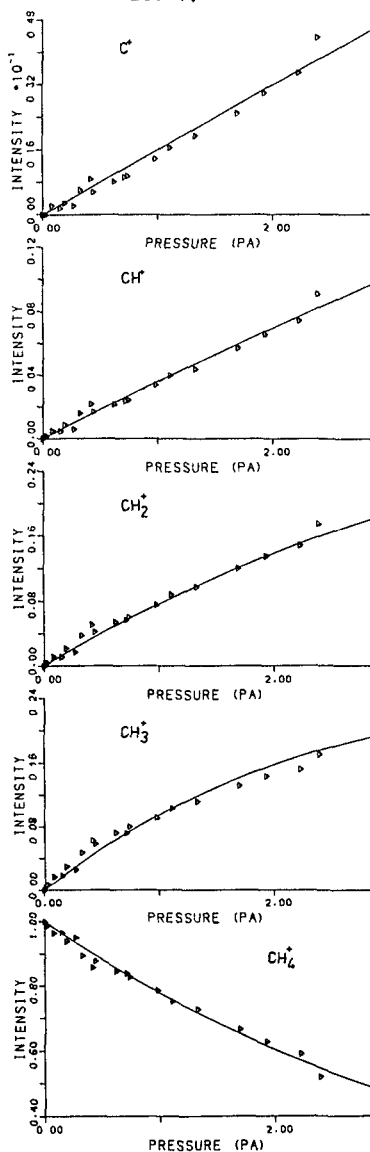
The methane molecular ion was chosen for the target pressure dependence measurements because it gives a relatively simple fragmentation pattern but one which can result from a combination of 10 pathways each of which is characterized by a decay constant α_{nm} (see scheme).



The decay constants are proportional to the respective fragmentation cross sections multiplied by the pathlength and have the dimensions of Pa^{-1} . According to this scheme, the fragment ion intensities are given by a sum of exponential functions $\exp(-\alpha_{nm}p)$. These functions are the solution of the set of simultaneous linear homogeneous differential equations describing the fragment ion intensity variations as a function of pressure. This approach is different from those reported by other workers [2-4]. The decay constants were derived from a series of measurements using CH_4^+ , CH_3^+ , CH_2^+ and CH^+ as precursor ions. In the evaluation it is assumed that the fragmentation probability of a fragment ion CH_n^+ ($n < 4$) formed in the collision cell is identical to the fragmentation of a CH_n^+ precursor ion analyzed at the appropriate collision energy.

We have measured the decay constants for 6 keV CH_4^+ precursor ions using three different gases (He, Ar, Xe). Figure 1 shows the comparison between the theoretical model using these constants and the experimental data for the CH_4^+/He system. It can be seen that the pressure dependence of the fragment ion intensities is reproduced fairly well in the model as was also observed for the CH_n^+/Ar and CH_n^+/Xe systems. As observed previously [2,3], He appears to

FIG. 1.

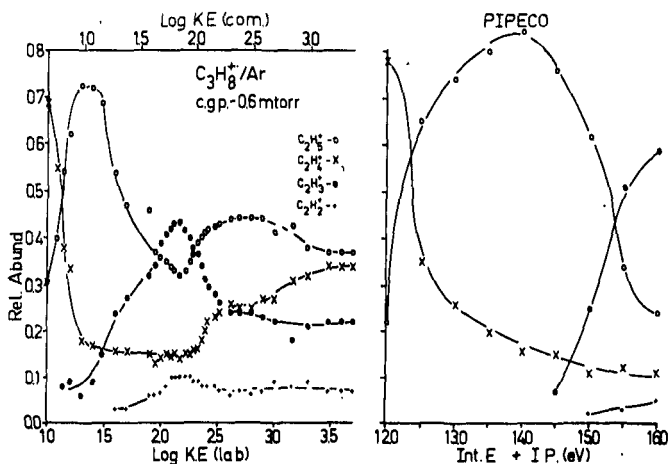


be the most efficient target gas at this collision energy in terms of the degree to which the CH_3^+ ions are excited. This is indicated by consistently higher decay constants found for the highest energy pathways using He as the target than when Ar or Xe were employed. The total fragmentation cross section measured for CH_4/He is $\sigma_{\text{CD}} = 10 \text{ \AA}^2$.

MS/MS spectra of ionized methane, propane and n-butylbenzene have been collected as a function of collision energy over the range of ~ 10 -6000 eV. All spectra were collected using low target gas pressures to avoid contributions from multiple collisions. The emphasis in this work is the degree to which polyatomic ions are excited as a function of collision energy. Collision energy effects are demonstrated by plotting relative fragment ion abundances (or ratios) as a function of collision energy and comparing these curves with the corresponding plots given as a function of internal energy. For example, Figure 2 shows a comparison of the collision energy-resolved MS/MS data for four selected fragment ions from the propane molecular ion with the internal energy-resolved plot derived from PIPECO measurements [5]. Examination of the two plots indicates that over the collision energy range of ~ 150 -400 eV, the average internal energy of the sampled ions actually decreases. The collision energy-resolved MS/MS spectra of the n-butylbenzene molecular ion also show a collision energy region where the average internal energy deposited on collision appears to decrease. Collision energy-resolved MS/MS data for the methane molecular ion, however, indicate a smooth increase in energy deposition with increasing collision energy over the entire range studied. The former results are interpreted as reflecting a change in the relative importance of direct vibrational and electronic excitation as the collision energy increases (and hence, as the interaction time decreases). These observations are expected only when the degrees of inelasticity associated with direct vibrational excitation and electronic excitation differ significantly in the collision energy region where they cross over in importance. The methane molecular ion may be an example where this situation does not prevail.

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FIG. 2.



A MOLECULAR BEAM STUDY OF THE COLLISION INDUCED DISSOCIATION REACTIONS
OF CH_4^+ AND C_3H_8^+ AT ELECTRON VOLT COLLISION ENERGIES

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The collision-induced-dissociation (CID) reactions of organic ions are of great practical interest in that these phenomena are the basis of the analytical technique of mass spectrometry-mass spectrometry (MS-MS). The introduction of tandem triple quadrupole MS-MS spectrometers and their recent application to analytical problems is focusing attention on CID reactions of organic ions in the 1-10 eV range rather than the kilovolt energy regime in which these reactions have generally been investigated. The low energy reaction dynamics of this class of ion-molecule collisions for organic ions is also of considerable fundamental interest. For this reason, we have initiated a study of the CID reactions of CH_4^+ and C_3H_8^+ . This progress report presents the first results of this exploratory study.

EXPERIMENTAL - These experiments were carried out using an apparatus whose characteristics have been described elsewhere(1). Ions are generated by electron impact, accelerated and mass-analyzed by a permanent magnet momentum analyzer, decelerated or accelerated to the final collision energy and crossed at right angles by a modulated supersonic beam. Elastically scattered primary ions and product ions are energy-analyzed by a retarding field analyzer and mass analyzed by a quadrupole mass filter and recorded by an electron multiplier operated in pulse-counting mode. A multi-channel analyzer operated in add/subtract mode referenced to the neutral beam modulation frequency serves as a data buffer for an IBM-9000 computer.

RESULTS AND DISCUSSION - Methane: The most succinct way to display kinematics data for a given reaction is by means of Newton diagrams (2,3), which present the angular scattering profiles for the reaction in center-of-mass coordinates. The relationship between the center-of-mass and laboratory coordinate frames is shown schematically in Fig. 1. The velocity of the center-of-mass is conserved in the collision, and the velocity of the reactants and products relative to this vector are readily computed from conservation of energy and momentum considerations. (It is noted parenthetically that both energy and angle must be measured in the laboratory frame to effect the required transformation.)

Also shown in the Figure are two limiting case models which could plausibly be invoked for the CID reaction $\text{CH}_4^+ + \text{He} \rightarrow \text{CH}_3^+ + \text{H} + \text{He}$. The circle inscribed by the longer vector labelled u' , originating at the head of the CM vector and passing through the laboratory vector $v(\text{CH}_4^+)$ is the locus of elastically scattered CH_4^+ . If the decomposing ions already contain sufficient energy to dissociate, collision with He which deposits no energy in the ion gives products formed along this circle. If, on the other hand, CH_4^+ ions undergoing the CID process are ground state species and the full 1.64 eV energy required must be provided by collision, the thermochemically limited case inscribed by the inner vector u , properly describes the locus of product vectors. As expected, and as observed, products are bounded by these two circles.

This reaction was investigated over the kinetic energy range 1.2 - 4.01 eV relative energy (4.8 to 16 eV laboratory energies). At all energies the maximum intensity is found at 0° (forward-scattering); however, extensive scattering at all laboratory angles is noted. The forward-scattered products are readily interpreted as the (dominant) large impact parameter collisions with little energy transfer, while the back-scattered ions result from small impact parameter "hard-core" collisions. At higher energies the ratios of forward to back scattered product increases.

Of special interest is the fact that the maxima of the contours of product intensity curl inward with increasing angle, reflecting a smooth increase of translational endoergicity with scattering angle. The energy transferred depends linearly on angle, and the zero-angle intercept is common to all relative energies investigated. The intercept of 0.07 eV represents the the minimum energy required to drive the dissociation reaction.

Propane: We attempted to study the propane molecular ion decompositions to give propyl, ethyl, and ethylene ions. These reactions involve, respectively, simple bond breakage (as in methane) with large and small mass ratios of the ion and neutral product and a 4-center elimination reaction. Unfortunately, it was impossible to study the propyl

ion reaction because the intensity of the long-lived metastable decay overwhelmed the CID reaction. Accordingly, results were obtained only for the ethyl ion and ethylene ion CID reactions.

In contrast with the $\text{CH}_4^+ + \text{CH}_4^+$ reaction, the intensity maxima are found at non-zero center-of-mass scattering angles. At 4.2 eV the peak maxima are found at 15° for ethylene and at 26° for ethyl ions. At lower kinetic energies the angles increase monotonically, reaching 27° and 40° for C_2H_4^+ and C_2H_5^+ , respectively, at 1.89 eV collision energy. Also noteworthy is the fact that the maxima are located well within the u_{el}^+ circle for these reaction products, indicative of significant translational endoergicity at all scattering angles. These results correlate with experimental and theoretical breakdown graphs via the linear angle-endoergicity relationship already discussed.

CONCLUSIONS - A limited resolution preliminary study of the scattering of CH_4^+ and C_2H_5^+ from rare gas atom targets suggests the following general features: (1) A two-step mechanism of bimolecular excitation accompanied by relatively slower unimolecular dissociation is followed at very low collision energies (to 1 eV CM). (2) Threshold energies for CID may be measured quite accurately. (3) Extensive angular scattering is observed. (4) Translational endoergicity correlates approximately linearly with scattering angle in C.O.M. coordinates, providing a basis for deducing breakdown graphs for complex ions.

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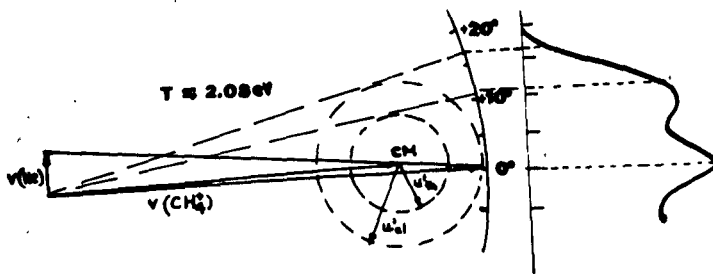


Figure 1: Boundary conditions for the CID reaction of CH_4^+ with He scattering gas. Energetics of the reaction, the range of energies investigated and the relationship between laboratory and center-of-mass scattering angles are shown. The two dashed circles in the CM frame delineate the boundaries for reaction defined by the elastic scattering (u_{el}^+) and thermochemical (u_{th}^+) models, respectively.

CHARGE-EXCHANGE CHEMICAL-IONIZATION MASS SPECTROMETRY:
AN ANALYTICAL TOOL FOR STRUCTURAL ISOMER DIFFERENTIATION

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Many polycyclic aromatic hydrocarbons (PAH) are carcinogenic, and this activity is highly dependent upon the specific structure of the compound. The conventional electron impact (EI) and chemical ionization (CI) mass spectra of isomeric PAH are often indistinguishable. Differentiating between structural isomers has usually been based on their GC retention indexes (1). Charge-exchange chemical-ionization mass spectrometry (CE/CIMS) offers an alternative, predictive method for PAH isomer elucidation. Different mass spectra of PAH isomers are generated by the use of argon mixed with methane as a reagent gas for chemical ionization. This creates a competitive charge-exchange chemical-ionization effect in the ion source. Protonated molecular ions $(M+1)^+$ are formed by proton transfer from CH_5^+ and $C_2H_5^+$ while M^+ ions are formed primarily from charge-exchange reactions between the analyte and Ar^+ . The ratio of the $(M+1)^+/M^+$ has been shown to correlate with the first ionization potential for the PAH studied (2).

Although simple to employ, requiring only an argon/methane reagent gas mixture and a mass spectrometer equipped to do CIMS, (CE/CIMS) has had limited use in the past. This is attributed to the lack of standard operating conditions resulting in a lack of reproducible data in repetitive studies. The purpose of this research was to investigate the effect of operating parameters on the reactant ions in the ion source and hence on the $(M+1)^+/M^+$ ratios of selected PAH.

All experiments were performed on a Hewlett Packard 5985B GC/MS system equipped with a dual ionization source (EI and CI). The mixed reagent gases were introduced through a modified transfer line.

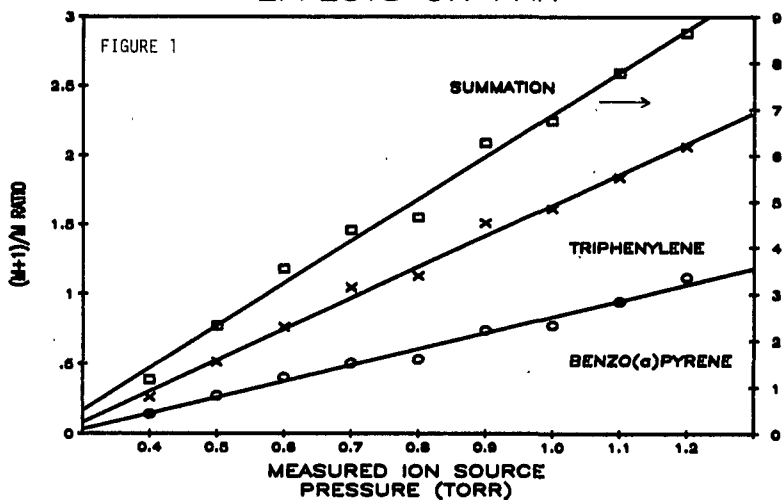
The data indicated that the GC parameters had little or no effect on the $(M+1)^+/M^+$ ratio. Varying the energy of the electron beam only effected the sensitivity not the relative amounts of reactive ions. The ion source temperature was observed to be a significant factor in the $(M+1)^+/M^+$ ratio of individual PAH. As the temperature was increased, the reactive methane ions ($C_2H_5^+$ and CH_5^+) decreased resulting in a decrease in the $(M+1)^+/M^+$ ratio. The most significant variable involved in both the relative abundances of reactive ions and $(M+1)^+/M^+$ ratio was the ion source pressure. As the source pressure was raised, the reactive methane ions increased yielding a corresponding $(M+1)^+/M^+$ increase (see Figure 1). Although the use of different percentages of methane in argon gave different relative abundances of reactive species, the $(M+1)^+/M^+$ ratios were only slightly effected. Higher methane percents gave larger ratios (with other conditions fixed). The optimum conditions were found to be an ion source pressure of approximately 0.8 torr at 250°C employing 15% methane in argon as the reagent gas. Early investigations showed that when the ion source becomes dirty, the ratio of the reactive methane ions to argon ions increases, therefore inflating the $(M+1)^+/M^+$ ratio. To compensate, the use of an internal standard [1,2,3,5 tetrafluorobenzene (TFB)] which mimicked changes in the ion source conditions was employed. By adjusting the flow of reagent gas, we can obtain the same $(M+1)^+/M^+$ for TFB. This results in reproducible $(M+1)^+/M^+$ ratios of analytes (<5% RSD).

Since coeluting isomers (e.g. chrysene and triphenylene) yield different $(M+1)^+/M^+$ ratios, we were able to generate a working curve (see Figure 2) from which the relative percentage of triphenylene and chrysene were determined in carbon black and liquefied coal samples. (CE/CIMS) also allows isomer structure elucidation when standards are not available. This was demonstrated when we determined acephenanthrylene ($M^+=202$) rather than aceanthrylene ($M^+=202$) to be the correct isomer in a carbon black sample.

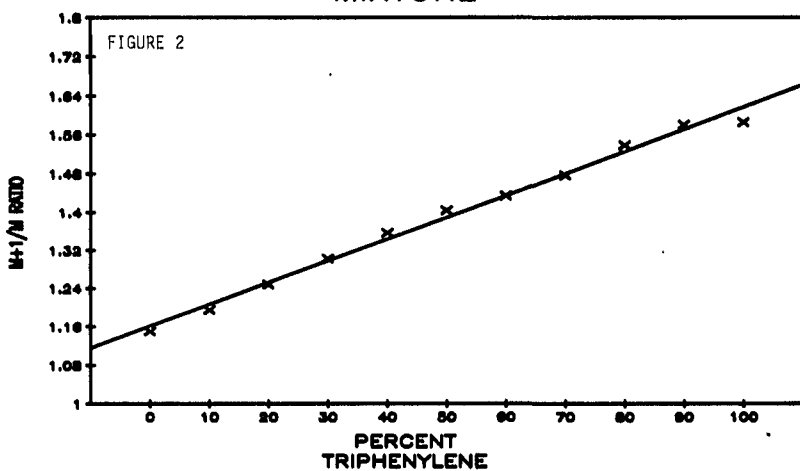
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ION SOURCE PRESSURE EFFECTS ON PAH



OBSERVED $M+1/M$ RATIO FOR TRIPHENYLENE/CHRYSENE MIXTURE



GENERATION AND DETECTION OF POSITIVE IONS IN A QUADRUPOLE MASS SPECTROMETER
OPERATING IN THE NEGATIVE ION CHEMICAL IONIZATION MODE

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In the course of experiments with a home made modification of a Finnigan 3300 for negative ion detection (1) it was found that very strong signals were detected when the high voltage power supply of the conversion dynode was switched off.

The ion source was operated at -5 V and was supposed to inject negative ions into the quadrupole. Negative ions are repelled by the electron multiplier and cannot be detected without a negative to positive ion conversion dynode.

The particles detected by the electron multiplier are therefore most probably positive ions.

In order to investigate the conditions for the unexpected positive ion formation and to obtain the relative proportions of positive and negative ions, the "Faraday cup" plate of the Finnigan 3300 was used to collect the ions. Electron multiplier systems show a widely different response to positive and negative ions in the low mass region (2).

Figure 1 shows that positive ions are observed with a poor peak shape. They are poorly focused and/or injected into the quadrupole with a high velocity. Formation of positive ions is clearly favoured by high positive voltages on the lens plate.

In the case of methane the ions observed are m/z 16, CH_4^+ and m/z 15, CH_3^+ . Positive ions above m/z 16 show a low abundance.

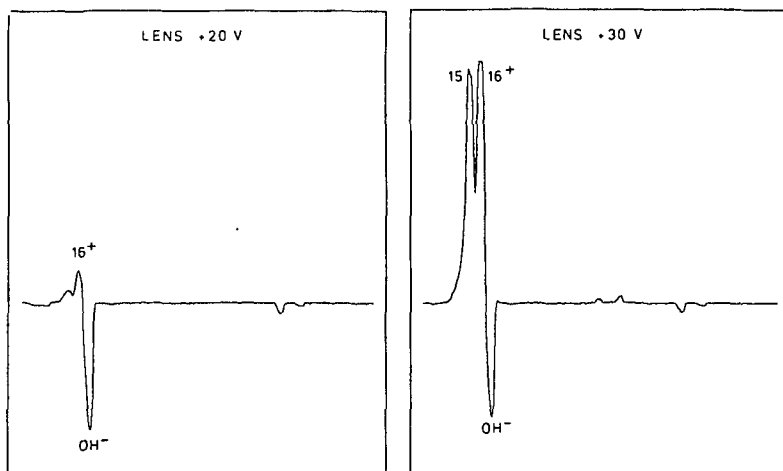


Figure 1

The generation of positive ions can be explained by the following sequence of events:

- electrons are thermalized inside the source by ionizing and non ionizing collisions.
- thermal electrons and reactant gas molecules leave the source through the ion exit hole.
- electrons are accelerated towards the lens plate and ionize gas molecules by electron impact.
- positive ions are accelerated and injected into the quadrupole by the potential difference between the lens plate and the entrance aperture plate (Figure 2).

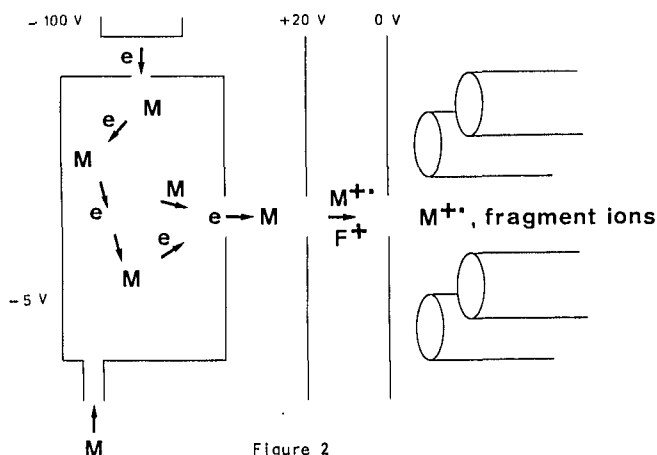


Figure 2

CONCLUSIONS

1. Positive ions are formed outside the source near the lens plate by electron impact of the reactant gas.
2. Both positive ions and "regular negative ions" are mass analyzed by the quadrupole.
3. A detector equipped with only one conversion dynode will repel positive ions and detect only negative ions, giving a true picture of the reactant ion region in the negative ion CI mode.
4. PPINICI systems will detect the positive ions and display them as "negative ions", together with "real" negative ions, giving a false picture of the reactant ion region in the negative ion CI mode (has indeed been observed in a Finnigan 4000).
Solution: disconnect the cable of the conversion dynode for positive ions before tuning on low mass negative ions.
5. No positive ions are observed above the molecular weight of the reactant gas:
PPINICI detectors can be used for measuring negative ion spectra of sample molecules, without interference from positive ions.

References

1. A.P. Bruins, Biomed. Mass Spectrom. 10, 46 (1983).
2. G.C. Stafford, Env. Health Persp. 36, 85 (1980).

The Practical Application of Methylene Chloride as a Negative Chemical Ionization Reagent Gas

Killmer, L. B., Mentzer, M. and Rottschaefer, S.
Smith Kline and French Labs. Phila., PA 19101

Introduction:

The use of methylene chloride (MeCl_2) as a negative chemical ionization reagent gas was described in a paper by A. Tannenbaum, J.D. Roberts, and R. Dougherty. The scope of this presentation is to demonstrate how MeCl_2 can be used to obtain molecular weight information on compounds that will not yield molecular ion or pseudomolecular ion species when they are ionized using conventional CI reagent gases such as methane, isobutane and ammonia.

Theory:

When MeCl_2 is used as a CI reagent gas in negative chemical ionization mass spectrometry $[\text{M}+\text{Cl}]^-$ ions are observed for compounds with "active hydrogens". To a much lesser degree $[\text{M}+\text{Cl}]^-$ ions can be observed for aldehydes, ketones, ethers, and esters. Many factors determine whether a compound will chloride attach such as its ability to form hydrogen bonds and its electron affinity as compared to the electron affinity of MeCl_2 . The source of the chloride anion results from the dissociative electron capture of MeCl_2 .

Instrumentation:

All of the negative chloride attachment spectra were obtained using a Finnigan 3600 GC/MS equipped with PPINICI. Methylene chloride contamination of the mass spectrometer can be eliminated by one of the following two simple equipment modifications:

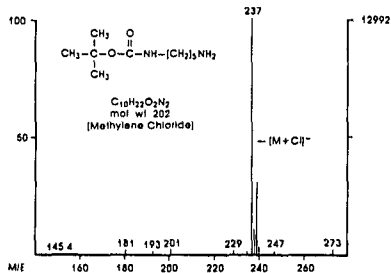
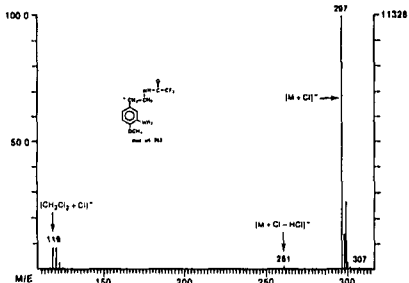
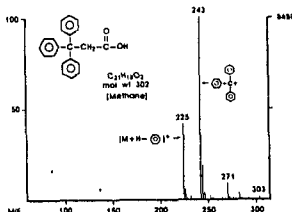
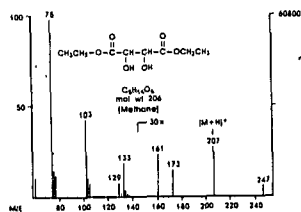
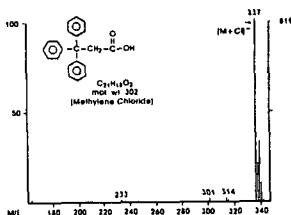
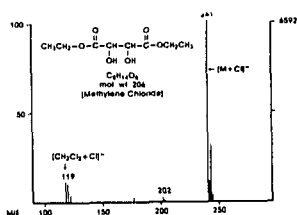
Option A - Installation of a dual glass lined metal transfer line and using one of the lines as the MeCl_2 inlet into the ion source. A glass bulb reservoir containing the MeCl_2 is connected to a fine metering valve and this assembly is attached to one of the injection ports of the GC via an 1/8" OD stainless steel tube. An empty short glass GC column is installed to connect the MeCl_2 reservoir assembly to its transfer line.

Option B - Installation of a stainless steel 1/8" Swagelok tee into the CI direct probe reagent gas line between the solenoid valve and the vacuum manifold. A length of 1/8" OD stainless steel tubing is attached to the Swagelok tee which then connects to a fine metering valve which is mounted on the right top front of the instrument. Both of these modifications eliminate the MeCl_2 from flowing through solenoid valves and flow controllers. These two devices contain rubber seals and/or diaphragms that tend to absorb MeCl_2 , thereby contaminating the mass spectrometer. This equipment modification is also useful with other "solvent" reagent gases and corrosive gases such as ammonia.

Experimental:

The following spectra will demonstrate the power and versatility of this little used and often overlooked reagent gas:

1. Analytical Chemistry 47:49-54 (1975)



Conclusions and Comments

1. MeCl_2 via chloride attachment is a very soft ionization process.
2. Chloride attachment is both pressure and source temperature dependent.
3. MeCl_2 will not chloride attach with all compounds, but it will generally attach with compounds that contain "active hydrogens".
4. Generally chloride attachment spectra are quite simple. In most cases one only observes the $[\text{M}+\text{Cl}]^-$ ions and sometimes $[\text{M}+\text{Cl}-\text{HCl}]^-$ ions.
5. Capillary GC/MS can be performed using MeCl_2 as the reagent gas and helium as the carrier gas.
6. Methylene chloride is inexpensive and 50 mls will last a very long time.

BEHAVIOR OF AROMATIC ESTERS UNDER AMMONIA CHEMICAL IONIZATION. D. Fraisse¹, R. Hass², and J. C. Tabet³; ¹Centre de spectrométrie de mass, SCA - C.N.R.S. - B.P. 22, 69390 Vernaison, France; ²NIEHS, Research Triangle Park, NC 27709, USA; ³Ecole Polytechnique, 91128 Palaiseau Cedex, France.

Introduction

In previous work under CI/NH_3^+ conditions, functional exchange reactions between NH_3 and the molecule being studied have been observed. These reactions compete with the unimolecular decomposition of $(\text{M}+\text{NH}_3)^+$ to produce the protonated molecule MH^+ (1). The pathway for MH^+ production is not favored when the difference $\text{PA}_{\text{MAS}} - \text{PA}_{\text{M}}$ is positive. On the other hand, MH^+ formation is endothermic. In particular alcohols and their derivatives react with NH_3 to form protonated amines via HALDEN inversion during an $\text{S}_{\text{N}}2$ process (2). In the case of aromatic compounds such as nitrobenzene, aromatic electrophilic substitution has been proposed (3).

Results and Discussion

In addition to MH^+ ions and their fragment ions (m/z 105 and m/z 77), the presence of peaks at m/z 94 and at m/z 122 have been observed in the CI/NH_3^+ mass spectra of various substituted phenyl benzoates. These peaks are respectively shifted to m/z 97 and m/z 125 in CI/ND_3^+ spectra showing the presence of an ND_3^+ group while the MH^+ ions become MD^+ . In the 2nd FFR under collision conditions the MH^+ ions formed in $\text{CI}/\text{C}_4\text{H}_9$ and in CI/NH_3^+ give rise to several fragment ions characterized by the same relative abundances shown in the CAD/MIKE spectra. These results indicate that the protonated sites are the same for these ions produced with both reagent gases. No important electronic effects have been observed in the fragmentation induced by collision.

The CAD/MIKE spectra of m/z 94 and m/z 122 ions have been compared to those of protonated aniline and benzamide ions. The similar spectra show that these ions have identical structures.

The formation of protonated aniline and benzamide ions in the source is not observed during the unimolecular decomposition of $(\text{M}+\text{NH}_3)^+$ ion in the 2nd FFR. Indeed, the MIKE spectra of the adduct ion is characterized by the presence only of MH^+ formed by loss of ammonia. The collision mass spectra (Table) show in particular the presence of $(\text{M}+\text{NH}_3 - \text{C}_6\text{H}_5\text{COOH})^+$ produced during intramolecular nucleophilic substitution (equations 1, 2 and 3). The lack of production of aniline can be interpreted by the spacial arrangement of the NH_3 group in the adduct. The $(\text{M}+\text{NH}_3 - \text{C}_6\text{H}_5\text{COOH})^+$ ions are favored in the case of halogenated esters and in their para isomers.

The study of ammonia pressure effects shows a linear relationship between the ratios $(\text{C}_6\text{H}_5\text{NH}_3^+)/(\text{MH}^+)$ and $(\text{C}_6\text{H}_5\text{CONH}_3^+)/(\text{MH}^+)$. These results indicate that m/z 94 and m/z 122 are formed in a process of higher order in ammonia pressure than formation of MH^+ .

The results of this study prove that the formation of protonated aniline and benzamide is consistent with a bimolecular reaction produced in the source (equations 5 and 6).

Conclusion

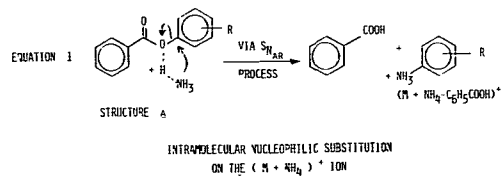
The aromatic esters have been studied under chemical ionization conditions with hydrocarbons (and H_2) as reagent gas. These gases, characterized by a low proton affinity, produced mainly the decomposition of protonated molecules of the structure MH^+ . In our work the formation of MH^+ ions by unimolecular decomposition of the $(\text{M}+\text{NH}_3)^+$ adduct ion is likely to be weakly exothermic (or athermic). Under these conditions other reactions such as ion molecule reaction can compete with MH^+ formation.

These reactions can be compared to aromatic nucleophilic substitution between $(\text{M}+\text{NH}_3)^+$ and NH_3 . Other workers have observed aromatic electrophilic substitutions.

No important substituent effects have been observed here. This can be explained by the charge localization being mainly on the carboxylic function. The pressure effects show the bimolecular character of the formation of protonated aniline and benzamide in the source.

Since the $(\text{M}+\text{NH}_3)^+$ adduct ions are not thermolyzed in the 2nd FFR where NH_3 is not present, these ions are not observed in CAD/MIKE spectra. Other biomolecular reactions such as the $(\text{M}+\text{NH}-\text{RH})^+$ ($\text{R}=\text{OCH}_3$, Br, Cl) are produced by analogous processes.

- 1) J. C. Tabet, M. Bertrame, J. C. Belocil and D. Stahl, *Org. Mass Spectrom.*, submitted.
- 2) J. Bastard, D. Do Khac Mauh, M. Fetizon, J. C. Tabet and D. Fraisse, *J. C. S. Perkin II* 1591, 1981.
- 3) J. Van Thuijl, W. C. M. Luijten and W. Onkenhout, *J.C.S. Chem. Commun.* 106, 1980.

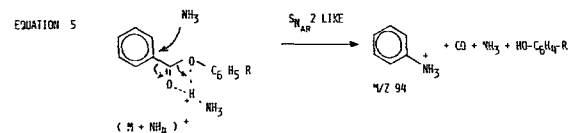


CAD / MIKE SPECTRA OF $(M + NH_4)^+$ ADDUCT IONS FORMED UNDER CI / $^{35}Cl^+$ CONDITIONS.

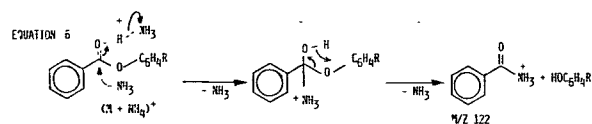
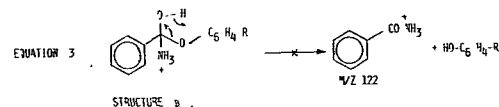
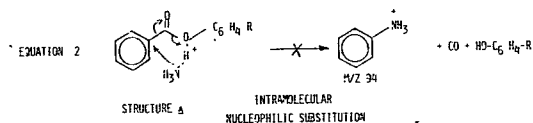
$C_6H_5COOC_6H_5R$ (M/Z MAIN BEAM)		FRAGMENTS IONS FORMED WITHIN COLLISIONS			
		$C_6H_5^+$ (M/Z 77)	$C_6H_5CO^+$ (M/Z 105)	$(M + NH_4)^+$ $C_6H_5CO_2H^+$ (M/Z)	MH^+ (M/Z)
R = H (1)		.8	7.9	.1	20.7 (199)
R = CH_3	P	.4	3.6	.3 (108)	95.7 (213)
(230)	M	6	4.2	.2 (108)	95 (213)
R = OC_4H_9	P	.2	3.5	.6 (124)	3 (214) 95.4 (229)
(246)	M	.3	3.2	.1 (124)	.1 (214) 96.3 (229)
R = Br	P	.2	12.9	2.6 (172)	.2 (214) 84 (277)
(294)	M	3.4	11.9	.5 (172)	.4 (214) 84.7 (277)
R = Cl (2)	P	2.2	12.2	3.0 (128)	.6 (215) 81.4 (233)
(250)	M	1.1	14.0	2.1 (128)	4 (215) 83.1 (233)

(1) $(MH - H_2O)^+$ IS ALSO OBSERVED (0.5 %).

(2) THE ION M/Z 111 IS DETECTED : 0.6 % FOR P CI AND 0.3 % FOR M CI.



BIMOLECULAR AROMATIC SUBSTITUTION INDUCED BY FORMATION OF THE ADDUCT ION $(M + NH_4)^+$



BIMOLECULAR ADDITION-ELIMINATION INDUCED BY FORMATION OF THE ADDUCT ION $(M + NH_4)^+$

CHEMICAL IONIZATION EFFICIENCY AS A FUNCTION OF PROTON AFFINITY:

STUDIES PERTINENT TO QUANTITATIVE MIXTURE ANALYSIS

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Department of Medicinal Chemistry
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Direct sample insertion mass spectrometric methods may be used to analyze mixtures of compounds that are introduced into the ion source by way of a probe inlet system. This approach has been shown to be useful for the identification of even minor mixture components and, in some cases, has been applied to the quantitative analysis of closely related compounds. This approach may be extended to provide an approximate quantitative measure of the amounts of material in the mixture if the chemical ionization efficiency of all mixture components were approximately the same. These studies were designed to determine the relative protonation efficiency of highly exothermic reactions of a series of compounds under conditions where there are no matrix effects present in sample evaporation or quenching effects of sample-sample proton transfer reactions.

The experiment determined the chemical ionization efficiency (Total Ion Current/Mole) of twenty-five compounds that have a wide range of proton affinities and structures (Table 1). The ionization efficiencies were measured using four reagent gases: Methane (Figure 1), Water (Figure 2), Isobutane (Figure 3), and Ammonia (Figure 4).

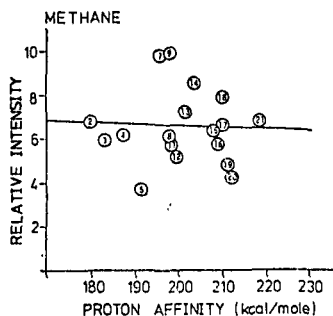
This experiment was designed to determine: the efficiency of proton transfer from a reagent gas to structurally dissimilar compounds relative to the proton affinity of those compounds; the efficiency of proton transfer relative to the proton affinity of the reagent gas employed; if the mild reagent gases, isobutane and ammonia, were capable of selectively protonating compounds that have different proton affinities.

Mass spectrometric data were obtained using a Finnigan 4023 gas chromatograph mass spectrometer interfaced to an Incos 2000 data system, compounds, 0.08 to 0.19 pmol/ μ l, were introduced into a standard chemical ionization source through an OV225 gc column without the use of a separator, column temperatures were programmed from 40 to 175°C at 10°/min with a methane, isobutane or helium carrier gas flow of 30 ml/min. Ammonia or water was added to the helium gas flow at a point between the gc column and ion source. The partial pressure was adjusted to optimize the NH_4^+ or H_3O^+ intensity. These "dilute" conditions favor protonation of the sample molecules rather than association of protonated molecules rather than association of protonated molecular ions with neutral reagent or sample molecules. Ion current from all peaks in the ci spectrum was integrated over the gc peak area and reported as total ion current per mole relative to other structures measured under the same conditions.

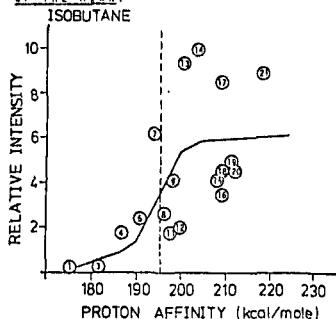
Conclusions:

- 1) Although it has been shown that chemically related molecules (homologs) may be ionized with equal efficiency, this does not appear to be true for dissimilar structures. Ionization efficiencies for highly exothermic (>10 kcal/mole) protonation reactions for all four reagent gases are characterized by a broad variation with percent standard deviations from the mean between 21 and 60%.
- 2) Selective protonation of sample molecules by ammonia and isobutane is observed for molecules that are ± 5 to 7 kcal from the reagent gas conjugate base. Under the non-equilibrium conditions of these chemical ionization experiments, there is an irregular increase in ionization efficiency within this range.
- 3) All four reagent gases had essentially similar behavior. There was no readily apparent correlation between sample or reagent ion structure and ionization efficiency for the compounds studied.
- 4) Quantitative analysis of unknown mixtures of chemically dissimilar compounds by direct sample insertion chemical ionization mass spectrometry would be subject to a large error.

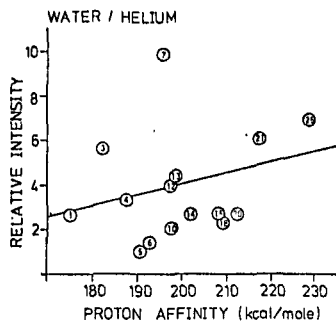
NO.	COMPOUND	PROTON AFFINITY (± 5 KCAL/MOLE)			
1	DECALIN	175	13	METHYLBENZOATE	201
2	TRICHLOROACETONITRILE	178	14	CYCLOHEXANONE	204
3	CHLOROBENZENE	182	15	BENZOPHENONE	208
4	TOLUENE	187	16	DIMETHYLSULFOXIDE	208
5	DIOXANE	191	17	DIMETHYLFORNAMIDE	209
6	1-OCTANOL	193	18	PYRROLE	209
7	BENZONITRILE	195	19	P-ANISALDEHYDE	211
8	TETRAHYDROFURAN	196	20	P-TOLUIDINE	212
9	2-BUTANONE	197	21	PYRIDINE	218
10	AMYL ACETATE	198	22	PIPERIDINE	223
11	MESITYLENE	198	23	CYCLOHEXYLAMINE	228
12	ANISOLE	199	24	TRIETHYLAMINE	229
			25	GUINUCRIDINE	229



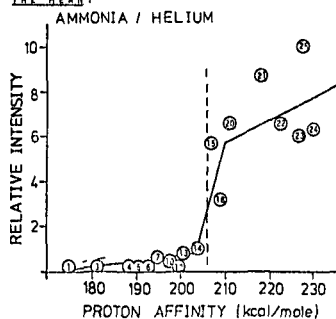
METHANE REAGENT GAS IONS CH_5^+ AND C_2H_5^+ , WITH CONJUGATE BASE PROTON AFFINITIES OF 127 AND 161 KCAL/MOLE, PRODUCED EFFICIENT IONIZATION OF SAMPLE MOLECULES WITH PROTON AFFINITIES BETWEEN 178 AND 218 KCAL/MOLE. THERE WAS NO TREND TOWARD GREATER IONIZATION EFFICIENCY OF THE MORE BASIC SAMPLE MOLECULES, SLOPE = -0.0056. THE MEAN RELATIVE IONIZATION EFFICIENCY WAS 6.51 WITH A 26% STANDARD DEVIATION AND A RANGE FROM 4.3 TO 10.0 OR 88% OF THE MEAN.



THE C_4H_9^+ ION FORMED FROM ISOBUTANE, CONJUGATE BASE PROTON AFFINITY 195 KCAL/MOLE, IS NOT A VERY SELECTIVE REAGENT GAS. NO IONIZATION WAS OBSERVED FOR MOLECULES 13 KCAL LESS BASIC THAN ISOBUTYLENE. THERE WAS A RANGE OF INCREASING IONIZATION EFFICIENCY BETWEEN 187 AND 201 KCAL CHARACTERIZED BY A RANGE OF 4.7 RELATIVE UNITS. ABOVE 200 KCAL/MOLE THE MEAN IONIZATION EFFICIENCY WAS 6.5 WITH A 37% STANDARD DEVIATION AND A RANGE FROM 3.5 TO 10.0 OR 100% OF THE MEAN.



THE HYDRONIUM ION, H_3O^+ , FORMED USING WATER, PROTON AFFINITY 165 KCAL/MOLE, AS A REAGENT GAS WAS ABLE TO PROTONATE ALL SAMPLE MOLECULES HAVING PROTON AFFINITIES BETWEEN 175 AND 229 KCAL/MOLE. THERE WAS A SLIGHT TREND TOWARD HIGHER IONIZATION EFFICIENCIES FOR MORE BASIC COMPOUNDS, SLOPE = 0.045. THIS WAS NOT SIGNIFICANT DUE TO THE WIDE VARIATION IN IONIZATION EFFICIENCIES AND SAMPLE DISTRIBUTION. THE MEAN RELATIVE IONIZATION EFFICIENCY WAS 4.0 WITH A 60% STANDARD DEVIATION AND A RANGE OF 0.9 TO 10.0 OR 228% OF THE MEAN.



AMMONIA, PROTON AFFINITY 207 KCAL/MOLE, WAS USED AS A REAGENT GAS UNDER CONDITIONS THAT FAVOR PROTON TRANSFER OVER ASSOCIATION. LITTLE OR NO SAMPLE IONIZATION WAS OBSERVED FOR MOLECULES WITH PROTON AFFINITIES 15 KCAL LESS BASIC THAN AMMONIA. A RATHER SHARP INCREASE IN IONIZATION WAS OBSERVED FOR SAMPLES BETWEEN 201 AND 212 KCAL. ABOVE THIS POINT, MOLECULES WERE IONIZED WITH A MEAN EFFICIENCY OF 7.5 WITH A 21% STANDARD DEVIATION.

EFFECT OF PROTONATION SITE IN CHEMICAL IONIZATION OF STEREOISOMERIC NORBORNENOLS

J. Jalonen

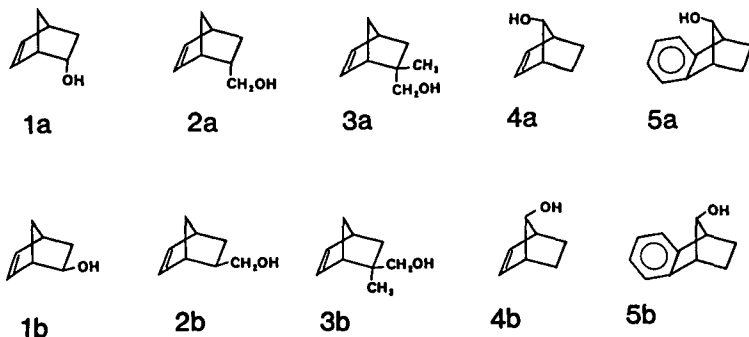
Department of Chemistry, University of Turku, SF-20500 Turku, Finland

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Orion Pharmaceutical Co., Research Laboratory, P.O.B. 19, SF-00101 Helsinki, Finland

Chemical ionization (CI) technique has recently emerged as a useful tool for analyzing stereochemical effects in organic molecules because it allows selective ionization and/or fragmentation of the substrate molecule by a proper choice of the reagent gas. In principle, the usability of chemical ionization as a potential stereochemical probe is based on the fact that CI process is a chemical reaction between an ion and a chiral molecule and that the formation of product ions as well as their relative stability may be stereosensitive.

Reagent gas can form either protonated molecules or adduct ions with substrates both of which can be utilized to probe spatial arrangements of molecules. However, there is no generally accepted model to explain the observed differences in CI spectra of isomeric molecules. Thus, in order to get better understanding on the interactions between the ionized species and substrate molecules, we chose a set of stereoisomeric norbornenols (1 - 5) as model compounds as a part of our project directed to develop selective ionization methods for organic analysis.^{1,2}



Jelus et al. studied recently CI mass spectra of 2-norbornanols using methane and isobutane as reagent gases.³ The CI mass Spectra of the corresponding endo- and exo-isomers were nearly identical in both CI conditions. Introduction of a double bond to the norbornanol skeleton dramatically changes the picture. In the isobutane CI mass spectrum of endo-5-norbornen-2-ol (1a) MH^+ peak is more pronounced compared to the corresponding ion in exo-isomer. On the other hand, exo-form shows a prominent $(MH - H_2O)^+$ ion. Similar but even more profound effects are found in the CI mass spectra of 7-norbornenols (4) as seen from Table 1. In fact, this table shows a very consistent type pattern: protonated molecules, MH^+ of a isomers (i.e. endo- or syn-) are more stable than the

Table 1.

	MH^+		$\frac{MH^+}{(MH-H_2O)^+}$	
	CH_4	$i-C_4H_{10}$	CH_4	$i-C_4H_{10}$
1a/1b	2	3	2	3
2a/2b	4	4	5	6
3a/3b	1	7	1	16
4a/4b	6	25	8	170
5a/5b	0.1	2	0.1	2

corresponding b isomers (i.e. exo- or anti-) in methane and isobutane CI conditions. The obvious exceptions from this rule are CH_4 CI mass spectra of benzonorbornen-7-ols (5). In all cases a isomers have a double bond (or aromatic ring) and hydroxyl group close to each other. This spatial arrangement allows interaction between the two functional groups. The observed stereochemical effects can be explained by assuming protonation on the hydroxyl group and the stabilization of the protonated molecules by intramolecular H-bonding in a isomers. Actually similar rationalizations has been used earlier for cyclic diols⁴ and applied recently also for unsaturated alcohols.⁵ The alternative interpretation is based on the assumption that both functional groups are protonated. If the double bond is protonated a stable MH^+ is formed. In the case of OH-group protonation immediate elimination of water is expected. In a isomers the stereochemistry allows proton transfer from hydroxyl group to the double bond which evidently has higher proton affinity (PA) (PA of norbornene is 202 kcal/mol; sec. alcohols: PA < 190) thus increasing the amount of MH^+ unable to loose water.

This interpretation on the importance of the protonation site in CI is further supported by our MNDO calculations on protonation sites and relative stabilities of MH^+ in 4a and 4b.¹ The results indicate that CI may enhance the stability differences between stereoisomers and that protonation site may play an important role in stereospecific control of chemical ionization.

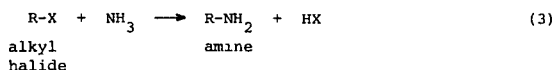
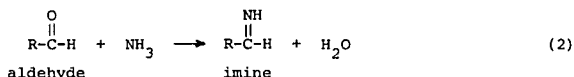
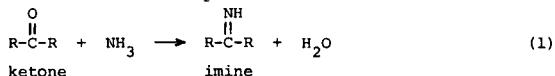
1. J.Jalonen, J. Taskinen and C. Glidewell, Int. J. Mass Spectrom. Ion Phys. **46** (1983) 243.
2. J.Jalonen, J.M. Tedder and P. Vidaud, Adv. Mass Spectrom. **8** (1980) 201.
3. B.L. Jelus, D.L. Dalrymple, J. Michnowicz and B. Munson, Org. Mass Spectrom. **13** (1978) 163
4. J. Winkler and F.W. McLafferty, Tetrahedron, **30** (1974) 2971.
5. J. Bastard, D. Do Khac Manh, M. Fetizon, J.C. Tabet and D. Fraisse, J.C.S. Perkin II, (1981) 1591.

NEUTRAL REACTIONS IN GC-CI MS

Patrick Rudewicz and Burnaby Munson, Department of Chemistry, University of Delaware, Newark, Delaware 19711.

In ammonia CI MS it has been reported that aldehydes and ketones form Schiff bases in the gas phase with mechanisms analogous to those occurring in solution. Tabet and Fraisse observed that cyclohexanone reacts with NH_4^+ to give an abundant ion at $m/z = 98$, corresponding to the protonated Schiff base or imine of cyclohexanone.¹ In very different experiments, Hunt and McEwen observed that mixing aldehydes with NH_3 and then leaking the mixture into a CI source gave a major ion corresponding to the protonated imine.² The imine was formed in a neutral reaction between NH_3 and the aldehyde.

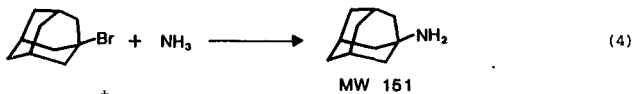
In an investigation of the formation of imine molecules by the reaction of ammonia with aldehydes and ketones we have used NH_3 as a carrier gas for GC-CI MS studies. The following neutral reactions have been observed on various packed columns:



The products of these on-column reactions elute from the GC into the source of a DuPont 492-B mass spectrometer and are protonated under ammonia CI conditions.

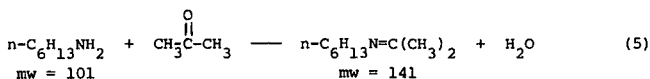
Figure 1 is a comparison of the NH_3 CI spectra of acetophenone (minus the reagent ions) using two different carrier gases, He and NH_3 . With He as a carrier gas the spectrum contains two major ions: $m/z = 138$, $(\text{M}+\text{NH}_4)^+$, and $m/z = 155$, $(\text{M}+\text{NH}_3\cdot\text{NH}_4)^+$. Using NH_3 as a carrier gas, the most abundant ion in the spectrum is $m/z = 120$. Precise mass measurements show that this ion is the protonated imine, $\text{C}_8\text{H}_{10}\text{N}^+$, not the molecular ion of acetophenone, $\text{C}_8\text{H}_8\text{O}^+$. Using single ion monitoring, it can be seen that the imine and the unreacted acetophenone have different chromatographic peak profiles.

Potential analytical utility of ammonia as a carrier gas in GC-CI MS can be demonstrated in the case of alkyl halides. Under normal NH_3 CI conditions with He as a carrier gas, 1-bromoadamantane does not react with NH_4^+ . With ammonia as a carrier gas, however, 1-bromoadamantane undergoes a neutral substitution reaction to give an amine,



which reacts rapidly with NH_4^+ to give a protonated species at $m/z = 152$.

In related experiments with He as the GC carrier gas, primary aliphatic amines react with acetone to form a small amount of imine on a 3% SP-2100 column. Injection of 5.0 μL of acetone immediately after injection 0.15 μL of a mixture of n-hexylamine, diallylamine, and tripropyl amine results in the following neutral reaction:



In this case, using He as a carrier gas, a flame ionization detector can be used in conjunction with the MS as a means of detection and an extra peak on the FID trace corresponding to the imine is found. The secondary and tertiary amines do not react with acetone. Similarly, when 5.0 μL of acetic anhydride is injected immediately after injection of an amine mixture

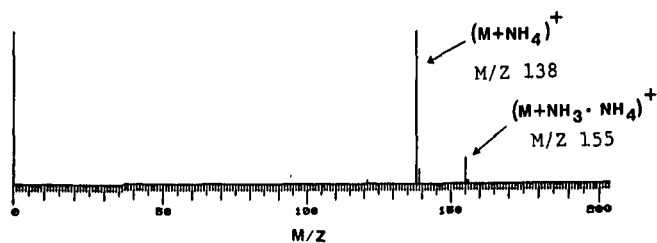
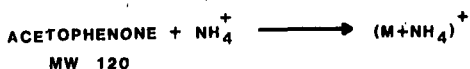
consisting of aniline, m-toluidine, N-ethylaniline, and N,N-diethylaniline, the primary and secondary amines are quantitatively derivatized on the column to form their corresponding amides.

¹J.C. Tabet and D. Fraisse, Org. Mass Spectrom., 1981, 16, 45-47.

²D.F. Hunt, in Prog. in Analytical Chem., Vol. 6, Ed. by I.L. Simmons and G.W. Ewing, Plenum Press, New York, 1973, p. 369.

ACETOPHENONE NH₃ Cl

GC CARRIER GAS: He



GC CARRIER GAS: NH₃

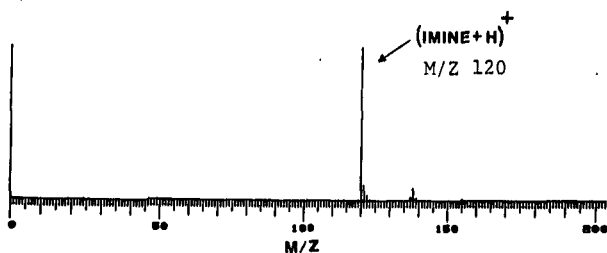
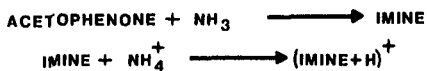


FIGURE 1

IONIZATION RANGE OF 100 to 600 eV ELECTRONS IN CHEMICAL IONIZATION REAGENT GASES:
Toshihiro Fujii and G.G. Meisels; Dept. of Chemistry, University of Nebraska,
Lincoln, NE 68588-0312.

Range is defined as the distance traversed by the impinging particle before it comes to rest in the absorbing material. Knowledge of the range of low-energy electrons used for the production of reactant ions in chemical ionization (CI) sources is important to CI ion source design, to the optimization of source performance, and to a better understanding of source characteristics.

We have previously reported the spatial distribution of primary ion formation in CI sources and the range R_i within which electrons of 100 to 600 eV energy produce 97% of the total ionization.(1) These measurements are based upon time resolved methods whose interpretation relies on known ion drift properties.(2)

In this communication we show that R_i can be correlated with the mean electron energy required to form an ion pair (W) and the ionization cross section (σ), quantities available from non-mass spectrometric measurements. A relationship is derived which can be used to estimate R_i provided that W and σ are known.

The probability that an ionization event will take place in the length element dx is proportional to the ionization cross section $\sigma(E)$. Therefore electrons have a larger number of interactions per unit path length when $\sigma(E)$ is larger if other parameters are equal; the electron ionization range R_i decreases as $\sigma(E)$ increases.

$W(E)$ is the mean electron energy required for ion pair formation and hence represents the average energy lost in forming an ion pair in the gas. Since electrons lose more energy per ionization event when $W(E)$ is larger, range also decreases with increasing $W(E)$ provided that other parameters remain unchanged.

The number of ion pairs formed per unit path length dx , di/dx is proportional to the ionization cross section $\sigma(E)$ at energy E and the gas density n

$$di/dx = \sigma(E) \cdot n \quad (1)$$

It is clear that the energy loss dE/dx is related through $W(E)$

$$dE/dx = di/dx \cdot W(E) = \sigma(E) \cdot n \cdot W(E) \quad (2)$$

Therefore the range is given by the integration over $dE/(W(E)\sigma(E))$:

$$n \int_0^{R_i(E)} dx = n \cdot R_i(E) = \int_{IP}^E \frac{dE}{\sigma(E) W(E)} \quad (3)$$

Figure 1 illustrates the functions $W(E)$, $\sigma(E)$, and $[W(E)\sigma(E)]^{-1}$ as a function of energy for Argon. The range is proportional to the area under the curve for $[W(E)\sigma(E)]^{-1}$ up to the energy of the electron. Clearly, range is not exactly linear with electron energy. It should be noted here that this approach incorrectly uses the average value $W(E)$ for electrons of energy E ; this is the energy E of the incident electron divided by the average total number of ion pairs it produces when it expends its energy completely. Instead, one should use the differential value of W , $dW(E)$, the average energy expended for the first ionization event of an electron with energy E .

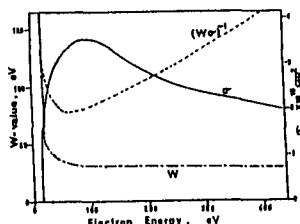


Fig.1 $W(E)$, $\sigma(E)$ and $[W(E)\sigma(E)]^{-1}$ vs. electron energy

The range derived for Argon using available data (3,4) may be compared with experimental values. Table I shows that calculated and experimental values are in reasonable agreement with each other, and do not differ by more than about 20%.

Estimates can be extended to gases where $\sigma(E)$ and $W(E)$ are not as well known if it is assumed that the functional form of $[W(E)\sigma(E)]^{-1}$ is the same for all species, and that the values of $\sigma(E)$ at a specific energy, such as 70 eV, and $W(\infty)$ at the high, energy independent limit may be used to scale the range (3,5). Table II gives the results of such an approach, and a comparison with experimental results, for the common CI gases methane, nitrogen, and iso-butane; it also gives the calculated estimates for ranges in ammonia. For these gases, calculated and experimental ranges are also within about 20 to 30% of each other, however, there appears to be a systematic underestimate of the range at lower energies and an overestimate at higher electron energies. This probably reflects primarily differences in the energy dependence of $\sigma(E)$ since $W(E)$ is functionally fairly similar for most substrates (4).

ACKNOWLEDGMENTS. We appreciate the support of this investigation by the Department of Energy under contract number DE-ACD2-76-ER02567.

LITERATURE CITED. 1. A.J. Illies and G.G. Meisels, Anal. Chem. 52, 325 (1980); 2. G.G. Meisels and A.J. Illies, Anal. Chem. 53, 2162 (1981); 3. J. W. Otvos and D.P. Stevenson, J. Chem. Phys. 78, 546 (1956); 4. D. Combecher, Radiation Res. 86, 89 (1980); 5. T.A. Stoneham, D.R. Ethridge and G.G. Meisels, J. Chem. Phys. 54, 4054 (1971).

Table I Ionization Range of Electrons in Argon

electron energy	electron ionization range (cm at 1 torr and 298K)	
	calculated	measured
100 eV	0.22	0.28
200 eV	0.47	0.51
300 eV	0.79	0.73
400 eV	1.20	0.96

Table II Measured and calculated electron ionization range data for CI reagent gases. Range is expressed in cm torr at 298K.

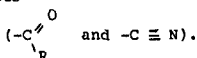
electron energy	N ₂		CH ₄		i-C ₄ H ₁₀		NH ₃	
	meas.	calc.	meas.	calc.	meas.	calc.	meas.	calc.
100 eV	0.26	0.21	0.24	0.17	0.087	0.061	-	0.22
200 eV	0.44	0.44	0.40	0.35	0.15	0.13	-	0.46
300 eV	0.69	0.74	0.57	0.60	0.21	0.22	-	0.78
400 eV	0.85	1.11	0.74	0.88	0.28	0.33	-	1.2
500 eV	-	-	0.91	1.20	-	-	-	-
600 eV	-	-	1.13	1.51	-	-	-	-

Electrophilic Reactions in Aromatic Systems
Using CI-MS Conditions

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A number of classic organic reactions occurring in the condensed phase have been studied recently using chemical ionization mass spectrometry.¹ The present study offers an example of the application of CI-MS to the investigation of electrophilic aromatic substitution reactions in which effects of the electron releasing and withdrawing substituents on aromatic systems are identified in the mass spectra. The analytical utility of methylene chloride CI-MS lies in its potential for distinguishing between these electronic effects.

Reagent ions generated from methylene chloride reacted with aromatic compounds under positive ion CI-MS conditions. Methylene chloride positive ions were observed at m/z 49, m/z 83, and m/z 133, corresponding to $[\text{CH}_2\text{Cl}]^+$, $[\text{CHCl}_2]^+$, and $[\text{C}_2\text{H}_4\text{Cl}_3]^+$, respectively. The aromatic reactants included benzene, naphthalene and naphthalened₈, and substituted benzenes with electron releasing substituents ($-\text{OH}$ and $-\text{OCH}_3$) and electron withdrawing substituents



The ion-molecule reactions appear to involve adduct formation between methylene chloride electrophilic ions and the aromatic compounds. The adduct may or may not undergo subsequent elimination.

Benzene, naphthalene, phenol and anisole exhibit ions at $[M + 83]^+$, $[M + 47]^+$, and $[M + 13]^+$, corresponding to $[M + \text{CHCl}_2]^+$, $[M + \text{CH}_2\text{Cl} - \text{H}_2]^+$, and $[M + \text{CH}_2\text{Cl} - \text{HCl}]^+$, respectively. The basis for the proposed mechanism, Scheme 1, lies in the use of deuterated naphthalene and phenol, which yield $[M + \text{CH}_2\text{Cl} - \text{DH}]^+$ and $[M + \text{CH}_2\text{Cl} - \text{DCl}]^+$.

The carbonyl and cyano substituents give rise to ions at $[M + \text{CHCl}_2]^+$, $[M + \text{CH}_2\text{Cl}]^+$, and $[M + \text{CH}_2\text{Cl} - \text{HCl}]^+$, as shown in Scheme 2. Ions at $[M + 49]^+$ are observed and undergo no loss of H_2 , i.e., no ions at $[M + 47]^+$ are observed.

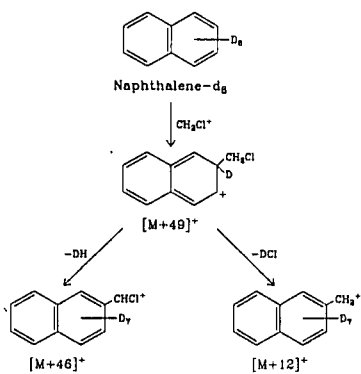
Although the methylene chloride adduct proposed for the electron releasing substituents is analogous to typical electrophilic aromatic substitution intermediates, attack by the methylene chloride reagent ions on the compounds containing electron withdrawing substituents may occur either at the substituent site or the aromatic ring. Attack at the substituent site (Scheme 2) seems more likely for the latter compounds since such a route explains the lack of ions at $[M + 47]^+$ due to the relative instability of structure e. This instability results from the proximity of an electronegative chlorine to the oxygen accommodating a high positive charge density. Loss of H_2 from structure c, on the other hand, does not lead to a similarly unstable structure. Attack at the electron withdrawing substituent site has been suggested for protonation of aromatic compounds.²

¹ C. Wesdemiotis and F. W. McLafferty, *Org. Mass Spectrom.*, **16**, 381 (1981).

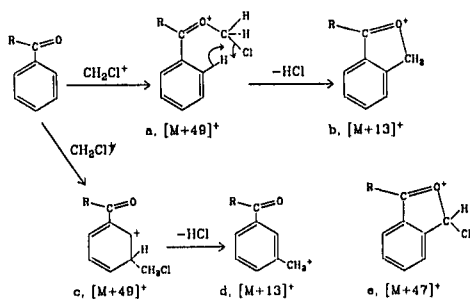
² Y. K. Lau and P. Kebarle, *J. Am. Chem. Soc.*, **98**, 7452 (1976).

* National Research Council Associate

NOTE: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.



Scheme 1



Scheme 2

A STUDY OF POSITIVE IONS SPUTTERED FROM BORIDES WITH 10 keV Ar⁺, Frank G. Satkiewicz,
The Johns Hopkins University, Applied Physics Laboratory, Laurel, Maryland 20707

In a previous study⁽¹⁾, a comparison of the relative yields of metal ions from six silicon-based NBS K-glasses with those from sixteen silicides (sputtered in the presence of added oxygen) showed agreement within a factor of two whereas the range of yields was about sixty. As an extension of this work, the following seventeen borides were studied: MgB₂, AlB₂, SiB₆, CaB₆, TiB₂, VB₂, VB, CrB₂, MnB₂, MnB, Fe_{0.51}B_{0.44}Si_{0.045}, NiB, Ni₂B, Ni₃B, ZrB₂, TaB₂, and TaB. Secondary ion energy distributions of X⁺ and B⁺ were obtained for each boride after steady sputtering conditions were established.

Polyatomic spectra of the borides showed different degrees of oxygen contamination so the effect of introducing additional oxygen on the ion intensities was determined. Energy distributions were also obtained at the highest O₂ pressure (10⁻⁵Torr). The following expression was used to define the relative ion yield, Y_{X⁺}, of the metallic ion species X⁺ in a binary boride (Y_B = 1):

$$Y_{X^+} = \frac{n_B}{n_X} \frac{(F I_m A_t)_{X^+}}{(F I_m A_t)_{B^+}} \quad [1]$$

where n's are the number of atoms of the elements in the borides, F's are isotope correction factors, I_m's are the maximum intensities in the ion energy distributions, and A_t's are areas under the distributions in the range 0 to 500 eV. The ion yields at 10⁻⁵Torr O₂ for the borides and those found previously for the silicides and glasses⁽¹⁾ are shown in Table I. The agreement is within ±29% on the average and supports the conclusion that suitably normalized relative ion yields obtained from families of materials can be used for semi-quantitative analysis of any homogeneous sample.

The relative ion yields of two elements which are not normally found together in a single matrix can be estimated by sputtering binary compounds containing one of the elements of interest. This is illustrated by estimating the yield of Cd in a Si or B matrix. The silicide or boride of cadmium may not exist (there are no powder diffraction patterns listed)⁽²⁾; however, sputtering data for CdS and Cu₂S is available⁽³⁾. The latter was used to establish the relationship to the silicides through the results for Cu₅Si, i.e., the yield of Cu from Cu₂S was normalized to Cu from Cu₅Si and then S from CdS to S from Cu₂S. The result is: Y_{Cd} = 0.042 Y_{Si} and Y_S = 0.018 Y_{Si} (major isotope, no addition of O₂, and E_w = 250 ± 25 eV).

Table I
Comparison of Relative Ion Yields from Sputtering
Borides, Silicides, and Glasses

Species	Glasses	Silicides(O_2)	Borides (O_2)
B	-	-	1.0
O	0.024	-	-
Mg	5.4	-	2.9 MgB_2
Al	6.5	5.0 $Al_{88}Si_{12}$	4.2 AlB_2
Si	1.7	1.0	1.1 SiB_6
Si			0.97 $Fe_{0.51}B_{0.44}Si_{0.045}$
Ca	10.2	17.1 $CaSi_2$	13.9 CaB_6
Ti	5.9	3.0 $TiSi_2$	5.0 TiB_2
V	-	1.9 V_3Si	3.6 VB
V	-	3.0 VSi_2	4.6 VB_2
Cr	-	3.5 Cr_3Si	4.5 CrB_2
Cr	-	2.1 $CrSi_2$	
Mn	2.7	2.4 $MnSi_2$	3.8 MnB_2
Mn			4.9 MnB
Fe	1.2	1.4 $FeSi_2$	1.6 $Fe_{0.51}B_{0.44}Si_{0.045}$
Fe		2.7 $FeSi$	
Ni	-	0.74 Ni_2Si	1.35 NiB
Ni	-		1.05 Ni_2B
Ni	-		1.28 Ni_3B
Co	0.97	1.2 $CoSi_2$	-
Cu	0.39	0.28 Cu_5Si	-
Zn	0.027	-	-
Zr	5.2	3.2 $ZrSi_2$	2.9 ZrB_2
Ba	9.2	10.2 $BaSi_2$	-
Ta	0.79	0.56 $TaSi_2$	0.51 TaB_2
Ta			0.63 TaB

- (1) F.G. Satkiewicz, "Relative Yields of Positive Ions Sputtered from Silicides", presented at the 30th Annual Conference on Mass Spectrometry and Allied Topics, Honolulu, HI, 1982.
- (2) Powder Diffraction File Search Manual, SMA-26, 1976
- (3) F.G. Satkiewicz, and H.K. Charles, Jr., "Sputter Ion Mass Spectrometer Analysis of Copper Sulfide/Cadmium Sulfide Solar Cell Samples", APL/JHU Technical Memorandum TG-1284, October, 1975.

HIGH ELECTRON AFFINITY ELEMENT PROFILES IN InP BY SIMS WITH Cs⁺ PRIMARY IONS

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Indium phosphide is becoming under consideration for electro-optical applications such as lasers, LED's and photodiodes for use with low loss optical fibers in the 1.1 - 1.6μm wavelength region. Among previous papers concerning this new material we can quote J.D. OBERSTAR et al. who studied depth distribution of Be, Mg, Si in as-implanted or annealed samples [1-3].

This paper reports the depth profiles of unannealed C, Si, O, S, Se implants in InP. Si, S and Se are of primary importance as n-type dopants, C, O and Si are the main residual impurities. Such a work has to be compared to the previous study of D.P. LETA et al. [4] but here the analytical conditions have been optimized. We used a CAMECA ims 3 f instrument equipped with a primary Cs⁺ ion source. The elements having strong electron affinities, like C, Si, O, S, Se, have also very high negative ion yields with Cs⁺ primary beams [5].

EXPERIMENTAL : The samples were <100> crystals, implanted at fluences and energies ranging from 10¹² to 10¹⁵ atom. cm⁻², from 400 to 1 000 KeV, respectively. They were tilted 7° from the incoming ion beam to prevent any channeling effect. The ¹³³Cs⁺ ion beam, of 1μA intensity and of 14.5 KeV impact energy, was scanned over areas of about 500μm². The secondary ion extraction optics of the microanalyzer was tuned either in the 150μm mode (imaged field diameter equal to 60μm with the 750μm field aperture), or in the 25μm mode (imaged field diameter equal to 10μm). As calculated, by recording the same low dose profile with the two experimental conditions, a conversion factor equal to 20 has been adopted. In all circumstances, the energy slit was fully opened for maximum sensitivity.

Either mono atomic negative ions or molecular negative ions of the studied elements were monitored (see table 1 and 2).

SUBSTRATE	ELEMENT	MONITORED ION	USEFUL YIELD (a)	BACKGROUND Cps (b)	DETECTION LIMIT ATOM-CM ⁻³
InP	C	¹³ C ⁻	7.5 10 ⁻³	250 M	2. 10 ¹⁶ (c)
	Si	²⁸ Si ⁻	1.6 10 ⁻³	80 M	2. 10 ¹⁵
	O	¹⁶ O ⁻	9.5 10 ⁻²	7 800 M	6. 10 ¹⁶
	S (d)	³² S ⁻	≈ 0.7	35 M	2. 10 ¹⁵
	Se	⁸⁰ Se ⁻	0.1	30 M	1. 10 ¹⁴
Ga As	C	¹³ C ⁻	2.3 10 ⁻²	340 M	2. 10 ¹⁶ (c)
	Si	²⁸ Si ⁻	3.5 10 ⁻³	76 M	1. 10 ¹⁵
	S	³² S ⁻	0.5	740 M	2. 10 ¹⁵
	Se	⁸⁰ Se ⁻	0.3	20 M	6. 10 ¹³

(a) - Useful yields are calculated for the 150μm transfer optics with a 750μm field aperture

(b) - The background is mentioned according to the experimental conditions :

* 150μm transfer optics, ** 25μm transfer optics,

*** 150μm transfer optics + high resolution conditions.

(c) these detection limits must be multiplied by the reverse of the isotopic ratio.

(d) Sulfur is monitored with high resolution conditions, so the useful yield determination is not very accurate.

TABLE 1 - USEFUL YIELDS AND DETECTION LIMITS FOR NEGATIVE SECONDARY IONS

SUBSTRATE	ELEMENT	MONITORED ION	USEFUL YIELD (a)	BACKGROUND Cps (b)	DETECTION LIMIT ATOM-CM ⁻³
InP	C	⁴⁴ PC ⁻	0.2	75 M	2. 10 ¹⁴ (c)
	Si	⁵⁹ PSi ⁻	0.3	300 M	6. 10 ¹⁴
		²⁹ SiH ⁻ (e)	1.8 10 ⁻⁶	1 M	4. 10 ¹⁵
	O	⁴⁷ PO ⁻	3.5 10 ⁻²	3 380 M	5. 10 ¹⁶
	S	⁸² PS ⁻	-	-	-
	Se	⁸² Se H ₂ ⁻ (e)	7.9 10 ⁻³	4 M	8. 10 ¹⁴
GaAs	C	⁸⁸ As C ⁻	8.6 10 ⁻²	37 M	5. 10 ¹⁴ (c)
	Si	¹⁰³ As Si ⁻	0.2	500 M	2. 10 ¹⁵
	S	¹⁰⁷ As S ⁻	5.1 10 ⁻²	120 M	3. 10 ¹⁵
	Se	⁸² SeH ₂ ⁻ (e)	2.8 10 ⁻³	50 M	1. 10 ¹⁵
		¹¹¹ PSe ⁻	6.8 10 ⁻³	4 M	2. 10 ¹⁴

(a)-(b)-(c)-(d)-same remarks as in table 1

(e) - if the implanted ions are ⁸²Se H₂⁺, or ²⁹Si H⁺

TABLE 2 - USEFUL YIELDS AND DETECTION LIMITS FOR SOME MOLECULAR NEGATIVE IONS

After SIMS analyses the X-axes were calibrated by measuring the depths of the craters with a taly-step stylus type surface profiler. The SIMS technique, performed from implanted samples, allows to determine : $1/R_p$ and ΔR_p , the projected ranges and the straggling of the atomic distributions, respectively ; ii/the actual maximum concentrations and the background levels of the profiles ; iii/the useful yields of the analyses. The Y-axes were calibrated (in atom.cm^{-3}) by setting the integrals of the depth distributions equal to the implant fluences, accurate to within about 5% [6]. The useful ion yield is defined as the ratio of the number of collected ions, for the implant under investigation, respect to the atoms sputtered from the actual sampling area.

RESULTS AND DISCUSSION : The useful yields calculated either for monoatomic ions or for molecular ion species are displayed in table 1 and 2. The background levels, in counts per second, and the corresponding detection limits, in atom.cm^{-3} are also mentionned. The useful yields have been normalized for the same experimental conditions (150 μm transfer optics with a 750 μm field aperture) while the background levels are given without any correction. Carefull examination of table 1 and 2 results suggests the following remarks :

1 - The useful yields for the 4b-6b group elements (C, Si, O, S, Se) are very high, in the range 10^{-3} - 10^{-1} , several orders of magnitude higher than those calculated by D.P. LETA et al. [4] obtained with oxygen primary ions. Therefore, better sensitivities and, consequently, lower detection limits can be expected. Mono atomic useful yields (table 1) increase accordingly with electron affinities of the elements, except for carbon. The electron affinities are equal to 1.27, 1.39, 1.46, 2.02, 2.07 for C, Si, O, Se, S, respectively. In the case of carbon it can be suspected that the useful yield calculation is not accurate due to the high background level registered ; the same remark holds for oxygen.

2 - The useful yields calculated for molecular ions are also very high (table 2), even higher for PC^- and PSi^- , AsC^- and AsSi^- than those found for the corresponding single atomic ions. The molecular 4b group elements have higher useful yields than the 6b group elements. As a consequence, these molecular ions can be monitored to depth profiling analyses ; they don't correspond to higher background levels and allow as low detection limits as monoatomic ions. This case is well illustrated by carbon whose the detection limit is in the range of 2.10^{16} atom.cm^{-3} when $^{44}\text{PC}^-$ ions are monitored.

3 - At least for monoatomic ions the useful yields obtained from GaAs substrates are higher than those calculated for InP. This result is in agreement with V.R. DELINE et al. measurements [6] who described a general matrix effect.

Finally, as mentionned in table 1 and 2, the detection limits, or the background levels for C, Si, O, S, Se in InP are equal to 2.10^{16} ($2.10^{14} \times 100/1.107$), 6.10^{14} , 5.10^{16} , 2.10^{15} , 1.10^{14} , respectively. These limits are two or three orders of magnitude lower than previous data [4]. In GaAs, the limits are not markedly lower, except for Selenium. Besides the fact that the background level can be the actual residual impurity content, for such or such element, two other secondary effects can be encountered : i/interferences with matrix molecular ions or vacuum contaminants ; ii/contribution of secondary ions coming from other parts than the center of the craters (edge effects, neutral sputtering, secondary ion emission from the extractive lens).

The high dynamic range depth profiles are well fitted by the PEARSON IV model. Nevertheless the experimental four first moment values are significantly different from the theoretical ones.

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REPRODUCIBILITY OF FAB SPECTRA OF PHARMACEUTICALS; D.V. BOWEN,
L.A. BROAD, J. THORPE; Analytical Chemistry Department, Pfizer Central
Research, Sandwich, Kent, CT13 9NJ, UK.

Objectives:

1. To test the reproducibility of the Fast Atom Bombardment mass spectra of pharmaceuticals.
2. To explore the potential application of FAB to the routine analysis of bulk pharmaceuticals.

Introduction:

Are Fast Atom Bombardment mass spectra reproducible enough for routine qualitative use? In routine pharmaceutical analysis it is difficult to keep constant conditions either within a lab. or between laboratories. We have varied:

1. The mass spectrometer/data system.
2. The operator.
3. The liquid matrix.
4. The sample concentration.

Experimental:

Five compounds were examined by FAB (or related techniques) on up to eight different instruments.

The FAB probes were made of copper or stainless steel. In most cases glycerol was used as the liquid matrix. The resolution was unit mass (for quadrupoles) or about 1000 to 3000 (for double focussing instruments). The FAB sources were all operated at or near room temperature. Magnetic sector instruments were operated at their maximum accelerating voltages for most runs.

Results:

Since parameters were not varied individually, it is not possible to say which parameters contributed most to the variations we observed.

The spectra of azalomycin were not reproducible from lab. to lab., but were reproducible within each lab.

The spectra of the other four compounds were reproducible enough for qualitative analysis.

AZALOMYCIN B

The results of FAB runs with azalomycin fell into two different groups. Six experiments did not show any peaks near the molecular weight, but had a series of intense peaks around m/z 537. Three experiments (one negative and two positive) did not show this series of peaks at m/z 537, but did have weak peaks in the molecular ion region.

Repeated introductions of azalomycin in the same matrix on the same mass spectrometer gave reproducible spectra.

However the spectra obtained on different mass spectrometers were not reproducible. It would not be possible to identify azalomycin reliably in a series of FAB experiments.

CEFALOGLYCIN

Six positive FAB experiments were in good agreement. The intensity of cefaloglycin peaks ($M + 1$ and $M + 1 - 60$) compared to glycerol peaks (notably $3G + 1$ AT m/z 277) varied widely, but these spectra would allow the identification of cefaloblycin. Two negative FAB experiments also gave similar spectra.

NEOMYCIN SULPHATE

Five positive FAB experiments gave intense spectra with strong M + H peaks and clusters of M with H₂SO₄. The relative intensity of the glycerol spectrum (e.g. G5 + H at m/z 461) varied widely. A sixth positive FAB run gave no useful spectrum at all. Three negative FAB runs gave weak spectra in which glycerol peaks were generally stronger than the peaks from neomycin. The spectra from the three runs were similar. With one exception, the FAB spectra of neomycin sulphate were reproducible and useful.

OLEANDOMYCIN⁹

Seven positive FAB experiments with oleandomycin gave strong spectra. The intensity of the major fragment (M + 1 - 144) varied widely, but the spectra were suitable for qualitative analysis. Three negative FAB experiments with oleandomycin also gave strong, reproducible spectra.

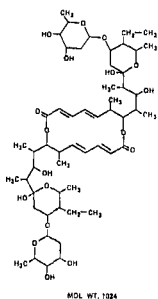
BENZYL PENICILLIN, SODIUM SALT

Six positive FAB runs all gave strong spectra. Most of the intensity was in the molecular ion region. The relative intensities of M + 1 and M + Na varied, but the spectra were suitable for qualitative analysis. Five negative FAB runs gave weaker spectra. These were also reproducible enough for qualitative analysis.

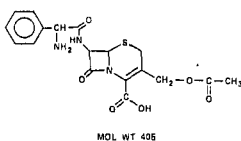
Conclusions:

The FAB spectra of four of these compounds are sufficiently reproducible for qualitative analysis. As azalomycin shows, some compounds that give strong FAB spectra are not reproducible from lab. to lab. This implies that routine application of FAB to unknowns or to new compound classes will require care in the selection of the matrix, and may be affected by the mass spectrometer used.

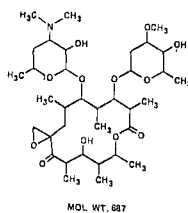
AZALOMYCIN B



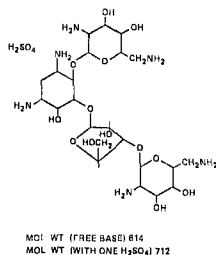
CEFALOGLYCIN



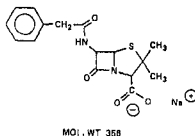
OLEANDOMYCIN



NEOMYCIN B SULPHATE



SODIUM BENZYL PENICILLIN



TPA 14 | FAB MASS SPECTRA OF HIGH MOLECULAR WEIGHT
COMPOUNDS; L. Baczynskyj & D. J. Duchamp; Physical and
Analytical Chemistry Research, The Upjohn Company,
Kalamazoo, Michigan 49001.

The mass range of most mass spectrometers until very recently was limited to 1,000 or 1,500 daltons. In the case of sector instruments this range could be extended to 2,000 or 3,000 by lowering the accelerating voltage with some loss of sensitivity. The recent advent of soft ionization techniques (FD, PD and especially FAB) as well as the introduction of high field magnets allows us to record mass spectra above 1,500 daltons. This new capability has prompted us to change our data acquisition and data processing system to record mass spectra up to mass 6,000. The problems of finding the appropriate compounds for calibration of the mass scale will be presented. Our new scheme allows various marker compounds to be combined to form a single calibration curve. In addition the presentation of mass spectra, which have ions above 1,500, cause additional complications. Finally the storage of such large mass spectra in our mass spectrometry library has caused some problems. Examples of how these problems were solved in our UPACS-II data system will be presented.

TPB 8 | MICROCOMPUTER-BASED FOURIER TRANSFORM MASS
SPECTROMETER; T. J. Buckley, S. Tehrani, R. T. Su, R. R.
Weller, and J. R. Eyler; Department of Chemistry, University
of Florida, Gainesville, Florida 32611.

We have developed a microcomputer-based Fourier transform ion cyclotron resonance mass spectrometric detection system utilizing an Apple II and a KIM I microcomputer in a master-slave configuration (1). Recent experimental work has been devoted to increasing the speed of transformation by: 1) use of a fast floating point processor board, 2) use of a BASIC compiler, and 3) use of a 6502 machine code fast Fourier transform routine. Results of these and several other system improvements will be presented. While our instrument can study only a limited mass range and a relatively small number of data points (when compared to commercial instruments) it can still be applied to the solution of a number of interesting problems. Initial results will be presented for the detection of trace quantities of one specie in the presence of much larger quantities of another specie of similar mass.

1. R.J. Doyle, Jr., T.J. Buckley, and J.R. Eyler, Lecture Notes in Chemistry, in press; 28th ASMS Conference, paper TPMP11.

MIDDLE MOLECULE MOLECULAR WEIGHT DETERMINATION AT VARIOUS RESOLUTIONS: EXPERIMENTAL AND THEORETICAL CONSIDERATIONS; J.A.Vergey, D.Heller, R.J.Cotter and C.Fenselau, Middle Atlantic Mass Spectrometry Facility, Johns Hopkins School of Medicine, Baltimore, MD 21205.

INTRODUCTION

Mass spectrometric analysis of middle molecules, with molecular weights between 1,000 and 10,000 daltons, yields molecular ion isotopic distributions which are quite different theoretically and experimentally from isotopic distributions for smaller molecules. New approaches are needed to obtain molecular weights from spectra in which monoisotopic mass ions have become vanishingly small, and a multiplicity of isotopic isobars contribute to peaks at each nominal mass.

Theoretical and experimental aspects of using monoisotopic and average masses to designate molecular weights have been examined for a number of middle molecular weight compounds. Sensitivity, accuracy and precision have been studied as a function of resolution, to assess the utility of average and monoisotopic mass determinations.

EXPERIMENTAL

Experiments were carried out using a Kratos MS-50 mass spectrometer, equipped with a 23 kGauss high-field magnet and a fast atom bombardment source. Five commercially available peptides were examined, as well as clusters of an alkyl-sulfonate and a perfluoro-alkyl sulfonate. Samples were dissolved in an appropriate volatile solvent followed by solvation in the non-volatile liquid matrix. Approximately 1 μ l of sample, containing on the order of 1-5 μ g of sample, was deposited on the 3 mm diameter copper tipped sample probe, and bombarded with 8 kV xenon atoms.

Data were acquired using the Kratos DS-55 data system by narrow scanning the molecular ion region of each sample at 300 ppm/sec. Mass assignments were made by acquiring additional scans with the accelerating voltage peak switched to the bracketing C_8I_2 clusters. Data were processed using in-house programs for manipulating uncentroided data.

Experimental and theoretical data are presented for each sample at unit resolution and low resolution ($R=1500$). Intensities are presented in arbitrary units, but can be compared for unit vs. low resolution for a given sample, since data were acquired under identical experimental conditions.

RESULTS AND DISCUSSION

Upon examining the experimental and theoretical data, including that illustrated in Figures 1-2, a number of observations can be made concerning the designation of monoisotopic masses in the middle molecule mass range:

- 1) Typical organic compounds above m/z 3000 will probably not produce an easily observable monoisotopic mass ion when ionized by techniques such as FAB.
- 2) Compounds which contain a high proportion of anisotopic elements can produce observable monoisotopic mass ions throughout the middle molecule region.
- 3) Using theoretical distributions may not be helpful in assigning monoisotopic masses from unit resolution data in the middle molecule region, due to a combination of effects observed in FAB type ionization mechanisms, such as peaks immediately below the molecular ion cluster, and solution chemistry problems like partial disulfide bond reduction.

Average mass calculations for the experimental and theoretical data suggests that:

- 1) Accuracy of average molecular weight determinations are quite good at both high and low resolutions, with the exception of the first sample, which apparently underwent partial disulfide bond reduction.
- 2) Precision, as determined for the porcine insulin sample, was better for the low resolution case as might be expected from a statistical standpoint.
- 3) Absolute intensities can be expected to be far larger for low resolution data, therefore making it both easier to observe peaks and to assign average masses.

CONCLUSIONS

A comparison of experimental and theoretical mass spectrometric data for a variety of compounds with molecular weights ranging from 3400 to 8100 daltons led to the following conclusions concerning molecular weight determinations in the middle molecule mass range:

- 1) Most compounds will not produce readily delineated monoisotopic mass ions, even at unit resolution, unless the compound contains a high proportion of anisotopic elements.
- 2) Theoretical distributions may not be useful for assigning monoisotopic masses, due to complicating effects observed for ionization mechanisms like FAB.
- 3) Average masses can be determined equally well at both high and low resolutions. However, better precision and sensitivity can be expected for low resolution determinations.

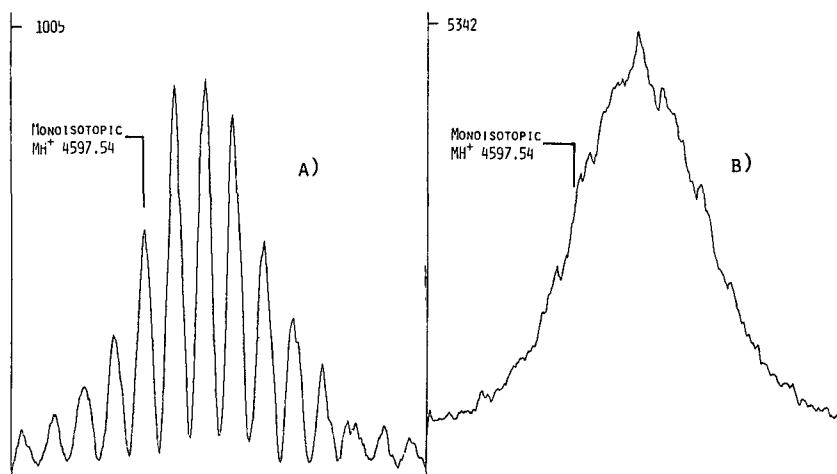


FIGURE 1: SAUVAGINE A) UNIT RES.
B) $R=1500$ C) SINGLE LETTER AMINO
ACID CODE

C) $\text{Pyr G P P I S I D L S L E L L}$
 $\text{[J Q K E K E Q K E I E I H K R]}$
 $\text{[A A H N R L L L D T I H H]}$

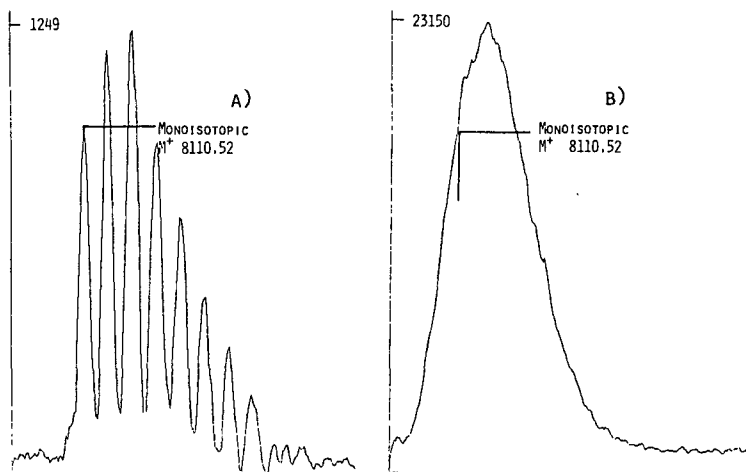


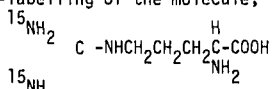
FIGURE 2: CESIUM PERFLUOROHXYLSULFONATE $N=15$ ($\text{C}_6\text{F}_{13}\text{SO}_3\text{Cs}$) $_{15}\text{Cs}$
A) UNIT RESOLUTION B) RESOLUTION= 1500

A ¹⁵N-LABELLING/FD/FAB STUDY OF THE LOSS
OF AMMONIA FROM PROTONATED ARGININE^x

J.J. Zwinselman¹, N.M.M. Nibbering¹, J. van der Greef² and M.C. Ten Noever de Brauw²

¹Laboratory of Organic Chemistry, University of Amsterdam, Nieuwe Achtergracht 129, 1018 Amsterdam and ²Central Institute for Nutrition and Food Research CIVO-TNO, Utrechtseweg 48, 3704 HE Zeist, The Netherlands.

The positive ion FD spectrum of arginine, taken at an emitter current of 15 mA, exhibits not only a peak due to (M+H)⁺ ions, but also a peak corresponding to (M+H-NH₃)⁺ ions. Specific ¹⁵N-labelling of the molecule, i.e.

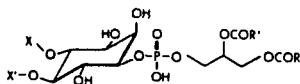


shows that the eliminated NH₃ molecule exclusively contains one of the terminal N-atoms of the guanidine group. This is true both for the (M+H)⁺ ions which decompose at the emitter and for the metastable decomposing (M+H)⁺ ions. The positive ion FAB spectrum of the ¹⁵N-labelled molecule contains peaks due to (M+H-¹⁵NH₂)⁺ and (M+H-NH₃)⁺ ions in the ratio of ~1.3. However, the metastable decomposing (M+H)⁺ ions generated by FAB eliminate exclusively ¹⁵NH₃, as observed in FD which shows that the loss of unlabelled NH₃ is the higher energy demanding process. The mechanisms of these eliminations will be presented. The negative ion FD and FAB spectra of arginine exhibit only a peak corresponding to the (M-H)⁻ ions.

^xOrg. Mass Spectrom., to be submitted.

THE FAB ANALYSIS OF PHOSPHOINOSITIDES ON A TRIPLE SECTOR INSTRUMENT,
W. R. Sherman, Department of Psychiatry, Washington University School
of Medicine, St. Louis, MO 63110 and R. H. Bateman, B. N. Green and
I. Lewis, VG Analytical Ltd., Tudor Road, Altrincham, Cheshire, England

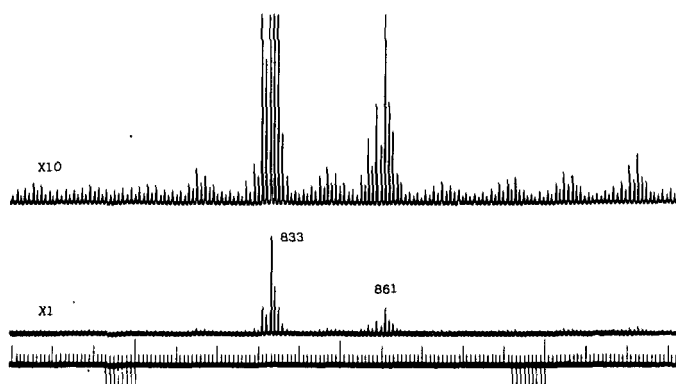
The phosphoinositides are phospholipids comprised of three parent species: phosphatidylinositol (PI), diphosphoinositide (DPI) and triphosphoinositide (TPI). Each of these lipids is heterogeneous in fatty ester composition where R' is predominantly made up of CIS-methylene interrupted unsaturated species and R is predominantly saturated species. Chain lengths in each instance vary, the composition being characteristic of the tissue source. Important information that is not easily obtained is the composition of each molecular species, i.e. the fatty acid mix of each of the phospholipid diesters. An important example of a metabolically active species of PI is the stearyl arachidonoyl lipid which, on stimulation in many tissues, releases free arachidonic acid, a precursor of prostanoid substances.



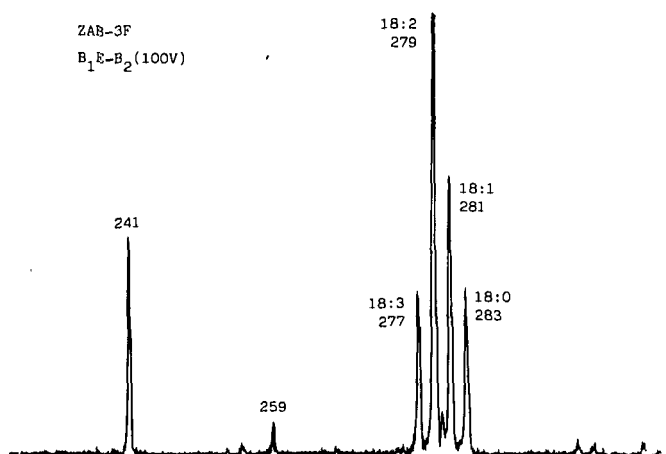
PI: X, X'=H; DPI: X = PO₃H₂, X'=H; TPI: X, X' = PO₃H₂

There is a need to accurately determine the distribution of the molecular species of these lipids in tissues, for example following stimulation by a specific agonist. Negative ion fast atom bombardment can provide a route to this information but only when using a triple sector instrument, MIKES having insufficient daughter ion resolution to separate isobaric species differing in their fatty ester composition. For example, in the case of soybean phosphatidylinositol, the spectrum shown in the first illustration was obtained from a total of 10 micrograms of the mixture dissolved in glycerol. The (M-H)⁻ ion at m/e 859 represents about 0.5 microgram of lipid. The fatty acid carboxylate ions obtained following collision induced dissociation and MIKES analysis were unresolved. When subsequently analysed on a VG ZAB-3F, a triple sector instrument of BEB configuration, it was found that there were two isobaric species consisting of 18:0, 18:3 and 18:1, 18:2. This is shown in the second illustration. Comparison of the total fatty acid composition of the soybean PI by triple sector analysis and by gas chromatography were in qualitative agreement.

DPI, TPI, lysophosphatidylinositol and phosphatidic acid are also amenable to analysis by FAB.



Molecular ion region of soybean phosphatidylinositol by negative ion FAB.



Daughter ion spectrum from mass 859 in soybean PI showing fatty acid fragments resolved. Ion energy in collision region = 100V.

ENZYME KINETICS BY FAB

Laurence A. Smith and Richard M. Caprioli

Analytical Chemistry Center and Department of Biochemistry and Molecular Biology, The University of Texas Medical School, Houston, Texas 77030

The development of fast atom bombardment (FAB) as a method for analysis of ionic and non-volatile compounds has led to a variety of applications in the biological sciences. These applications are possible due to the ability of FABMS to detect polar molecules without the necessity of chemical modification. In addition, FAB has the unique characteristic of employing a liquid phase (i.e. glycerol, water) containing the sample molecules, instead of the thin solid films utilized in other techniques. Recently, we have demonstrated that these characteristics, especially that of sample suspension in a liquid phase, can be used advantageously in maintaining enzymatic activity and that on-going reactions can be followed in real-time inside a FABMS by applying intermittent scanning to show progressive increase in the intensity of the $(M+H)^+$ ion of product(s) along with subsequent decrease in the $(M+H)^+$ ion of the substrate. Moreover, several examples demonstrating this monitoring technique have been shown for a variety of enzymes on their assay substrate and several peptides.

Since the completion of these experiments, we have addressed several questions concerning the kinetic behavior of enzyme reactions occurring during real-time monitoring such as determining if an enzyme can maintain a linear rate under FABMS conditions and how do FABMS monitored reaction rates compare to rates determined by established classical techniques. Results are presented for several studies in which trypsin and its assay substrate, tosyl-arginine methyl ester (TAME) have been utilized as an enzyme-substrate reaction model to answer these questions and to characterize reaction behavior under FABMS conditions.

Results from these studies show that several effects on the reaction system monitored by FABMS are evident. Effects such as response saturation of sample at high concentrations and reaction rate changes due to use of glycerol in the assay buffer are apparent. In addition, trypsin reactions monitored in real-time by FABMS exhibit slower rates (5% to 10%) when compared to rates determined spectrophotometrically in the same percent glycerol and same concentration of enzyme. These slower rates have been found to be equal to or less than rates determined spectrophotometrically in 90% glycerol and are likely due to evaporation of water from the sample matrix under vacuum (10^{-5} torr) resulting in an increase in glycerol. Approximate initial rates can be determined by real-time monitoring if the reaction rate is read at 0.3 minutes or less from a graph of $\mu\text{Mole TA Produced vs. Time}$. In order to see significant changes in substrate/product ions with time, however, real-time reaction monitoring requires approximately ten times more enzyme than the amount of enzyme used under spectrophotometric assay conditions.

On the other hand, accurate initial reaction rates using spectrophotometric assay conditions can be determined by FABMS if aliquots are taken at timed intervals from the reaction mixture and scanned. These experimental results obtained through the use of trypsin and TAME as an enzyme-substrate reaction model indicate that special considerations should be taken into account when exploring the possibility of monitoring a particular reaction by FABMS. These considerations are important aspects in developing an assay system using FABMS monitoring, especially since FAB could offer an alternative to following reactions using true substrates that can not be detected by other classical techniques.

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2. Williams, D.H., Bradley, C., Bojesen, G., Santikarn, S. and Taylor, L.C.E., J. Am. Chem. Soc., 103, 5700 (1981).
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ROUTINE FAB/MS OF BIOLOGICAL COMPOUNDS TO M/Z 2000
 Ian Jardine and Gale Scanlan
 Dept. of Pharmacology, Mayo Foundation, Rochester, MN 55905

Cesium iodide (CsI) in glycerol has been used to effect routine computer calibration of our Kratos MS-50 mass spectrometer to m/z 2000 at 6KV for FAB-MS (e.g., Fig. 1). We have thus recorded the FAB mass spectra of a number of biological compounds in this mass range. For example, Fig. 2 illustrates the computer recorded positive ion FAB mass spectrum from m/z 400-1800 of a synthetic eighteen amino acid peptide (obtained from Dr. Z. Atassi, Mayo Foundation) illustrating only those structurally significant ions which are clearly definable in the spectrum. The negative ion FAB mass spectrum of the molecular weight region of the sulfated octapeptide, cholecystokinin is shown in Fig. 3. This spectrum was obtained on 2 µg of material isolated from human brain for the first time by Dr. L. Miller of the Mayo Foundation. Structurally significant fragment ions were not readily apparent. Fig. 4 illustrates the positive ion FAB mass spectrum of a mixture of three underivatized mannose oligosaccharides (obtained from Dr. C. C. Sweeley, Michigan State University). Obvious fragment ions are noted. Useful ions were not readily apparent below m/z 1000. In contrast, the negative ion FAB mass spectra of complex glycolipids (obtained from Dr. C. C. Sweeley) (Figs. 5 and 6) produced a considerable amount of structural information.

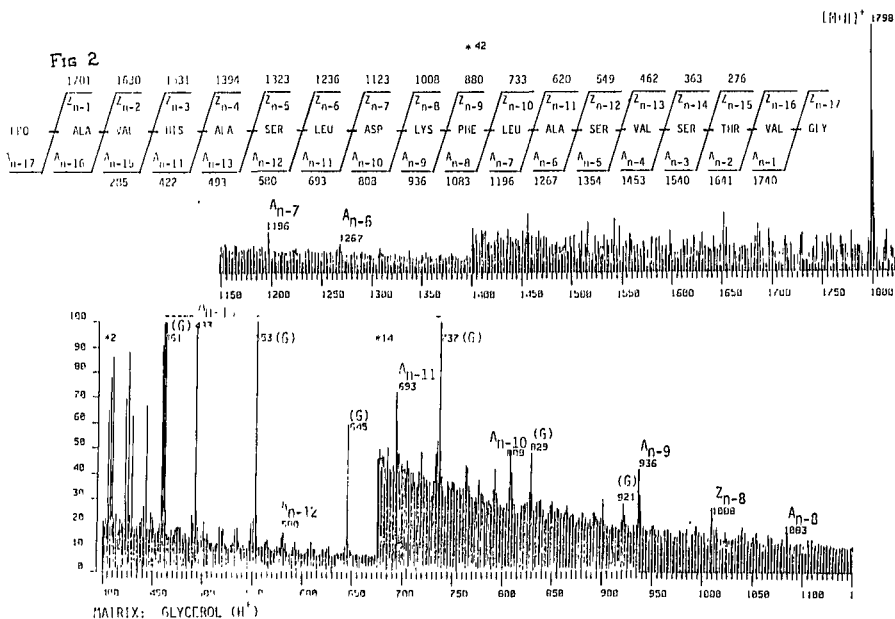
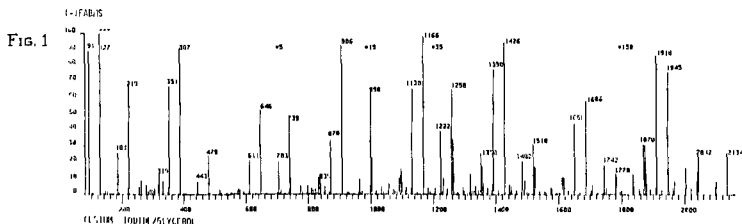
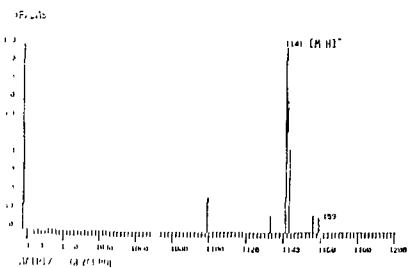


Fig 3



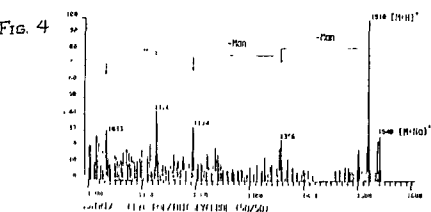
ASP-1YR(CO₂H)-HET-GLY-TRP-HET-ASP-PHE-III₂

m/z 1142

FORMIC ACID/ACETIC ACID

1500-1500 1500-1500 1500-1500

Fig. 4



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Fig. 5

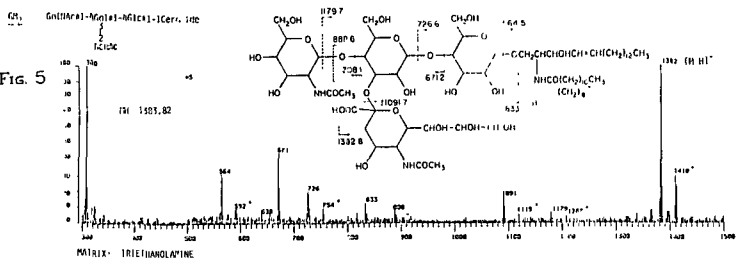
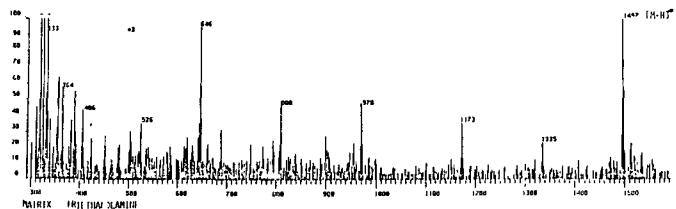
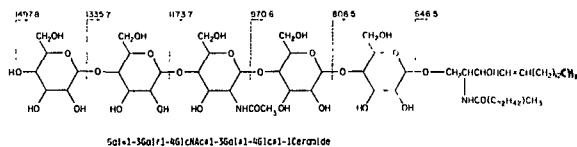


Fig 6

Blood Group B GL-5
M 1078,77



Characterization and Analysis of Acylcarnitines by FAB and B/E Linked Scanning: Application to Metabolic Disease. Millington, D.S., Roe, C.R., and Maltby, D.A. Duke University Medical Center, Division of Pediatric Metabolism, Durham, North Carolina 27710.

Acylcarnitines are an important group of metabolites that have thus far eluded characterization by mass spectrometry. In the FAB source, they exhibit very intense spectra (glycerol matrix) dominated by MH ions and fragments whose elemental compositions, summarized in Figure 1(a), were confirmed by accurate mass measurements. The spectrum of propionylcarnitine, Figure 1(b), is typical. The MH ions are further characterized by metastable ion analysis using the linked scan at constant B/E ratio (Figure 1(c)). These daughter ion spectra exhibit prominent M-59 ions and signals at m/z 144 and 85.

Recently, oral administration of L-carnitine to patients with propionic acidemia, methylmalonic aciduria and Reye's Syndrome has resulted in clinical and metabolic improvement accompanied by a large elevation in urinary acylcarnitine excretion as shown by radioenzymatic assay (see Table).

The acylcarnitines in these post-carnitine urine samples, partially purified by ion exchange chromatography, were identified by FAB mass spectrometry using high resolution peak matching and B/E linked scanning to confirm the identities of the proposed MH ions. Thus, the dominant acylcarnitine in propionic acidemia and methylmalonic aciduria was shown to be propionylcarnitine; while in Reye's Syndrome acetylacarnitine (MH at m/z 204) was the major metabolite.

A method for quantification of acetylacarnitine and propionylcarnitine was developed, based on isotope dilution using synthetic $^2\text{H}_3$ -acetyl and $^2\text{H}_5$ -propionyl analogues as internal standards. These were added to the raw urine samples in known amounts, then detected with the unlabelled counterparts by FAB by selected ion monitoring at high resolution ($> 7,000$). Relative signal areas were computed and the amounts of unlabelled acylcarnitine determined by reference to standard curves.

Some of the values obtained are summarized in the Table and indicate levels of acylcarnitine that are greatly elevated over normal. These data support the contention that L-carnitine therapy should be beneficial in these and related metabolic disorders by excretion of toxic short-chain acids as acylcarnitines.

TABLE 1
URINARY VALUES FOR ACYLCARNITINES DETERMINED
BY ISOTOPE DILUTION

Sample	Propionyl C (FAB)	Acetyl-C (FAB)	Total Acyl-C (FAB)	Total Acyl-C (Radioenzymatic)
MA-1	3.89*	—	3.89	3.08
MA-2	2.39	—	2.39	2.58
PA	5.24	2.47	7.71	8.47
RS-1	0.26	6.83	7.09	5.44
RS-2	0.12	3.72	3.84	4.48
Normal:	ND	ND	ND	0.07 $\pm$ 0.04

* Values are expressed as $\mu\text{mol/mg creatinine}$
ND = non detected

FIGURE 1(a)

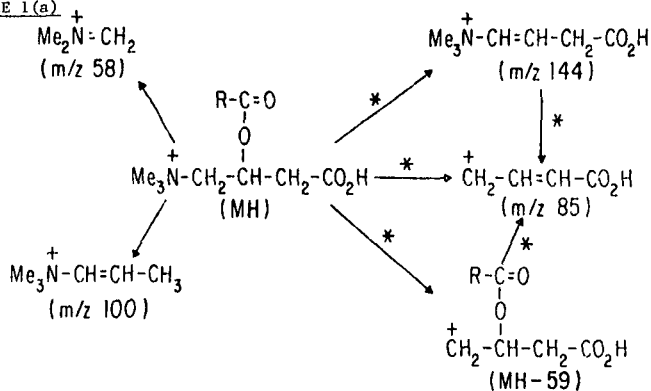


FIGURE 1(b)

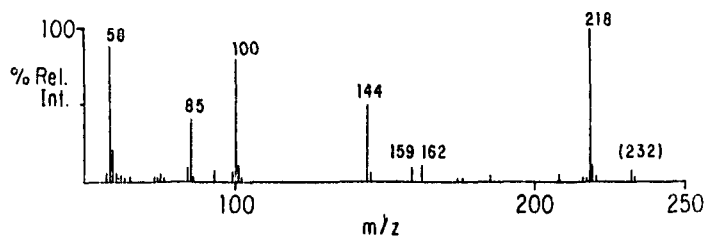
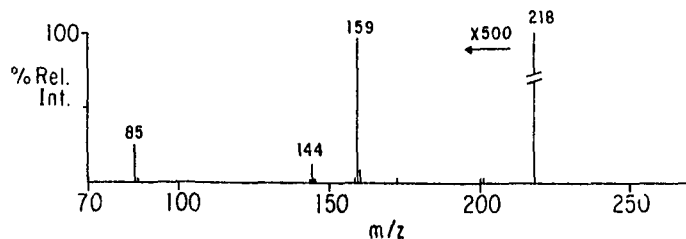


FIGURE 1(c)



MASS SPECTRAL CHARACTERIZATION OF MURAMYL PEPTIDES

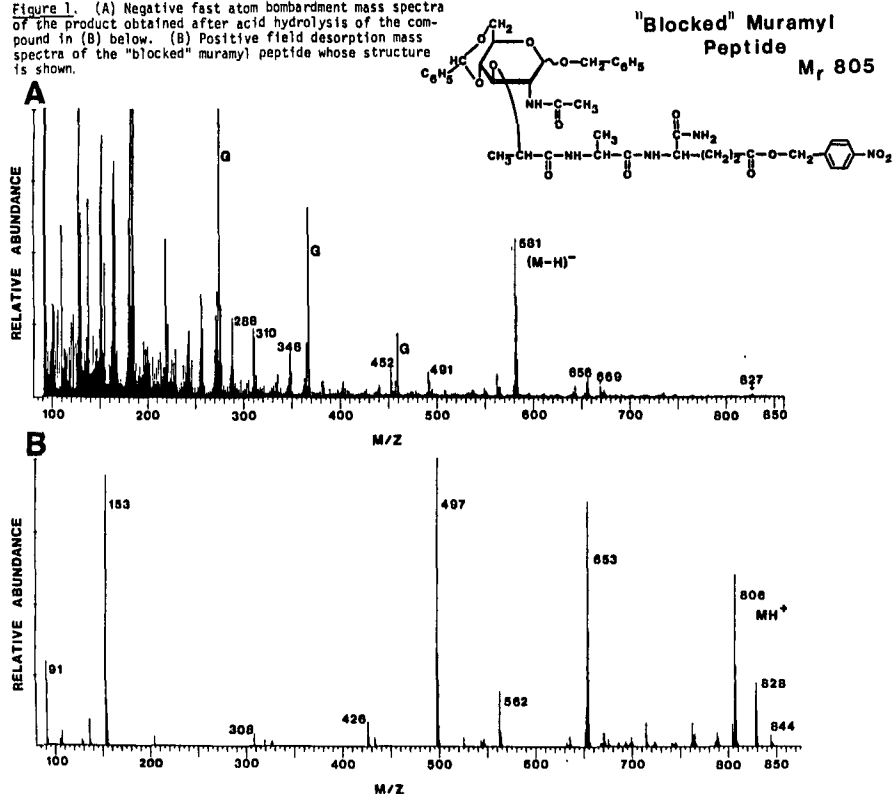
S.A. Martin, C.E. Costello and K. Biemann

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Muramyl peptides are the subject of much current interest among biochemists and physiologists because of their long-known immunoadjuvant activity and their more recently-recognized somnogenic effects. Because the type and extent of their biological activities vary with structure, it is important to establish the latter in detail. In order to explore the most effective mass spectral approaches to these structural determinations, we have examined the fast atom bombardment (FAB) mass spectra of 39 compounds which were muramyl peptides and their derivatives or represented the carbohydrate or peptide substructures. Field desorption (FD) mass spectra of many of these were obtained also. Mass shifts observed upon acetylation or methylation have been useful indicators of the number and type of reactive sites. In cases of possible ambiguity, exact mass measurements have been employed to verify assignments.

Figure 1(A) is the (-)FAB mass spectrum, measured in DMSO/glycerol (1:1), for the product obtained after acid hydrolysis of the sample whose mass spectrum is shown in Figure 1(B). The hydrolysis conditions were chosen to remove the *p*-nitrobenzyl ester and the 4,6-*O*-benzyl ether. They also caused some conversion of the amide group to the corresponding acid, (M-H)⁻ at *m/z* 582. Re-esterification with glycerol, probably catalyzed by the acid remaining in the

Figure 1. (A) Negative fast atom bombardment mass spectra of the product obtained after acid hydrolysis of the compound in (B) below. (B) Positive field desorption mass spectra of the "blocked" muramyl peptide whose structure is shown.



sample, is indicated by the peaks observed at m/z 656/7. Some of the hydrolyzed sample is present as the dibenzyl ether, $(M-H)^-$ at m/z 669. A peak of low abundance which appears at m/z 827, may correspond to the $(M + Na - 2H)^-$ of the dibenzyl ether *p*-nitrobenzyl ester in which the amide group has been converted to the acid. Figure 1(B) is the FD mass spectrum of a "blocked" muramyl peptide in DMSO. BAT 22 mA. Neither the positive nor negative FAB spectrum of this sample showed a molecular-weight-related ion and both positive and negative FAB spectra were rather unsatisfactory. When the unconjugated peptide was analyzed as a *p*-nitrobenzyl ester, it too gave an excellent FD spectrum but relatively poor FAB data. Hydrolysis of these esters in 0.1N HCl (4 hrs, 55 deg C) gave the free acid, for which good FAB spectra could be obtained (Figure 1a). Figure 2 and the accompanying structure represent the positive and negative FAB mass spectra of an immunoadjuvant muramyl peptide, M_r 1142. Finally, a muramyl peptide, M_r 1008 (not shown) whose structure required confirmation was derivatized with 3N HCl/MeOH for 30 minutes and acetic anhydride/pyridine (2:1) for 45 minutes at room temperature to identify functional groups and aid in sequence determination. After methylation the MH^+ ion at m/z 1023 is the methyl ester and indicates the major component has a single acid functionality. A peak at m/z 1038 corresponds to the dimethyl ester of a minor component which has two acid functionalities. The acetylated product of the methyl ester has a MH^+ ion at m/z 1275 which indicates there are six OH and/or basic NH moieties. Furthermore, water loss from the molecular ion is significant in the underivatized muramyl peptide and its methyl ester while the loss of an acetic acid moiety from Cl of muramic acid is the base peak, m/z 1215, in the molecular ion region of the acetylated product.

The samples used in this study were furnished by: M. Karnovsky and J. Pappenheimer, Harvard Medical School; J. Kreuger, The Chicago Medical School; and C.M. Gupta, Central Drug Research Institute, Lucknow, India. The M.I.T. Mass Spectrometry Facility is supported by Grant No. RR00317 from the NIH Division of Research Resources.

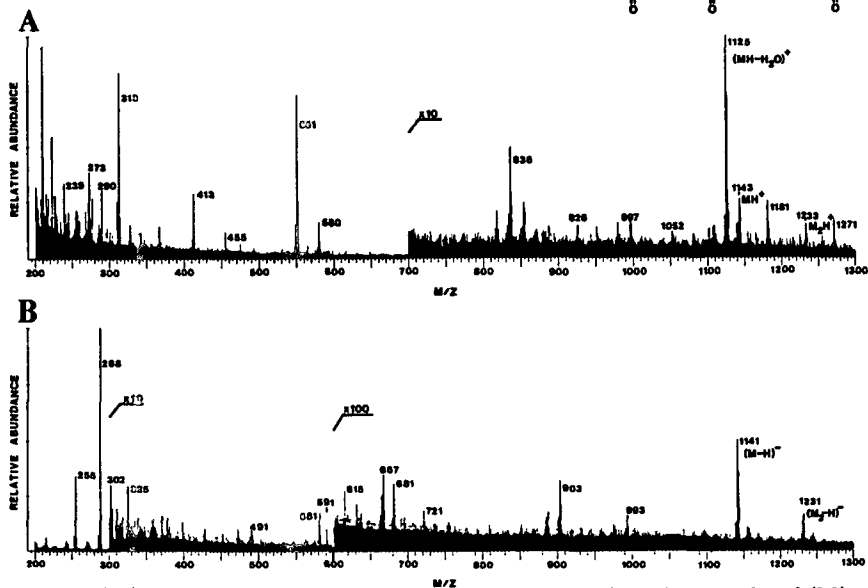
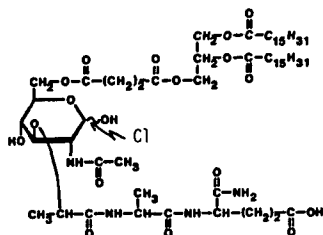


Figure 2. (+/-) Fast atom bombardment mass spectra of an immunoadjuvant muramyl peptide in DMSO/glycerol (1:1). Sample contains some of the analog having a Cl-O-benzyl ether, M_r 1232 (M_2). (A) Positive-ion mode FAB spectrum. MH^+ ions at m/z 1143 and 1233 are accompanied by $(M + K)^+$ at m/z 1181 and 1271, respectively. Base peak at high mass arises by loss of water (Cl-ether) from the molecular protonated ion. (B) Negative ion mode FAB spectrum. Water loss from molecular ion insignificant. Overall spectrum intensity greater than that obtained in positive-ion mode.

APPLICATIONS OF FAST ATOM BOMBARDMENT MASS SPECTROMETRY
IN NUCLEOSIDE AND NUCLEOTIDE STRUCTURE DETERMINATION

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Nucleosides which function as antimetabolites either alone or after anabolic activation comprise an important class of compounds in cancer chemotherapy. Several of these compounds are clinically useful antitumor agents while others are in varying stages of drug development. Structure determination of these nucleosides and their anabolites is thus important to delineating their mechanism of action and indispensable to any synthetic program seeking potential new antitumor drugs based on these models. An initial report of FAB MS suggested that it would be a very useful tool in the analysis of these polar molecules, since both molecular weight and structural information from fragmentation were available in spectra from nucleotides (1). We have applied both positive and negative ion FAB MS as a rapid and simple method for the analysis of synthetic and biologically-derived nucleosides and nucleotides.

Tiazofurin (NSC 286193, 2- β -D-ribofuranosylthiazole-4-carboxamide), a thiazole C-nucleoside which was synthesized as an analog of ribavirin, shows exceptional activity in the murine Lewis lung tumor model and has been assigned a high priority for clinical development by the NCI (2). Exposure of murine tumor cells to tiazofurin results in a depression of all purine nucleotides and concomitant inhibition of both DNA and RNA synthesis (3). A metabolite of tiazofurin which was a potent inhibitor of IMP dehydrogenase ($K_i=2 \times 10^{-7} M$) was isolated from murine P388 tumor extracts after drug treatment (4). Biochemical evidence suggested that this anabolite was a nucleotide pyrophosphate incorporating the elements of adenosine and tiazofurin. Both positive and negative ion FAB MS and high resolution FT-NMR were employed to demonstrate that this was indeed the case. The negative ion FAB spectrum (Figure 1) was especially diagnostic in indicating a dinucleotide structure in which the nicotinamide of NAD had been replaced by thiazole-4-carboxamide (i.e., TAD). In addition to a prominent M-H peak at m/z 668, two series of ions corresponding to cleavage of the phosphodiester linkage from either side of the molecule were present. These phosphate anions were observed to undergo dehydration, probably to cyclic phosphates, and minor cleavage of the sugar ring was noted (m/z 461, 468).

Chemical synthesis confirmed the structure of TAD since spectroscopic methods could not unequivocally demonstrate a 5'-5' diphosphate linkage (5). A synthetic program was also initiated to make more active analogs of TAD and to ascertain what effect base structure had on IMP dehydrogenase inhibition. Negative ion FAB MS was especially useful in this respect since it allowed a simple and rapid assessment of the structure and purity of synthetic nucleotides. Improper phosphorylation conditions or incomplete product isolation gave rise to the synthetic artifacts listed in Table 1. FAB MS was also useful for limited mixture analysis if the sample components were similar, being at least as indicative of mixture composition as HPLC analysis with UV detection at 254 nm. Figure 2 shows the negative ion FAB spectrum of the selenazole analog of TAD (SAD). It can be seen that the fragmentation pattern is quite similar to that of TAD and the presence of selenium in the M-H peak and the A series fragments is clearly indicated by its distinctive isotopic pattern. This dinucleotide is approximately 4X as potent an inhibitor of IMP dehydrogenase as TAD (6).

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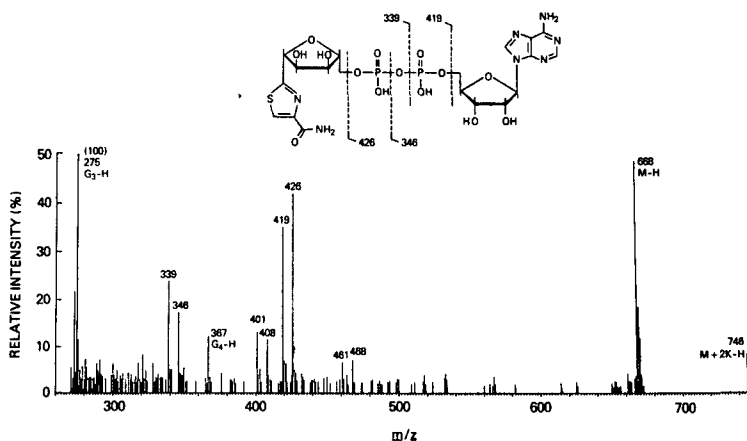


Figure 1. Negative ion FAB mass spectrum of biosynthetic TAD. Peaks designated with G correspond to glycerol adducts, e.g., G₃-H denotes the anion resulting from proton abstraction from the glycerol trimer.

Table 1. Artifacts of Nucleotide Synthesis Encountered in Negative Ion FAB

Component	Observed Masses	Comments
Cl ⁻	G _n +35,37	A,A+2 in a 3:1 ratio
H ₂ PO ₄ ⁻	G _n +97	
Na ⁺	G _n +21	
$\text{EtO}-\text{P}(\text{OH})_2$	M-H=125, G _n +125, M _n +125	
$(\text{EtO})_2-\text{P}-\text{OH}$	M-H=153, G _n +153, M _n +153	may form mixed dimers with other phosphates
carbonate	M-H+26, A _n +26	2',3'-carbonate formation with carbonyl diimidazole
isopropylidene	M-H+40, A _n +40	incomplete removal of 2',3'-isopropylidene

The abbreviations used are: G, glycerol; M, molecular weight; A_n, fragment ion.

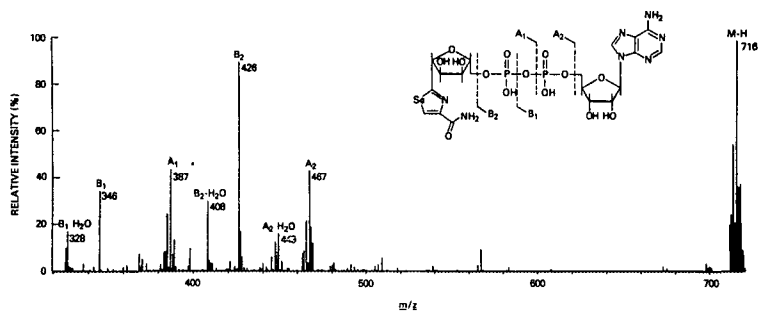


Figure 2. Negative ion FAB mass spectrum of synthetic SAD. The glycerol background has been computer subtracted and the above spectrum is the weighted average of five repetitive scans.

FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF SULFATED AND PHOSPHORYLATED GLYCOCONJUGATES.
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The sulfated glycosaminoglycans, acidic polysaccharides obtained by mild degradation of glycoproteins and proteoglycans, are widely distributed in connective tissues such as skin, collagen and bone. Speculation concerning the role of sulfate esters has ranged from simple augmentation of the anionic character of the macromolecule, which conveys emolliency, lubricity and water retaining capability, to more subtle physiological effects such as charge distribution and conformational changes that alter the cell surface and thus may affect antigen-antibody interaction. Sulfate and phosphate esters have also been found in glycosphingolipids, another important class of compounds found in high concentration in cell membranes.

Structural elucidation of sulfated or phosphorylated glycosaminoglycans and glycosphingolipids is hindered by irreversible degradation which occurs when conventional chemical or mass spectrometric methods are employed. FAB has previously been shown to be of value for structural analysis of neutral and acidic oligosaccharides and glycosphingolipids (GSL) (1). For these reasons we have now utilized fast atom bombardment mass spectrometry (FABMS) to analyze intact, underivatized sulfated and phosphorylated glycoconjugates.

The sulfated di- to octasaccharides yield intense cationized molecular ions and abundant fragment ions from which sugar sequence and sites of sulfation can be readily determined. These materials were prepared by hyaluronidase digestion and separated according to their degree of polymerization by gel permeation chromatography (2). The FAB mass spectra provide sequence specific fragments by cleavage at the glycosidic oxygen with charge retained on both the terminal and reducing ends of the molecule (e.g., Fig. 1-2). Other commonly observed fragment ions arise by the loss of $18u$ ($-H_2O$) and $120u$ ($-NaSO_3H$) from cationized parent and fragment ions. Since these oligomers are composed of repeating disaccharides, terminal or reducing-end fragments containing an odd number of sugar units have unique masses, whereas fragments with an even number of sugar residues are isobaric. Reduction with NaBD₄ shifts all ions containing the reducing end by $3u$ upward, thereby eliminating this mass redundancy and facilitating structure assignment. Our preliminary studies also indicate that the 4-O-sulfate and 6-O-sulfate structural isomers may be distinguished by (+) and (-) ion FABMS. This is because the 6-SO₄ isomer more readily loses H₂O from the reducing end fragment, R₁.

One unexpected result of this study was the observation of structural homologs of chondroitin sulfate that contain more than one covalently bound sulfate moiety per two sugar units. The additional sulfate is probably not randomly distributed in intact chondroitin, but enzymatic hydrolysis generates overlapping fragments of otherwise identical sequence which "scrambles" the apparent position of the sulfate. These "oversulfated" chondroitins are indicated by the cluster of ions $102u$ above the parent ions (particularly of the 6-SO₄ material) which corresponds to the replacement of an H with NaSO₃.

Thioglycerol is the preferred matrix for FAB analysis of sulfated sugars. The yield of sample-related ions is two- to three-fold greater for oligomers dissolved in thioglycerol and matrix related ions are reduced by approximately one-half. The overall result is a four- to six-fold increase in sensitivity. However, thioglycerol is more volatile than glycerol which shortens observation time and, since it is more susceptible to oxidation, the mass spectrum of the matrix may change with time.

Molecular ion abundances are enhanced in the (-) versus (+) ion FAB mass spectra of the sulfated oligosaccharides. In addition, matrix related ions and interferences from salt cluster ions (such as $Na_2SO_4(+Na)^+$) are substantially reduced in the (-) ion mode which also contributes to the apparently increased signal/noise ratio. The cation concentration also exerts a strong influence on the appearance of the mass spectra. The highest sensitivity and greatest abundance of MW-related ions are obtained when a molar equivalent of Na⁺ to carbohydrate-bound sulfate is present. In contrast, samples desalted by cation exchange show a two- to three-fold decrease in overall sensitivity. Furthermore, ions due to loss of sulfate from molecular and fragment ions are increased in the latter which may complicate interpretation of the mass spectra.

Previous work from this laboratory has demonstrated the utility of FABMS for the structural elucidation of phosphorylated GSL (3). We have now extended this work to the analysis of novel phosphorous-containing GSL's from shellfish. For example, the FAB mass spectrum of the novel compound 1-O-[6'-O-(N-methylaminoethylphosphonyl)Gal] ceramide (4) exhibits a series of (M+H)⁺ ions as well as fragments corresponding to the N-methylaminoethylphosphoryl Gal, and a series due to loss of the fatty-acyl moiety, (MH-RCO+H)⁺.

The results presented in this report clearly demonstrate the great potential of FABMS for the analysis of highly polar sulfated and phosphorylated glycoconjugates.

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- (4) Sample kindly provided by A. Hayashi, Kinki Univ., Osaka, Japan.

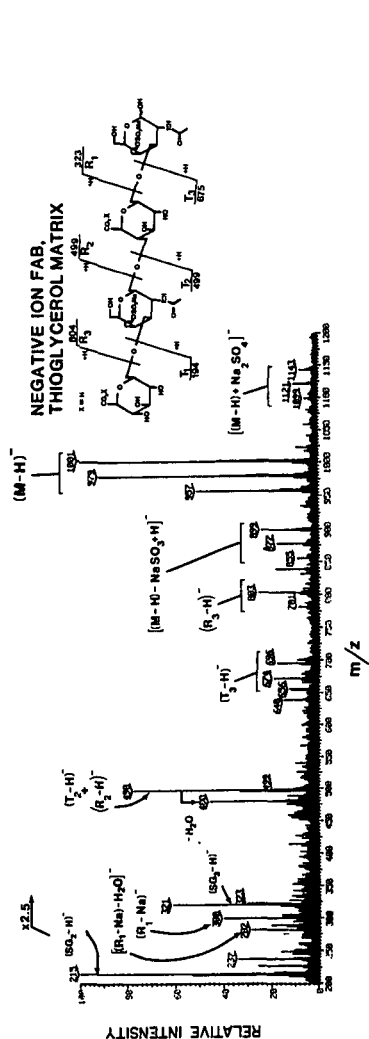


Fig. 1. Negative ion FAB mass spectrum of chondroitin 4-SO₄ tetrasaccharide, thioglycerol matrix.

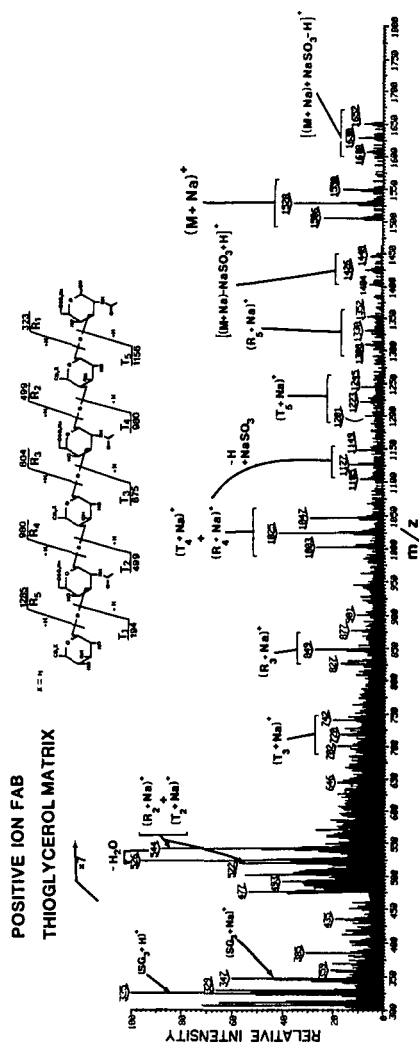


Fig. 2. Positive ion FAB mass spectrum of chondroitin 6-SO₄ hexasaccharide, thioglycerol matrix.

Analysis of Copper Complexes of Biological Interest by Fast Atom Bombardment Mass Spectrometry

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Copper-containing proteins serve vital functions in many biological systems. Hemocyanin is a binuclear copper protein involved in the transport of dioxygen in the tissues of arthropods and mollusks (1). Other copper proteins are important because of their ability to transport electrons in biological systems (2).

While the d^9 Cu(II) ion is accessible to a variety of spectroscopic techniques (for example EPR, optical spectroscopy, and Magnetic susceptibility) the spin-paired d^{10} configuration renders the study of Cu(I) complexes quite difficult, due to the spectroscopic invisibility of the Cu(I) ion. Other problems such as disproportionation in solution (especially aqueous systems) (3) and autooxidation in the presence of air (4) make the analysis of these complexes a difficult task.

Fast atom bombardment (FAB) (5) has been mostly applied to large polar molecules such as peptides and nucleosides. However, there has been some use of the technique for characterization of coordination complexes (6,7). Because of the problems associated with the analysis of copper complexes mentioned above, we were interested in applying FAB to a variety of copper complexes to investigate its effectiveness as a characterization tool.

A VG 7070 mass spectrometer equipped with a modified saddle-field ion source obtained from Ion Tech Ltd. to serve as the atom beam gun was used in this work. Xenon was used as the gas for the primary beam which was maintained at 1 mA at an energy of 4 to 8 keV. The stainless steel probe tip was covered with a small piece of aluminum foil to provide a clean surface for each sample and to minimize "memory effects" sometimes encountered in FAB. No problems were encountered with this procedure. Samples were either dissolved or suspended in glycerol. In cases where low solubility seemed to result in poor spectral quality, the sample was first dissolved in DMF. This proved quite effective.

Several 2-, 3-, 4-, and 5-coordinate copper(I) and copper(II) complexes involving both monodentate and multidentate ligands were investigated. Informative spectra were obtained for almost all samples. The existence of the two isotopes of copper (63 and 65) made assignment of copper containing ions quite easy.

Copper (I) Complexes

Complexes Containing Monodentate Ligands. Many two-coordinate monodentate ligand complexes such as bis-(N-methylimidazole)copper(I) tetrafluoroborate (BF_4^-) were studied. The spectra of these complexes were quite simple. The two major species present were the complex cation, CuL_2^+ , and the fragment CuL^+ obtained by simple cleavage of the copper-ligand interaction. Of interest were peaks which showed interaction of the complex with the glycerol matrix, such as attachment of a glycerol molecule to the two major ions. This effect was increased when DMF was used to dissolve the sample. No other peaks of reasonable intensity were observed.

Complexes Containing Multidentate Ligands. A three-coordinate complex, bis[2-(3,5-dimethyl-1-pyrazolyl)ethyl]ether copper(I) (BF_4^-), produced a FAB spectrum

consisting of the complex cation only. This is logical since the binding sites are in close proximity; the ether oxygen bridge serves as the third coordination site along with the two pyrazolyl nitrogens. The related tripod complexes tris[(3,5-dimethyl-1-pyrazolyl)ethyl]amine copper(I) and tris[(3,5-dimethyl-1-pyrazolyl)ethyl]methane copper(I) differ only in the apical position being either N or CH. Their FAB spectra display the complex cations but also contain several rearrangement fragment ions. These appear at m/z values differing by one mass unit between the two complexes. This indicates that the rearrangement mechanisms do not involve the apical position and probably originate by protonation of a nitrogen on one of the pyrazole groups.

Copper(II) Complexes

The copper(II) complex spectra did not display a molecular cation. The major ion seen corresponded to loss of one ligand, usually a monodentate group such as azide or a halide such as chloride. The spectra also included peaks due to rearrangement processes as seen for the copper(I) examples usually originating from protonated species. The analysis of bridged dimer species proved successful with the major peaks observed corresponding to the intact bridged species and the cleavage of the bond to the bridging ligand.

Exact Mass Measurement

By using the method of peak matching, exact mass measurements were made on copper-containing ions in the spectra. By mixing the sample with a small amount of a high molecular weight salt, such as tetraphenylarsonium chloride, strong reference ions could be produced and good results were obtained. The ion at m/z 249 in the spectrum of bis(3,5-dimethyl-N-benzylpyrazole) copper(I) (BF_4) was determined to have an exact mass of 249.050. The theoretical value for $\text{CuC}_{12}\text{H}_{16}\text{N}_2$ is 249.045, a difference of 5 millimass units. This accuracy is good enough to distinguish the formula from logical alternatives, such as $\text{CuC}_{13}\text{H}_{16}\text{N}$ (249.058), $\text{CuC}_{11}\text{H}_{12}\text{N}_3$ (249.033) and $\text{CuC}_{10}\text{H}_{10}\text{N}_4$ (249.020).

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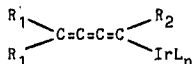
**FAB-MS of Iridium, Rhodium and Platinum Organometallic Complexes
Containing a Cumulene Ligand**

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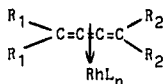
The potential of FAB-MS as a useful tool for the structure determination of nonvolatile compounds has prompted us to examine its application to the study of several classes of unusual organometallic compounds currently under study in our laboratory. The results reported here indicate that FAB-MS is the most useful method for structure determination short of X-ray crystallography, and the mass spectra obtained are more readily analyzed and potentially less ambiguous than various optical and magnetic spectra¹. The compounds reported here contain a variously substituted cumulene ligand attached to either iridium (via sigma-bonding, structure 1), rhodium (pi-bonding, structure 2a) or platinum (pi-bonding, structure 2b). In addition, spectral data are reported for Wilkinson's catalyst, Vaska's compound, the rhodium analog of Vaska's compound, and bis(triphenylphosphine)(ethylene)-platinum. These are synthetic precursors or analogs of the other complexes being studied. Several mass spectrometer systems have been used to collect the data: A KRATOS MS950⁴, a VG Micromass ZAB-2F with DS2035⁵, and a Varian MAT 731⁶. Argon (MS950) or xenon (ZAB-2F and MAT 731) fast atoms at 6-8 KV were used as the bombarding particles. Oscillographic chart recordings were made on the MS950 and MAT 731. Data system acquisition, using a mixture of RbI and CsI as mass calibrant, was used on the ZAB-2F. Only positive ion spectra at a resolving power of appx. 1000-3000 were recorded. The dispersing matrix used was the 18-crown-6 crown ether and tetraglyme mixture reported by Minard & Geoffroy². Expected isotope patterns were calculated by a program courtesy of Mr. J. Bill³, using polynomial expansions based upon natural abundances of the isotopes.

The spectra of Wilkinson's compound and rhodium Vaska's compound show very low intensity molecular ions. Minard & Geoffroy² indicate M⁺ for Wilkinson's cmpd., but we do not observe it. Our data indicate the Cl ligand is very labile. The base peak in the spectra is the Rh(P(C₆H₅)₂)⁺ ion. Vaska's cmpd., by contrast, shows M⁺ to be base peak, with (M-Cl)⁺ almost as intense. (M-CO)⁺ and Ir(P(C₆H₅)₂)⁺ are 30% and 70%, respectively. Spectra for the iridium-cumulene sigma-complexes show 0-10% M⁺. The most labile ligand here is the trifluoromethanesulfonate (triflate, OTf) ligand, which is subject to replacement by H₂O upon exposure to humid air for some of the compounds. The next most labile ligands are the CO and Cl. The cumulene ligand is lost as a single unit. The rhodium-cumulene pi-complexes show little or no M⁺, the Cl ligand being the most labile, as with Wilkinson's catalyst and rhodium Vaska's cmpd. The Rh(P(C₆H₅)₂)⁺ ion is used as base peak. The cumulene ligand is also lost here intact. The bis(triphenylphosphine)(ethylene)platinum spectrum shows 8% M⁺. Base peak is the Pt(P(C₆H₅)₂)⁺, analogous to that of the rhodium compounds. This compound is the only one of all compounds reported here which shows fragmentation of the organic ligand. A low intensity cluster (5%) corresponding to loss of a CH₂ unit from M⁺ is seen. It is not unreasonable to postulate a platinum-carbene ion, resulting from loss of CH₂, since many platinum-carbene complexes are known⁶. The structures for the platinum-cumulene pi-complexes are different from that of the rhodium complexes by other evidence, but the fragmentation patterns are similar. Base peak is the Pt(P(C₆H₅)₂)⁺ ion. M⁺ intensities are as high as 30%. The cumulene ligand is lost intact, as before.

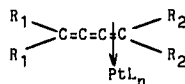
Isotope patterns were examined for all of the M⁺ and structurally significant ions. Comparisons of the observed patterns with expected patterns containing the multiple isotopes of germanium, tin, silicon, chlorine, iridium and platinum showed very good agreement. Comparison of spectra obtained from different instruments did not introduce significant difficulties. Our observations are consistent with a more careful study by Murphy & Clay⁷, which indicate that FAB spectra from different instruments will in general be as comparable as electron impact spectra. The 18-crown-6 crown ether matrix² has also proved to be an ideal matrix for these series of organometallic compounds.



1



2a



2b

Compound	Mol. Wt.	M ⁺	Significant Ion Clusters				
$\begin{array}{c} \text{PPh}_3 \\ \\ \text{PPh}_3\text{-Rh-PPh}_3 \\ \\ \text{Cl} \end{array}$	924	889, 16 14 10	662, 26 17 13 9	627, 100 56 22 11	Wilkinson's catalyst		
$\begin{array}{c} \text{CO} \\ \\ \text{PPh}_3\text{-Ir-PPh}_3 \\ \\ \text{Cl} \end{array}$	778	778, 55 79 100 59 46	743, 51 43 93 52 26	750, 17 31 30 41 25	713, 45 31 68 58 37	Vaska's Compound	
$\begin{array}{c} \text{CO} \\ \\ \text{PPh}_3\text{-Rh-PPh}_3 \\ \\ \text{Cl} \end{array}$	690	690, 13 9 7	665, 98 46 21	662, 44 20 22 11	627, 100 54 28		
$\begin{array}{c} \text{H} \\ \\ \text{Ad-C=C=C-Ir(PPh}_3)_2\text{COC10Tf} \end{array}$	1098	1098, 3 4 6 4 4 2	949, 35 25 76 42 34 15	778, 16 23 39 43 25 16	750, 6 9 13 13 6 6	743, 56 32 100 40 19 7	713, 19 18 33 30 14
$\begin{array}{c} \text{H}_3\text{C} \quad \text{C}=\text{C}=\text{C} \quad \text{CMe}_3 \\ \quad \quad \\ \text{H}_3\text{C} \quad \text{Rh(PPh}_3)_2\text{Cl} \end{array}$	824		789, 43 25 9 3	662, 45 19 18 7	627, 100 55 15		
$\begin{array}{c} \text{Ph} \quad \text{C}=\text{C}=\text{C} \quad \text{GeEt}_3 \\ \quad \quad \\ \text{Ph} \quad \text{Rh(PPh}_3)_2\text{Cl} \end{array}$	1022	1024, 4 3 4 3	987, 10 8 16 13 23 15	662, 45 20 19	627, 100 44 15		
$\begin{array}{c} \text{H}_2\text{C}=\text{CH}_2 \\ \\ \text{Pt(PPh}_3)_2 \end{array}$	742	745, 4 8 7 5 5 3	481, 4 7 13 8 15 9 11	717, 27 60 100 74 45 22			
$\begin{array}{c} \text{Ph} \quad \text{C}=\text{C}=\text{C} \quad \text{Ph} \\ \quad \quad \\ \text{Ph} \quad \text{Pt(PPh}_3)_2 \end{array}$	1074	1074, 3.5 7 7 6 4.5	813, 4 3.5 4 100 79	717, 25 66 100 79			

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DETERMINATION OF ACTIVE HYDROGEN IN AMINO ACIDS AND NUCLEOSIDES BY LIQUID IONIZATION MASS SPECTROMETRY

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Structure elucidation of a complex product is often aided considerably by knowledge of the number of active (or acidic) hydrogens which are O-H, N-H, N-H₂, S-H and COO-H, present in the molecule. The qualitative determination of active hydrogen is accomplished by equilibrating the sample with a labelled compound, such as D₂O and measuring the resulting change in molecular weight¹⁾.

This technique is applicable to involatile compounds by using liquid ionization (LI) mass spectrometry we have developed²⁾. The ion source of the LI mass spectrometer is at atmospheric pressure, providing easy sample handling. As the production of ions from a microgram scale of a sample like amino acid lasts generally for 2-5 minutes, a labelled compound can be introduced even when a mass spectrum of the sample is being measured. Besides, only a small amount (1-5 μ l) of D₂O is required.

As an example, a part of mass spectrum of alanine is shown in Fig. 1. At first, the mass spectrum around the MH⁺ peak (m/z 90) was being recorded repeatedly (one scan per 30 seconds) using about 1 μ g of alanine (10⁻¹ M aqueous solution) without D₂O. Then 1 μ l of D₂O was deposited on the glass wall near the port for sample introduction inside the ion source. Soon after the D₂O introduction, the mass spectrum changed as shown in Fig. 1. The increase in the number of peaks and in their mass number (91 to 94) indicates that three hydrogens in the molecule are replaced (MH⁺ (90) $\rightarrow$ MD⁺-d₃ (94)) by deuterium atoms. This number, 3, just corresponds to the N-H₂ and COO-H. D,L-alanine, β -alanine, proline, valine, serine, threonine, cysteine and lysine were examined by the same procedure and showed the expected changes in the mass number for each MH⁺ ion. In the case of lysine, one small extra peak was observed at m/z 154 as shown in Fig. 2, but the peak was small enough to distinguish the number of active hydrogens. These results suggest that the number of active hydrogens for most amino acids would be measured by this technique with D₂O.

In the case of adenosine and citydine, however, one extra peak was clearly observed as shown in Fig. 3 for adenosine. Adenosine gives very stable MH⁺ ion peak at m/z 268 with no fragment ion by this method (LI). The MH⁺ ions were observed by the same technique described for amino acids. Results obtained with 5 μ l of D₂O (Fig. 3) indicate that the mass number of protonated molecules (MH⁺) increases up to 275 and later it goes up to 278 (Fig. 3a). The peak height at m/z 275 is lower than that at m/z 274, but relatively intense compared with the peaks at m/z 276-278. Since the expected number of active hydrogens is five (N-H₂ and 3 O-H), four extra hydrogen atoms were replaced by deuterium atoms. Chemical ionization (CI) with D₂O produced only MD⁺-d₅ ions (m/z 274) and no extra hydrogen exchange was observed for the protonated molecule of adenosine³⁾. That is the case in NMR spectrometry, too. Figure 3b shows a part of the mass spectrum of base fragment (BH₂⁺) produced by increasing the voltage applied to the aperture up to 70 V which causes so called collision activated dissociation (CAD or CID). The result indicates that the increase in mass number of the fragment ions (BH₂⁺) which are directly produced from the MD⁺-d₆ ions by the CAD technique corresponds to the active hydrogens. Namely, the increase in mass number from 136 to 140 (BH₂⁺ to BD₂⁺-d₂) means that there is two hydrogens in the base (B), i.e. N-H₂. The same result has been observed by CI (D₂O). Therefore the extra four hydrogen atoms are likely to be four H of CH in ribose (sugar) of adenosine and one of such hydrogens might exchange quite rapidly with D₂O. When glycerol was used as a matrix, one extra increase in mass number of the fragment ion (m/z 136 $\rightarrow$ 141) was observed probably due to the reactions between deuteriated glycerol and adenosine. In the case of cytidine, one extra intense peak was observed at m/z 251 for the protonated molecule and at m/z 117 for the fragment ion (BH₂⁺), respectively. The reactions in detail have not yet been quite clear. However, the results are somewhat different from that obtained by the

CI technique³⁾ which is a gas phase reaction. NMR spectrometry provides the knowledge of reactions between D₂O and neutral molecules, while mass spectrometry provides information in the ionized state. This technique is useful not only for the structure elucidation but also for obtaining information about the H-D exchange reactions between ions and molecules probably in a condensed phase as well as in a gas phase at atmospheric pressure.

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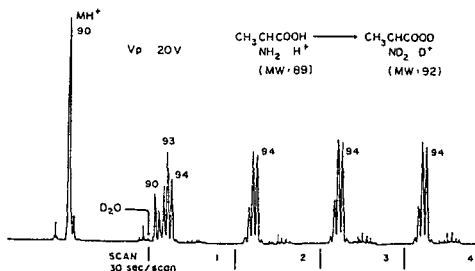


Fig.1 Mass Shift of MH⁺ for alanine

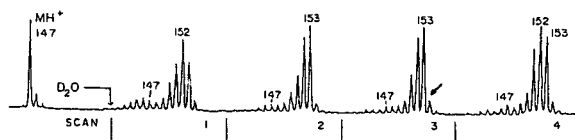


Fig.2 Mass shift of protonated molecules for lysine

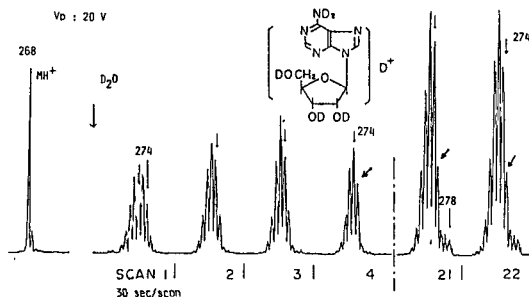


Fig.3a Mass shift of protonated molecules for adenosine

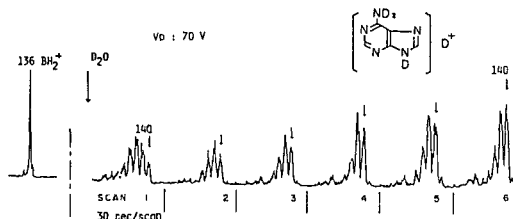


Fig.3b Mass shift of BH₂⁺ ions from MH⁺ of adenosine caused by H-D exchange reactions

STRUCTURAL CHARACTERIZATION OF NATURALLY OCCURRING OLIGOSACCHARIDES
BY AMIDE MATRIX-ASSISTED MOLECULAR SECONDARY ION MASS SPECTROMETRY

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Normally, the glycerol matrix is essential in sputtered ion mass spectrometry such as fast atom bombardment (FAB) and molecular secondary ion mass spectrometry (SIMS).¹⁾ Very recently, we have successfully applied the amine matrix, diethanolamine (DEA) and triethanolamine (TEA), instead of glycerol for the determination of molecular weight of neutral oligosaccharides by molecular SIMS.²⁾ When DEA is used, a stable molecular adduct ion species, $[M+DEAH]^+$ ion is formed as a sole ion derived from a neutral oligosaccharide molecule and no fragment ion is detected. Moreover, the $[M+DEAH]^+$ ion decomposes scarcely even under collision induced dissociation (CID) condition.

In order to obtain both molecular ion species and structurally informative fragment ions, we selected several amides as the matrix material, which possess intermediate proton affinity between glycerol and diethanolamine. Among amide compounds studied, N-(2-hydroxyethyl)-formamide (HEF), α -pyrrolidone (PR) and α -piperidone (PP) are available for our purpose. We attempt to measure secondary ion (SI) mass spectra of some neutral oligosaccharides [raffinose (1), stachyose (2)], acetyl-aminosugar containing oligosaccharides [viridopentaose A, B, C (3A, 3B, 3C)] and aminoglycoside antibiotics [amikacin (4), neomycin (5)].

When these amides are used, molecular adduct ions (e.g. $[M+HEFH]^+$, etc.) are observed together with several fragment ions in each SI mass spectrum. For example, Fig. 1(a) shows the SI mass spectrum of neutral tetrasaccharide (2) using HEF matrix. In the molecular ion region, the molecular adduct ion is recognized at m/z 756. Since the ions at m/z $[(89)_n+1]$ are HEF oligomer, the remaining ions at m/z 594, 505, 432, 414, 325 and 163 are derived from the sample molecule. They are assigned to tri-, di- and mono-saccharide sequence ions. Additionally, the linked scan (B/E) technique is available for further identifying these sequence ions. The metastable ion spectrum of $[M+HEFH]^+$ from 2 is presented in Fig. 1 (b). The molecular ion species decomposed readily to yield structurally informative ions. They are protonated molecule $[MH^+]$ and sequence ions which are produced by the cleavages of the glycosidic linkages.³⁾ The similar mass spectrometric behavior is shown in the PR and PP matrix-assisted SI mass spectra of neutral trisaccharide (1).

In the case of acetyl-aminosugar containing oligosaccharides, 3A, 3B and 3C,⁴⁾ their SI spectra show intense protonated molecule $[MH^+]$ with less abundant matrix-adduct ion in the high mass region as shown in Fig. 2. These phenomena are reasonably interpreted in consideration of proton affinity of sample and matrix molecules and their competition on the formation of molecular ion species.

The SI mass spectra of more basic pseudo-oligosaccharides, 4 and 5, display similar feature in their molecular ion region. Namely, both of amine or amide matrix-assisted

spectra are characterized by the appearance of fairly intense protonated molecule, like as glycerol-assisted spectrum. Thus, we can safely choose such clear $[MH]^+$ as a precursor ion in the linked scan technique.

As mentioned above, the effective use of selected matrix in molecular SIMS will be powerful technique for the structural characterization of naturally occurring oligosaccharides.

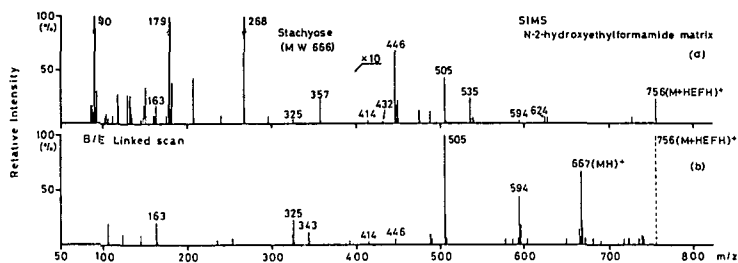


Fig. 1. (a) Secondary ion mass spectrum of stachyose (2) with HEF.
(b) Metastable ion spectrum of $[M+HEFH]^+$ from stachyose.

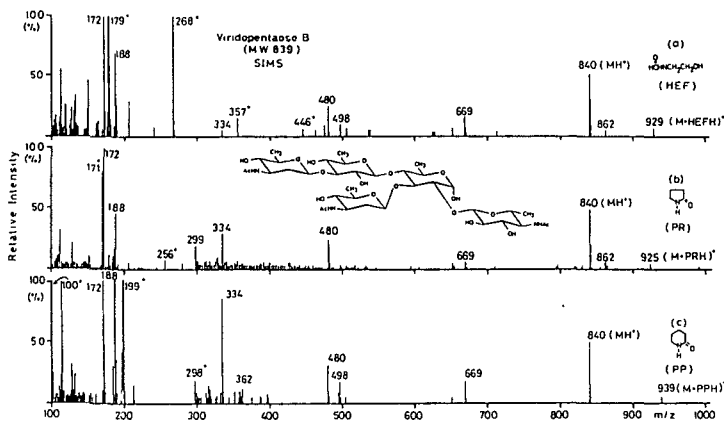


Fig. 2. Secondary ion mass spectra of viridopentaose B with amide matrices.

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FD and FAB Mass Spectral Studies of Technetium Complexes

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Technetium assumes a variety of oxidation states, each having interesting and unique chemistry. It is advantageous to exploit this wealth of synthetic possibilities, because Technetium-based radiopharmaceutical agents have become important clinical diagnostic agents. New Technetium agents are likely to be even more useful in the future, as their chemistry and physiological selectivity come to be better understood. In order to 1) analyze new synthetic compounds and radiopharmaceutical kits and 2) to follow chemical and metabolic transformations, we are extending our earlier studies and are making use of both field desorption and fast atom bombardment mass spectrometry.

A series of hexakis alkyl and aryl isocyanide complexes of Technetium(I) have been prepared by direct reduction of the pertechnetate ion by sodium dithionite in the presence of the isocyanide ligand. FD(+) has proved useful for characterizing these cations, as is shown in Fig. 1. When prepared with the metastable 99m Tc, this class of air- and water-stable cations has been shown to have potential use in lung perfusion, myocardial imaging, cell, platelet and liposome labeling studies. Similar uses are proposed for the compounds shown in Figs. 2 and 4. Tc-DTPA, shown in Fig. 3, is a commercially-available imaging agent, but the Tc/ligand ratio and even the oxidation state of Tc in this complex have been open to speculation. The Figures presented here illustrate the range of mass spectral approaches we have employed to solve the analytical problems arising during these investigations.

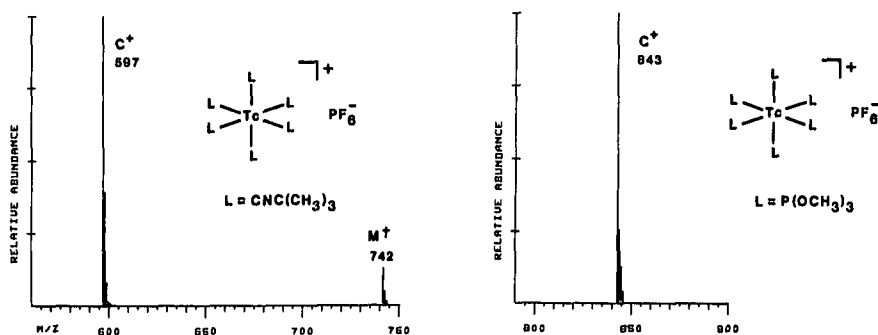


Fig. 1. (A) FD(+) mass spectrum of hexakis(*t*-butyl isocyanide) technetium(I)hexafluorophosphate. BAT 18 mA. (B) FD(+) mass spectrum of hexakis(trimethyl orthophosphate) technetium(I)hexafluorophosphate. BAT 0 mA. At higher emitter currents, sequential losses of ligand were observed.

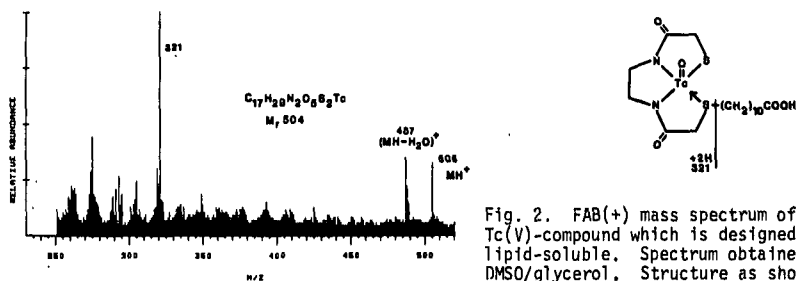


Fig. 2. FAB(+) mass spectrum of a neutral Tc(V)-compound which is designed to be lipid-soluble. Spectrum obtained in (1:1) DMSO/glycerol. Structure as shown.

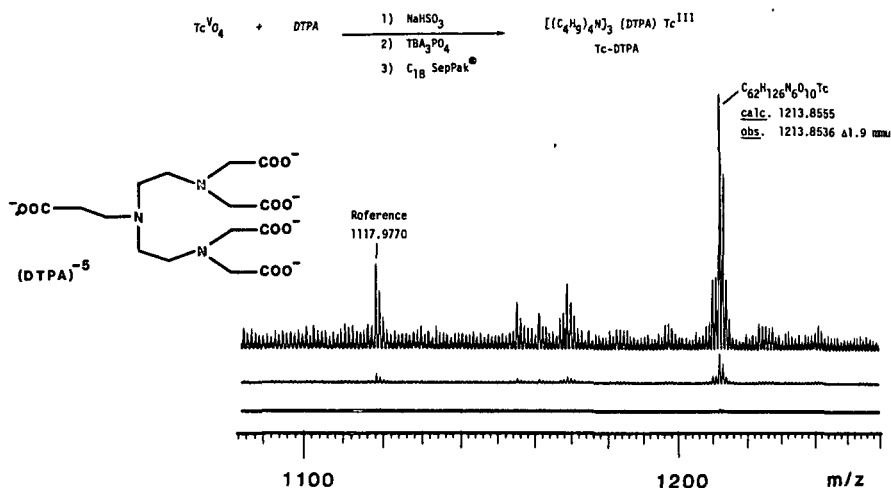


Fig. 3. FAB(+) mass spectrum of Tc-DTPA in (1:1) water/glycerol after being prepared by the sequence illustrated. The peaks assigned to the Tc-complex match those observed in a FAB(+) mass spectrum of the mixture of products obtained upon addition of SnCl_2 to the commercial Tc-DTPA radiopharmaceutical kit (the method used for the preparation of the clinical agent). This result indicates that the oxidation state of Technetium in the Tc-DTPA complex is +3, not +4 as had been assumed.

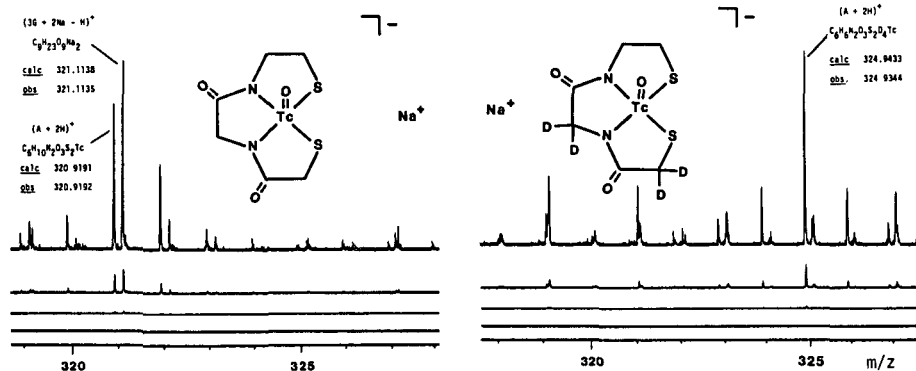


Fig. 4. FAB(+) mass spectra of Tc(V) complex in (1:1) water/glycerol before and after deuterium exchange of labile hydrogens. The high sodium chloride content of the samples caused an abundant glycerol-sodium ion series, including an ion at the same nominal mass as the $(A + 2H)^+$ peak of the unlabeled complex. To remove this interference, the spectra were obtained by scanning at 1:10,000 resolution. (A) Spectrum of the unlabeled complex. (B) Spectrum after complete exchange of the labile hydrogens.

Acknowledgements: The technetium complexes shown were prepared by M. Abrams (Figs. 1 and 2), J. Lister-James (Figs. 3 and 5), and J. Brodak (Fig. 4). This work was funded by the U.S. Dept. of Energy Contract No. DE-AC02-81EV10649. The M.I.T. Mass Spectrometry Facility is supported by the NIH Division of Research Resources Grant No. RR00317 (to K. Biemann).

APPLICATION OF FIELD DESORPTION MASS SPECTROMETRY TO THE ANALYSIS OF ORGANO-GERMANIUM, TIN, AND SILICON COMPOUNDS.

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Field desorption (FD) has been regarded as a suitable method for the analysis of non-volatile and thermally labile substances. We are now reporting the application of this technique for the study of organo-germanium, tin, and silicon compounds having metal to metal linkages. The utility of this methodology for evaluating the purity of the samples and confirming the structures of the reaction intermediates during the syntheses is also illustrated.

From the monogermene to the cyclotrigermane, the field desorption spectra are dominated by the molecular ions as shown in Figure 1. Similarly this characteristic is also observed for the organo-tin and organo-silicon compounds.

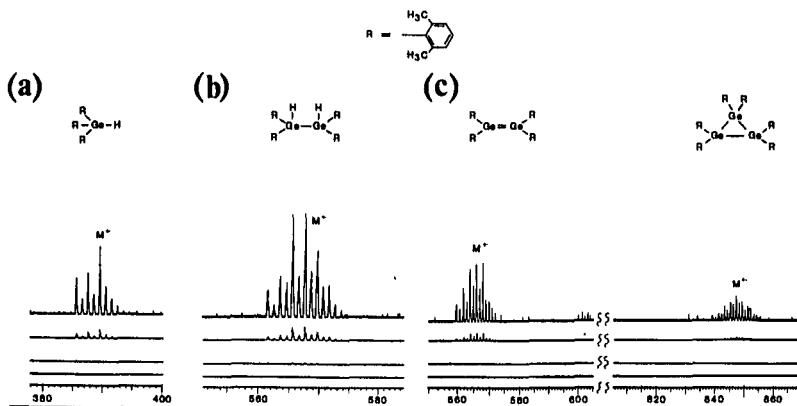


Figure 1. Field desorption mass spectra of (a) tris(2,6-dimethylphenyl)germane, ehc = 0 mA (b) 1,1,2,2-tetrakis(2,6-dimethylphenyl)digermane, ehc = 9 mA (c) a mixture of tetrakis(2,6-dimethylphenyl)digermene and hexakis(2,6-dimethylphenyl)cyclotrigermane, ehc = 20 mA.

During the synthesis of the hexakis(2,6-dimethylphenyl)cyclotrigermane (1), the structures of the reaction by-products -- 1,1,2,2-tetrakis(2,6-dimethylphenyl)-1-chloro- and 1,2-dichlorodigermane -- were confirmed by field desorption. In addition, the existence of the tetrakis(2,6-dimethylphenyl)digermene during its synthesis was further supported by the field desorption spectrum of its chemical reaction product with methanol (1). Analogously, field desorption mass spectrum produced additional evidence to support the structures of hexakis(2,6-diethylphenyl)cyclotristannane and the oxidized analog during their syntheses (2).

In addition to the molecular ion, ions formed by the cleavage of the metal-metal bond or metal-carbon bond are sometimes observed. Figure 2 is an example for these types of fragmentation.

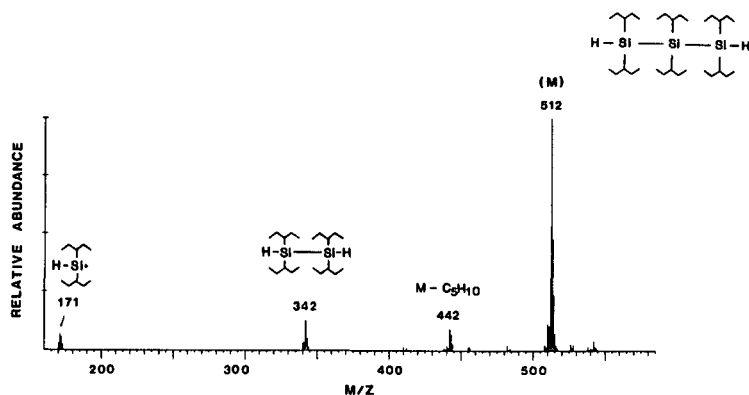


Figure 2. Field desorption mass spectrum of 1,1,2,2,3,3-hexakis(1-ethylpropyl)-trisilane, ehc = 0 mA.

Even though the organo-silicon compounds are sufficiently volatile for EI analysis, extensive fragmentation obscures important information. For example, most ions observed in the EI spectrum of a supposedly pure 1,1,2,2,3,3-hexakis(1-ethylpropyl)-1,3-dichloro-trisilane are explainable by such a structure, but its FD spectrum indicates that the sample also contains disilane and monosilane homologs.

Field desorption mass spectrometry thus is shown to be an excellent technique for unambiguously determining and confirming the molecular weight of organo-germanium, tin, and silicon compounds. Furthermore, the purity of the sample can also be estimated. In the case of the more volatile organo-silicon compounds, the use of chemical ionization may be comparable to that of field desorption, but with the non-volatile organo-metallic compounds, field desorption would be the method of choice. Although compounds containing three metallic atoms are used in this study, analysis of higher analogs should also be feasible by this method.

Acknowledgement

We would like to thank Prof. S. Masamune and his colleagues at M.I.T., Dr. Y. Hanzawa, Dr. H. Tobita, Mr. S. Murakami, and Mr. L.R. Sita for supplying the samples.

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Fast Atom Bombardment MS of Some Prostaglandins. A. N. Tyler, Dept. Psychiatry, Stanford University, Stanford, CA 94305; R. D. Sedgwick, Allied Corporate Labs, Colombia Park Ave., Morristown, NJ 07960; M. Barber and R. S. Bordoli, Dept. Chemistry, U.M.I.S.T., P.O. Box 88, Sackville St., Manchester M60 1QD, England

Introduction

Prostaglandins (PGs) are widely studied because of their diverse range of physiological roles. Mass spectrometry was used in the original determination of PG structure (1) and since then has been widely used in their estimation.

Electron (EI) impact GC/MS studies have usually been performed upon derivatives such as silyl ethers of their esters (2). EI studies of underivatized PGs produce highly complex spectra, with multi-step rearrangements involved in the formation of many ions (3).

FAB spectra have been obtained from PGs A_1 and A_2 , B_1 and B_2 , E_1 and E_2 , and F_{1a} and F_{2a} , using an AEI MS-902 instrument fitted with a FAB source.

Table 1 shows the major ions observed in the positive ion spectra of the free acids dissolved in glycerol.

Table 1. Major Ions Normalized to Largest Peak Above m/z 200

PG	(MH) ⁺	(MH-H ₂ O) ⁺	(MH-2H ₂ O) ⁺	(MH-3H ₂ O) ⁺	(MH-2H ₂ O-28) ⁺	(MH-H ₂ O-44) ⁺	Other Ions (Int)
A_1		100	33				
B_1	100	59	68				
B_2	80	100	67				
E_1		78	45	32	20	11	265 (100) 275 (16)
E_2		100	86	39	15	24	263 (82) 273 (17)
F_{1a}		48	71	100		60	277 (74) 266 (44) 265 (33)
F_{2a}		30	59	100		11	275 (70) 265 (24) 264 (20) 263 (26)

Except for the B group PGs, protonated molecules are not observed and the spectra are similar to those obtained by CI-MS (4). However, molecular weight information is obtained by the addition of alkali halide salts to the sample, causing formation of (M+Cat)⁺ species. In most cases the major fragments arise from the loss of water molecules from (M+H)⁺. On the basis of metastable V scanning and peak matching data, some other losses are assigned to fragmentations of the five-membered rings.

The major ions in the negative spectra are shown in Table 2.

PG	Table 2. Ions in the Negative Ion Spectra			
	(M-H) ⁻	(M-H-H ₂ O) ⁻	(M-H-2H ₂ O) ⁻	Other Ions (Int)
A ₁	100	44		
A ₂	100	46		
B ₁	100			
B ₂	100			
E ₁	51	18	100	281 (14) 235 (53) 271 (12)
E ₂	100	53	40	
F _{1a}	100			
F _{2a}	100			

Apart from the E PGs, these are very simple, showing only (M-H)⁻ ions and, in the case of the A group the loss of one water therefrom.

Monitoring the intensity of the (M-H)⁻ ion of PG F_{2a} for decreasing sample size indicated that this compound can be readily detected at the picomolar level.

In conclusion, each sample produced spectra unique to its structure, enabling unambiguous determination on a qualitative basis. Further studies with isotopically labelled PGs is required to establish the structures of certain fragment ions.

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Acknowledgment

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SPECTRA OF SOME DRUGS NOT IN THE EPA/NIH/NBS DATA BASE

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In the drug detection laboratory, mass spectrometry is used to identify drugs, their metabolites, and derivatives. Our identification of unknown substances often begins with one or more computerized searches of library mass spectral data in the hope that someone else has previously obtained a spectrum and entered it into the library. In our experience in drug analysis we have found that only occasionally does this yield a hit and result in an identification. Usually it fails and the identity of the substance must be inferred from spectral evidence (including molecular weight data), sample history, and molecular weight indexes such as the Merck Table of Molecular Weights (1). After successful identification we search the library to determine why the library did not identify the unknown. We find that the spectra of many common drugs are not included in the largest libraries.

We have begun an explicit effort to incorporate into our mass spectral library both electron impact and chemical ionization spectra from drug standards and drug metabolites which are of interest to us. We obtained their Chemical Abstracts Service registry numbers from either USAN (2) or MERCK (3). We checked the Chemical Abstracts Service number of each drug against the Chemical Abstracts Service numbers in the supplement one EPA/NIH/NBS index volume (4).

Of the 297 drug standards in our laboratory, 65 of them are not represented in the EPA/NIH/NBS data base. From these we obtained 35 EI and 30 CI spectra. Including metabolites and derivatives, we obtained over 100 spectra. To obtain the spectrum of metabolites, we used compounds of synthetic or biologic origin, provided that, in the case of biologic origin, the structure had previously been determined and reported in the literature by an independent investigator. EI spectra were obtained from the drugs listed below. For most drugs CI (methane) spectra were obtained as well. Mass spectrometric and, where applicable, gas chromatographic parameters are clearly stated in the text preceding each sample. Sources for metabolites are noted in the text. All silyl derivatives were prepared by adding 20 microliters of BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide, Pierce) to the dry residue in a screw capped tube, mixing on a vortex mixer briefly and heating for 20 minutes at 60 degrees. All methylations were done in the gas chromatographic injector by adding 20 microliters of TMAH (trimethylphenylammonium hydroxide, 1% in methanol, Eastman) to the dry residue in a tube. A portion of the mixture was then injected, and the drug methylated on injection.

Benoxaprofen	Diclofenac	Isoxsuprine	Oxymorphone
Buprenorphine	Diflunisal	Ketamine	Piperacetazine
Butaperazine	Doxapram	Meclofenoxate	Primaquine
Cicloprofen	Fenclozac	Mefenamic acid	Propionylpromazine
Cinchonidine	Fenoprofen	Mepivacaine	Ritalinic acid
Clenbuterol	Flurbiprofen	Metaxalone	Ritodrine
Chloroprocaine	Flumixin	Naloxone	Tetrahydrozoline
Dantrolene	Flufenamic acid	Naphazoline	Tuaminoheptane
Dexamethasone	Hordeine	Oxymetazoline	

Our small sampling indicates that the EPA/NIH/NBS data base does not contain the spectra of some common drugs of interest to us. For this reason the identification of unknown drugs by the mass spectroscopist must be based on more than just a search of the data base. In particular, molecular weight and structural information from CI spectra are very helpful.

- 1) Windholz, Martha, editor, Table of Molecular Weights, 1st edition, Merck & Co., Rahway, N.J. 1978.
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ANALYSIS OF BUTORPHANOL AT 1 ng/ml USING CAPILLARY GC/MS
WITH STABLE ISOTOPE LABELLED STANDARDS

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A mass spectrometry method for routine analysis of butorphanol, a synthetic nonaddictive morphinan, was developed to provide the specificity and sensitivity needed to demonstrate bioavailability after administration. To achieve the sensitivity necessary, capillary GC/MS was done and d_5 -labelled butorphanol was added to provide an internal standard. The chromatography of butorphanol was improved by forming the TMS-ether. To reduce the cost of analysis, efforts are under way to modify the method so GC/ECD could be used for routine analysis with verification done by GC/MS. An alternative to silylation being investigated is the use of EC sensitive reagents which improve chromatography. Both trifluoroacetic anhydride (TFAA) and pentafluorobenzyl (PFB) bromide can be used to make ECD sensitive derivatives of butorphanol and they are under investigation.

The method for analysis we have developed uses a capillary GC/MS with electron impact (EI) ionization and selected ion monitoring. The base peak in mass spectra for butorphanol and all its derivatives in EI results from loss of 55 daltons (C_4H_7). This ion is monitored in the SIM sequence used for the butorphanol (butor)-TMS-ether and the d_5 -butor-TMS-ether. With aqueous solutions a linear calibration was acquired from 25 to 0.5 ng/ml. The curve shows the intercept of the line was greater than zero, though blank samples contain no peaks in the ion chromatogram for butor-TMS-ether. The limit of sensitivity was not 0.5 ng/ml; this was the limit for linear quantitation. The limit of sensitivity was lower than 0.2 ng/ml. This sample had a signal to noise ratio of 26:1. At 0.2 ng/ml, 15 pg was injected on column from the solution assuming quantitative extraction and derivatization. Thus, the limit of quantitation may be lower than 0.5 ng/ml since it is not sensitivity which has initially limited this work.

Analytical Conditions:

A 1 ml aliquot of sample (aqueous or plasma) spiked with d_5 -butorphanol is placed in a 15 X 100 mm tube to which 6 ml of 2% isomyl alcohol in hexane is added. Next, 0.15 ml of saturated carbonate buffer is added to bring the solution to pH 9.5. The organic layer is removed and dried with Na_2SO_4 (anhyd.). The solution is then blown down to dryness. The sample is transferred to a 2-ml vial with derivatization grade acetonitrile.

Before derivatization the solvent is removed with $N_2(g)$. Silylation is accomplished by adding 50 μ l of acetonitrile and 25 μ l of BSTFA and then reacting the mixture for 15 minutes. The sample is then blown down and redissolved in 40 μ l of toluene.

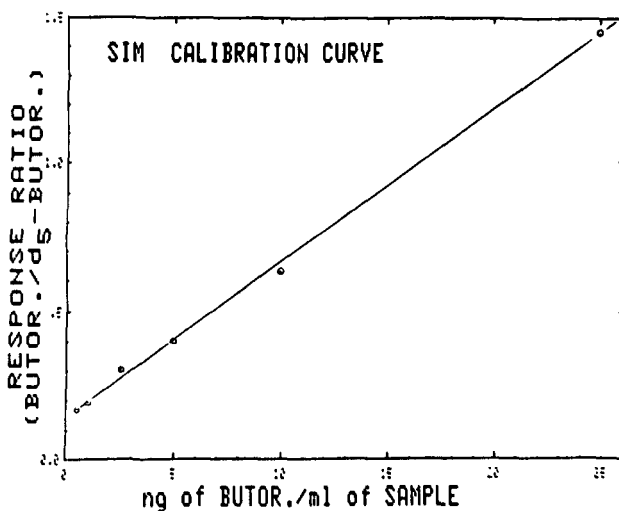
GC/MS Conditions:

GC: Initial temp. = 100° held 2 min.	MS: Source temp. = 200°
Final temp. = 300° held 3 min.	EM = 2200V
Program rate = 20°/min.	70 eV
Col. - .25 mm X 15 M WCOT DB1	Ions 344.3 and 349.3 in SIM
12 psi head pressure	
Splitless mode	

Analytical Data:

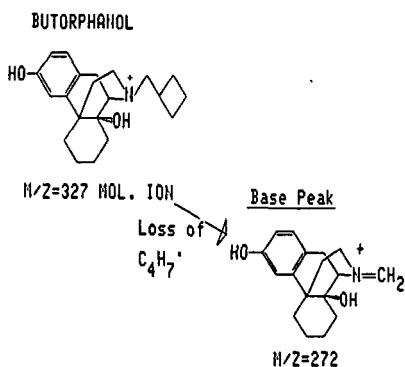
It was found in this work that butorphanol can be analyzed as the butor-TMS-ether using capillary GC/EIMS below the level of 1 ng/ml. It may be possible to refine the analysis to provide sensitivity below 0.5 ng/ml with this system. The calibration curve was not limited to 0.5 ng/ml by instrument response. The 0.2 ng/ml sample had a signal to noise ratio of 26:1. The ion chromatogram of this sample contained a strong peak. The sample at 0.2 ng/ml was not found to fall on the calibration curve. The standard curve generated is shown below and it shows how well the points fall on the line with a coefficient of linearity of .998.

Figure 1: Standard curve for the butorphanol



The ions monitored for the SIM data in the calibration curve are the result of a loss of C_4H_7 (M-55) for the butor-TMS-ether and d_5 -butor-TMS-ether. (Fragmentation shown below.) This cleavage is very efficient and yields the base peak for all butorphanol derivatives formed including methylation, acylation (TFA and HFB) and alkylation with PFBBR. Otherwise, only small fragment ions are formed and a weak molecular ion. Similarity of the mass spectra is expected because all derivatives can form this stable N-based ion.

Figure 2: Fragmentation to form the base peak



NEGATIVE CHEMICAL IONIZATION GAS CHROMATOGRAPHY/MASS SPECTROMETRY
(NCI GC/MS) FOR SENSITIVE ANALYSIS OF SOME ANTIDEPRESSANT DRUGS

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BIOAVAILABILITY AND PHARMACOKINETIC STUDIES OFTEN REQUIRE THE USE OF EXTREMELY SENSITIVE ANALYTICAL METHODS, WHICH HAVE TO BE ALSO SPECIFIC, ACCURATE, AND PRECISE. FOR DRUGS WITH POSITIVE ELECTRON AFFINITIES, THE NCI GC/MS APPROACH MAY PROVE MOST SUITABLE AND VERY PRACTICAL.

PROPER DERIVATIZATION CAN BE USED CONVENIENTLY FOR INCREASING THE ELECTRON AFFINITY OF MANY DRUGS. FOR EXAMPLE, SECONDARY AMINES CAN BE READILY ACYLATED TO YIELD HEPTAFLUOROBUTYRYL DERIVATIVES WITH HIGH ELECTRON AFFINITIES. THE ACYLATION OF SECONDARY AMINES IS WELL KNOWN AND MUCH USED BECAUSE OF GAS CHROMATOGRAPHIC CONSIDERATIONS. IT HELPS OVERCOMING COLUMN ADSORPTION AND TAILING CHARACTERISTIC OF THE UNDERIVATIZED COMPOUNDS. ACYLATION HAS BEEN USED SUCCESSFULLY IN ELECTRON IMPACT AND POSITIVE CI GC/MS METHODS FOR THE ANALYSIS OF LOW NG/ML DRUG CONCENTRATIONS.

IN THIS WORK, WE USED A RIBERMAG R 10-10 QUADRUPOLE INSTRUMENT EQUIPPED WITH SIDAR 111B DATA SYSTEM FOR COMPARING THE RELATIVE SENSITIVITY ACHIEVABLE FOR SEVERAL ANTIDEPRESSANT DRUGS OR METABOLITES BY MEANS OF NEGATIVE AND POSITIVE CI GC/MS. INCLUDED WERE BENZOCTAMINE, DESIPRAMINE, N-DESMETHYLCLOMIPRAMINE, MAPROTILINE, AND NORTRIPTYLINE.

IN ALL CASES, NCI GC/MS ALLOWED AT LEAST ONE ORDER OF MAGNITUDE BETTER SENSITIVITY WHILE MAINTAINING THE LINEARITY OF INSTRUMENTAL RESPONSE. THIS SHOULD AFFORD A CONVENIENT APPROACH FOR THE ANALYSIS OF THESE AND SIMILAR DRUGS AT CONCENTRATIONS IN BIOLOGICAL FLUIDS GOING DOWN TO THE PG/ML LEVEL.

ANALYSIS OF ACETAMINOPHEN METABOLITES BY FAST ATOM BOMBARDMENT MASS SPECTROMETRY.
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48824.

Acetaminophen (APAP), the active ingredient in Tylenol[®], has found widespread use as an aspirin substitute. However, reports of hepatic necrosis and renal failure following ingestion of large dosages of APAP have stimulated the development of methodology to characterize molecular species responsible for this toxicity.

Mass spectrometry has been useful in identifying the APAP metabolites involved in hepatic and renal toxicity. Baillie et al. (Biomed. Mass Spectrom. 8:244, 1981) analyzed the major APAP metabolites from both synthetic and biological origin using EI, CI, and FD. EI and CI were suitable for the analysis of the relatively nonpolar metabolites but required large amounts of sample. FD provided molecular ion species on metabolites intractable to the other methods but failed to grant molecular information on the polar sulfate conjugate. Recent work in our laboratory indicates that FAB is useful in obtaining molecular weight information for all the major APAP metabolites. Spectra have been obtained from both synthetic samples as well as samples isolated from reversed phase HPLC. A protocol for the isolation and preparation of urinary metabolites for analysis by FAB-MS has been developed.

Figure 1a is a mass spectrum of APAP-SO₄ isolated from urine. Figure 1b is the net spectrum resulting from subtraction of the spectrum of a control urine sample from the spectrum shown in Figure 1a. The control urine was used to represent the non-drug-related background constituents in the subject's urine. This control urine was obtained from the same subject (as provided the urine for Figure 1a) under drug-free conditions; the control urine was processed under the same protocol.

Background present in FAB spectra often obscures assignments of both molecular and fragment ion species. Therefore, extensive purification is required to minimize the background present in urinary samples prior to analysis by FAB-MS. Criteria are needed to assess the recognizability of analyte ions observed in FAB spectra. To facilitate this assessment, a set of criteria has been proposed to estimate signal to background (S/B). In this ratio, (S) is composed of the relative intensity of the peak of interest less the background. The background (B) is composed from the average intensity of the ions 3 to 15 u above the candidate peak and 3 to 14 u below. This set is chosen to avoid contributions from analyte isotope peaks, to select against likely neutral losses, and to remove interferences from other molecular ion adducts. Exceptions must be made when a known analyte ion falls within the range set for the background (i.e., Li⁺ adducts). Also it may be desirable to correct the calculated S/B for known large peaks coming from the matrix. Potential applications of the S/B index include: 1) a quick and realistic way to assess sensitivity, 2) a means to estimate the effects of matrix spiking, 3) a way to judge the results of additional purification, and 4) a measure of the effectiveness of background subtraction.

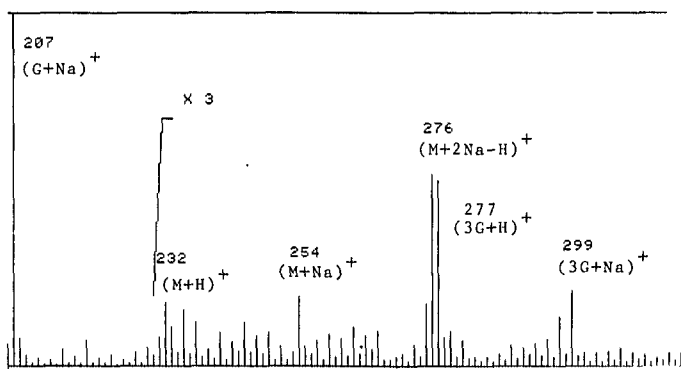


Figure 1a. 35nmol Urinary APAP-SO₄ (mw 231)

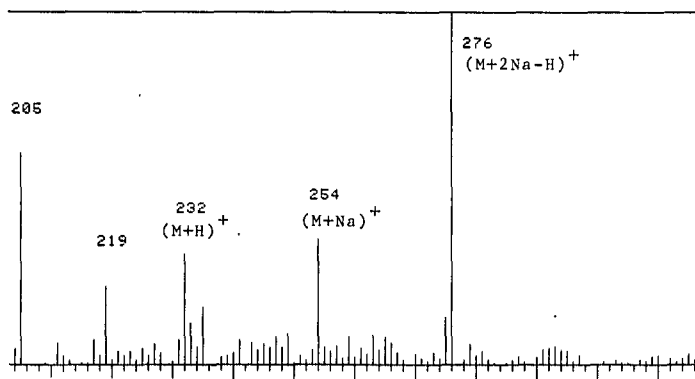


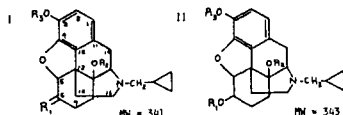
Figure 1b. 35nmol Urinary APAP-SO₄ (control subtracted)

THE QUANTITATIVE MEASUREMENT OF NALTREXONE AND ITS MAJOR METABOLITE IN PLASMA AND URINE

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Naltrexone ($I, R_1=O, R_2=R_3=H$) is a potent narcotic antagonist which has been investigated for use in the treatment of opioid dependence. Administration of the drug in the form of biodegradable beads implanted subcutaneously is currently undergoing clinical trials. In order to determine the bioavailability of naltrexone following the intravenous and subcutaneous modes of administration, a new assay was developed which is capable of measuring plasma concentrations of naltrexone and its major metabolite 6 β -Naltrexol ($II, R_1=R_2=R_3=H$), as low as 100 pg/ml. The basic procedure consists of addition of deuterium-labeled analogs of naltrexone and 6 β -naltrexol to 1 ml of body fluid, extraction, conversion of naltrexone to its methoxime bis-pentafluoropropionate derivative ($I, R_1=NOCH_3, R_2=R_3=COC_2F_5$), and 6 β -Naltrexol to its tris-pentafluoropropionate derivative ($II, R_1=R_2=R_3=COC_2F_5$), followed by GC/MS analysis using capillary column chromatography and negative ion chemical ionization.



Internal standards prepared by Research Triangle⁴ Institute, Research Triangle Park, North Carolina 27109, @ 15, 15, 16

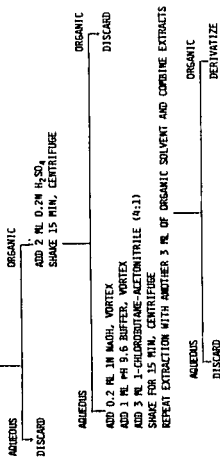
The extraction and derivatization schemes are given below in Figures 3 and 4. Analysis of the resultant residues were performed on a Finnigan 4500 operated in the negative chemical ionization mode by selected ion monitoring with methane as the reagent gas. Figures 5 and 6 are the respective mass spectra of methoxime bis-pentafluoropropionate naltrexone and tris-pentafluoropropionate naltrexol obtained with the source temperature at 100°C.

Gas chromatographic analysis was performed using a 10M, 0.32 mm i.d. SE 52 with a standard film thickness (0.25 μ m). Hydrogen (6 psi) was used as a carrier gas. The injection port and interface oven temperatures were set at 250°C. The temperature program used was 180–250°C per minute. The injections were made in the splitless mode, and the column effluent passed through an open/split interface.

Quantitations were performed automatically by the 4.07.82 INCOS software release. A single composite calibration curve consisting of four separate runs with nine different concentrations was used to quantitate the unknowns. It was found through examination of the standard curves that quantitations closer to theoretical values and lower percent CV's (percent coefficient of variation) could be achieved if height calculations were used for naltrexol and area calculations for naltrexone. Localizing the calibration curve to the seven closest points (or the minimum of the three closest concentrations) minimized the bias in the regression analysis imparted by incorporating high standards in the quantitation of small amounts of analyte. Calibrations were linear from 95 pg/ml to 62.5 ng/ml. Correlation coefficients were 0.999 for the curve. Intra-run percent CV for both naltrexone and naltrexol was 4% at 16 ng/ml and inter-run percent CV was approximately 8% at the 2 ng/ml level.

EXTRACTION AND DERIVATIZATION OF MALTREXONE AND 6 α -MALTREXOL FROM PLASMA AND URINE

1 mL SAMPLE + 5 NG α -MALTREXONE- 2 H $_3$ AND 6 α -MALTREXOL- 2 H $_3$, VORTEX.*
 EQUILIBRATE OVERNIGHT
 ADD 1 ML pH 9.6 BUFFER (50% K $_2$ HPO $_4$)
 ADD 1 ML 10% SODIUM CHLORIDE
 SHAKE FOR 30 MIN, CENTRIFUGE
 REPEAT EXTRACTION WITH ANOTHER 3 ML OF ORGANIC SOLVENT AND COMBINE EXTRACTS



*OPTIONAL ENZYMATIC HYDROLYSIS PERFORMED HERE

FIGURE 3

DERIVATIZATION

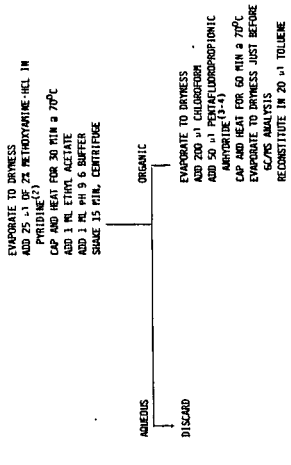


FIGURE 4

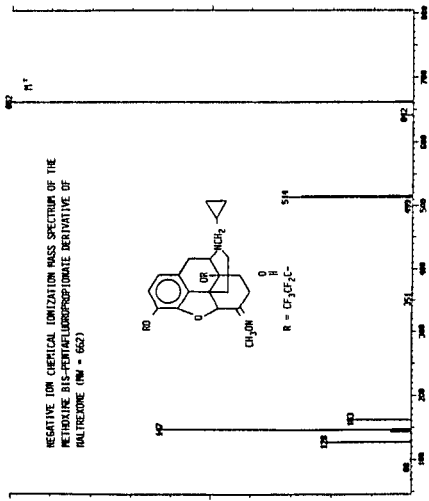


FIGURE 5

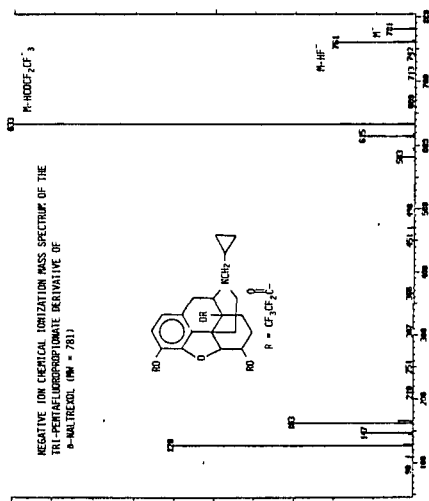


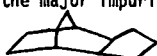
FIGURE 6

IDENTIFICATION OF CRUISE MISSILE FUEL METABOLITES FROM RAT ORGANS
BY GC/MS, SEX-RELATED DIFFERENCE IN KETONE FORMATION IN RAT KIDNEY
R. C. INMAN, K. O. YU, AND M. P. SERVE

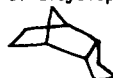
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JP-10, 2,3,3a,4,5,6,7,7a-octahydro 4,7-methanoindene is one of the high energy fuels used by the US Air Force and Navy. JP-10 is a synthetic saturated hydrocarbon fuel which gives higher BTU (15-20% more) than conventional petroleum-derived fuels. Fuel grade JP-10 is 98% pure and is synthesized from catalytic hydrogenation of dicyclopentadiene; the endo-isomer is the major impurity.

JP-10, exo-isomer



endo-isomer, impurity



JP-10 has a pungent, unpleasant odor. Therefore, it is unlikely that humans would be exposed to JP-10 for long periods. The fuel can be expected to occasionally produce environmental contamination in accidents and low level occupational exposure to workers during production and handling. This study seeks to develop an analytical tool to identify JP-10 metabolites in experimentally exposed rats.

Sex-related differences in ketone formation in rat kidneys will be studied because chronic exposure to JP-10 enhances renal damage in male rats but not in females.

METHODS: Male Fischer rats were injected intraperitoneally with 0.3 ml JP-10/kg. These rats were placed in metabolism cages to collect urine and feces. At the end of a 24 hr period, rats were sacrificed by halothane overdose. Tissues (kidney, liver), cecal contents, and feces were collected and homogenized. Samples were treated with beta-glucuronidase from *E. coli* at 37°C for 16 hours, and then placed on a disposable silica column and extracted with hexane. The hexane fraction was concentrated under nitrogen. Each concentrated sample was injected (1 μ l) into a Hewlett Packard (HP) 5880A GC and a HP 5985 GC/MS.

In a separate study, four male and four female Fischer rats were injected with JP-10 as in the above procedure. Kidney tissue was collected to identify and quantitate metabolites. Quantitation of JP-10 metabolites from the kidney extract was done by GC with a FI detector. The ketone and alcohol metabolites did not separate well on a non-polar column (OV-101 or SE-30), but a more polar column (SP-1000) gave good separation for quantitation. Each GC peak was identified by GC/MS using known compounds as references.

RESULTS AND DISCUSSION: 5-Hydroxy JP-10 (alcohol) was the major metabolite in the urine of JP-10 treated rats (1). Bile duct cannulation confirmed that monohydroxy JP-10 was excreted as the conjugated glucuronide. Cecal contents had the monohydroxy alcohol but feces did not have it. Liver showed trace amounts of alcohol. Kidney extract contained 5-keto-JP-10 (ketone) which was not detected in the other tissues or urine. The ketone metabolite was confirmed by GC/MS comparison with the independently synthesized compound. Sex-related differences in excretion in the rat have been reported: differences in perfluorooctanoate excretion in the rat (2) and effect of Stoddard solvent on kidney function (3). In the current study, there was a sex-related difference in ketone formation in rat kidney. Male rat kidney (n=4) had a much higher concentration of ketone than kidneys from females (n=4). An average of 22 μ g ketone/gram kidney was recovered from males while 0.06 μ g ketone/gm kidney was found in females. Extraction efficiency of the ketone was 50%. Further studies will be performed to determine whether this ketone causes the renal damage observed in male rats. Monohydroxy JP-10 was present in both male and female kidney samples.

CONCLUSION: A JP-10 metabolite, 5-hydroxy JP-10, was present in the urine, cecal contents, kidney, and liver of treated rats. Male kidney extract contained 5-keto-JP-10.

REFERENCES

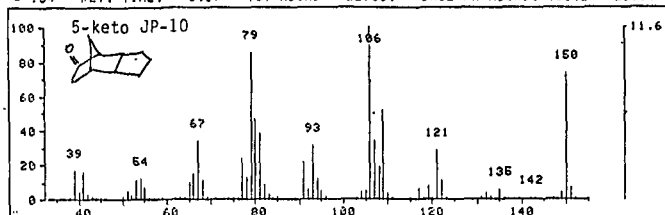
1. R. C. Inman, K. O. Yu, and W. C. Keller, 30th Annual Conference on Mass Spectrometry & Allied Topics, p. 104, 1982.
2. H. Hanhijarvi, Proceedings of the Society for Experimental Biology and Medicine, 171:50, 1982.

3. R. D. Phillips, 13th Annual Conference on Environmental Toxicology, Nov 1982, AFAMRL-TR-82-101, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH 45433.

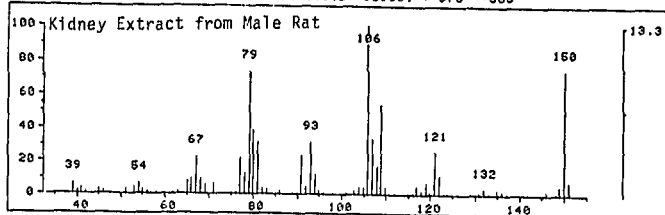
Keto JP-10 Mass Spectra
Principal Fragmentations and Their Relative Abundances in the 70eV Mass Spectra of 1-keto-JP-10, 5-keto-JP-10 and extract from rat kidney

m/z	1-keto	5-keto	kidney ext.	m/z	1-keto	5-keto	kidney ext.
150	68	78	75	94	31	12	11
122	44	11	12	83	32	3	1
121	16	28	26	80	68	48	40
109	5	53	54	79	52	86	73
107	10	35	34	68	17	12	9
106	9	100	100	66	100	15	11
95	41	5	4	65	17	10	8

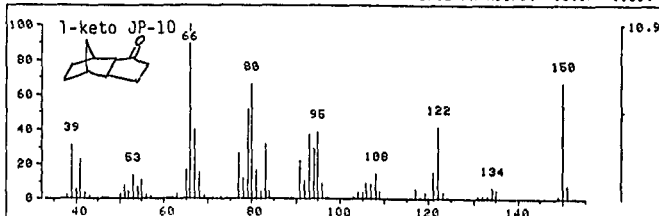
134 RET. TIME: 6.57 TOT ABUND= 22983. BASE PK/ABUND: 106.2/ 2655.



AVERAGED SPECTRUM * BASE PK/ABUND: 106.2/ 32000. + 378 -365



317 RET. TIME: 6.37 TOT ABUND= 10653. BASE PK/ABUND: 66.1/ 1166.



High Mass Measurement Accuracy by Fourier Transform Mass Spectrometry

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Parts-per million mass measurement accuracy is necessary for calculation of exact elemental compositions. A compound having a nominal mass of 500.15 ± 0.02 amu (40 ppm error) could be one of 20 different organic compounds containing C, H, N and O. If the measurement could be made with 1 ppm accuracy the number of possible compounds is reduced to a single composition, $C_{32}H_{22}NO_5$.

In Fourier transform mass spectrometry (FT-MS) the m/z of an ion is calculated from its measured cyclotron frequency. In principle this can be done very accurately because frequency is a physical parameter which can be measured with sub-ppm accuracy using standard laboratory equipment. In addition, the superconducting magnets and frequency synthesizer-based detection circuits in FT-MS instruments are very stable.

The simplest relationship between the measured resonant frequency of an ion and its mass is $\omega = qB/m$, where q/m is the charge to mass ratio and B is the magnetic field strength. Several experimental factors cause the observed frequency to be shifted from this simple relationship (1). The first of these factors, the voltages applied to the cell plates was modeled by Beauchamp and Armstrong in 1969 for a drift cell (2). More recently the effect of the potentials was modeled for a trapped ICR cell by Hunter and coworkers (3). The trapping voltage can shift the apparent mass of an ion by as much as one amu. The second factor, ion space charge, was modeled in 1979 by Hunter and McIver using a calibration equation which qualitatively included the number of ions (4). Their experiments demonstrated that the presence of ions affects the observed frequency in the same way as the applied voltages.

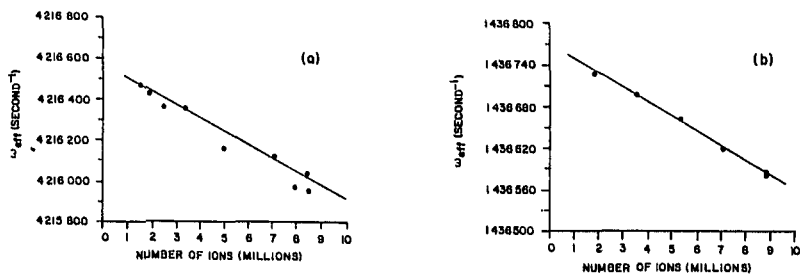
The mass measurement error introduced by the space charge term is approximately one part in 10^5 . However, FT-MS which relies only on accurate measurement of frequency, possesses sub parts-per-million capability. Unless the space charge term is characterized the full potential of the FT-MS is not realized.

In this presentation an analytical model for the ion space charge effects is presented. This is incorporated into the equations of motion for an ion in crossed electric and magnetic fields. The solution to these equation in the x and y direction gives an effective frequency

which depends on the number of ions, the shape of the ion cloud, the geometry of the analyzer cell and the applied voltages. Each of these factors cause substantial shifts in the measured frequency.

The changes in the ion cloud shape and density as the ions experience collisions has been shown to have a large effect on the measured frequency. Mass measurement errors as large as 43 ppm are observed if the number of collisions is not kept constant. In addition, the effective frequency has a linear dependence on the number of ions, if all other experimental parameters are kept constant. In Figure 1, the effective frequency is plotted as a function of number of ions for (a) an elongated cell and (b) a cubic cell.

Since the ion space charge term is demonstrated to be independent of mass, a new calibration equation for high mass measurement accuracy is obtained. This relative mass measurement technique has been shown to be accurate to within 1 ppm of the calculated mass. High mass measurement accuracy is essential for the FT-MS system since the resolution and sensitivity is much higher. A resolution of 110,000 at m/z 156 was recently obtained in our laboratory. The peak width at this resolution is approximately 1 millimass unit wide. For comparison, an electron has a mass of 0.5 millimass units. This calibration equation is dependent on the magnetic field strength. At 7.1 Tesla the theoretical error is 0.0011 ppm for measuring the frequency difference between m/z 78 and m/z 158.



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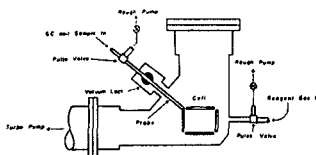
APPLICATION OF A PULSED VALVE IN FOURIER TRANSFORM MASS SPECTROMETRY

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Lincoln, NE 68588

Fourier transform mass spectrometers are capable of extremely high resolving power at low pressures (ca. $< 10^{-7}$ torr) (1). However, operation at these lower pressures precludes some useful mass spectrometric techniques that require higher pressures (10^{-7} - 10^{-5} torr), such as GCMS and chemical ionization. Therefore, to use the high resolving power of the FTMS for CI or GCMS, some method of operation of the mass spectrometer at low pressure while retaining the useful properties of both sub-techniques is required.

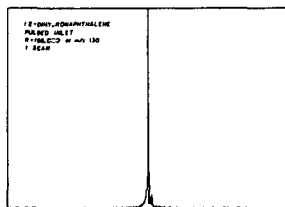
In our laboratory, we have solved this dilemma by the use of a small solenoid valve as an inlet port. The valve arrangement is shown in Figure 1. With the valve inlet, the pressure in the cell is increased by a gas pulse of 2-5 milliseconds during the ion formation period (i.e. electron ionization or CI reaction) after which the pressure is allowed to decrease while the ions are trapped in the FTMS cell. When the neutrals are pumped away, ion detection is performed in a low pressure environment, yielding high mass resolution spectra with excellent signal-to-noise due to the fact that the pressure is high during ion formation.

Figure 1



When used as a simple pulsed inlet for volatile samples, the pulsed valve allows a relatively straightforward means of resolution enhancement. Figure 2 illustrates the high resolution and high sensitivity of pulsed inlet, showing one scan of the molecular ion of 1,2-dihydronaphthalene with $R = 195,000$ (FWHH) at m/z 195.

Figure 2



In the chemical ionization experiment, the reagent gas is pulsed in at a high pressure (10^{-5} torr), ionized, and allowed to react with a low pressure of sample (5×10^{-8} torr) from the batch inlet or solid probe. Because the pulsed CI reagent pressure during the electron beam is high, the sensitivity is enhanced over conventional or self-CI in FTMS. Typically, CI sensitivity is increased by a factor of 3-10 times that for

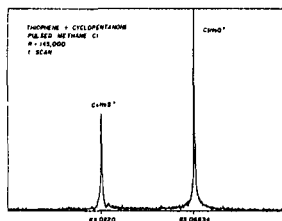
conventional methods. See Table 1. Figure 3 shows a typical high resolution pulsed CI spectrum of a mixture of thiophene and cyclopentanone. The $(M+H)^+$ ions at m/z 85 are easily separated with a resolution of 145,000.

Table 1

COMPOUND	RELATIVE TIC		
	STATIC	SELF	PULSED
<chem>c1ccsc1</chem>	0.065	0.00	1.00
<chem>O=C1CCCC1</chem>	0.36	0.022	1.00
<chem>CCCCC</chem>	0.23	0.0062	1.00

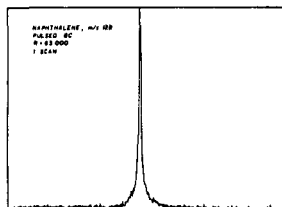
*SAMPLES AT 5×10^{-6} TORR
 *REAGENT GAS = METHANE
 *FOR STATIC CI. REAGENT P = 1×10^{-6} TORR
 *FOR PULSED CI. INSTANTANEOUS REAGENT
 P > 1×10^{-6} TORR

Figure 3



In GCMS, the valve is used as the interface as opposed to a jet separator or direct inlet. The effluent from a capillary GC column is directed to the valve which either sends the effluent to the FTMS via the probe (Figure 1) or into a mechanical pump. The electron beam is pulsed immediately after the valve pulse. The excess neutrals are allowed to be pumped away for 500 milliseconds before excitation and detection of the ion signals. Including the real-time disk storage of the time domain GC spectra, scan cycle time is about 1 second. Figure 4 is one scan of the molecular ion of naphthalene at m/z 128 with $R = 63,000$ at m/z 128. This new interface has also enabled an increase in GC/FTMS sensitivity. We report here that at a resolution of 32,000, the molecular ion of naphthalene could be detected at the level of 4 nanograms with a S/N of 4:1. This represents a factor of 5 improvement over the previously published results of Wilkins and Gross (2).

Figure 4



The pulsed valve GC interface may also be operated as a continuously variable splitter between the GC and FTMS. In this higher pressure, low resolution mode, the delay between the valve pulse and the electron beam is 50 milliseconds and the pump-out delay is reduced to 1 millisecond, giving a scan cycle time of about 50-100 milliseconds for wide-band, low resolution spectra of capillary GC eluants.

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Rapid advances are being made in Fourier Transform Mass Spectrometry (FTMS), yet the technique is not routine. This may be due to the lack of a complete functional theory which predicts ion signals to make possible the reasonable, accurate and rapid interpretation of results. The use of swept frequency excitation for wideband multichannel operation in FT-MS makes the theory difficult by compounding the complexity of modeling the ion motion and signal response. In an attempt to simplify matters, a circuit has been implemented for balanced impulse (an approximation to the mathematical 'Dirac') excitation of ions in a cubic cell [1]. Here we test the utility of impulse excitation in a cubic cell by a comparison with chirp excitation [2], and demonstrate some of the effects which complicate the use of the cubic cell in a routine FT-MS experiment.

Experimental: The comparison is made by displaying a figure of merit, "the average of the 3 points at a peak maximum in the magnitude spectrum" for one mass ($m/z = 131$) as a function of two normally used tuning parameters, excitation amplitude and trap voltage (selected to reveal information about the electric fields in the cell). The average, as a measure of peak height, approximates the product of signal intensity and resolution. The excitation amplitudes were measured as a peak-to-peak voltage on one of two plates for the chirp (0 Hz to 2 MHz at 2.105 kHz per μ sec) and as a base-to-peak voltage on one of two plates for the pulse (exponentially damped sinusoidal, 1.66 μ s to first zero, 8% overshoot). The experiment was conducted with a 2.5 cm cubic cell positioned in the 3" wide by 12" diameter gap of a Varian 7300 iron core magnet; the cell was driven by a Nicolet FT/MS 1000 console and operated in EI/positive ion mode. Other parameters were $B=1.2$ Tesla; pressure $\approx 2 \times 10^{-7}$ Torr of 1,1,1,2 tetrachloroethane; electron energy = 50 eV; beam-to-excitation delay = 1 msec; beam = 15 msec at 30 or 150 nA for 20,000 or 100,000 ions, respectively. Each data point is the result of a 16K FFT of 100 accumulated transients taken in heterodyne mode with a bandwidth of 30.075 kHz.

Results and Discussion: The figure of merit, displayed as a function of trap voltage and excitation amplitude, presents us with a surface with a ridge defined by the loci of maxima of the "3 pt magnitude" which occur in each excitation amplitude scan. For both the impulse and chirp at the higher trap voltages, the 3 pt magnitude generally does not respond in proportion to the amplitude of the excitation for amplitudes up to the position of the ridge, as one might expect from a linear system, suggesting that deconvolution may not work routinely. Only at the lower trap voltages (Trap = .305 V) can one imagine a proportional response (see Figures 1 and 3), suggesting that the trap electric fields adversely influence the coherence of ion motion. Perhaps the use of the hyperbolic Penning trap with its linear electric fields is a solution to this problem. Changing the number of ions from 20,000 to 100,000 produces a shift in the position of the ridge for both the impulse and chirp. At a trap of .506 Volts this shift is 40% (see Figures 3 and 4) for the impulse excitation, compared to 13% (see Figures 1 and 2) for the chirp. This shift indicates the coulombic forces of those 100,000 ions make a significant contribution to the electric fields in the cell. Also, if one uses peak heights, the movement of the surface with respect to the number of ions is problematic for such applications as GC/MS where the number of ions may be uncertain. Finally, for the chirp, increasing the number of ions by a factor of 5 produces an increase of approximately a factor of five in the 3 pt magnitude surface maxima, as one might expect. In contrast, for the impulse excitation, increasing the number of ions from 20,000 to 100,000 produces an increase of, at most, a factor of only two in the surface maxima. This contrast, together with the amplified movement of the surface with numbers of ions, suggests that the impulse excitation is more susceptible to coulombic effects.

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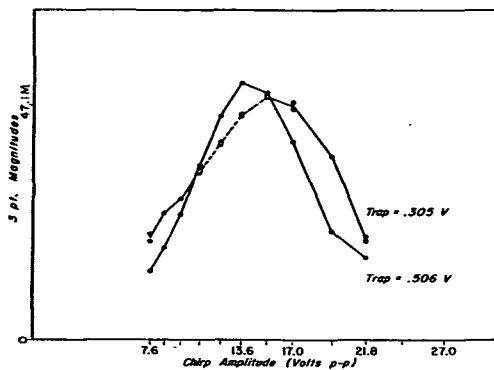


Figure 1. Chirp excitation amplitude scan; 20,000 ions

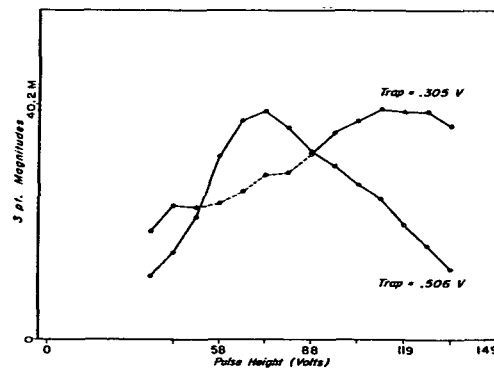


Figure 3. Impulse excitation amplitude scan, 20,000 ions

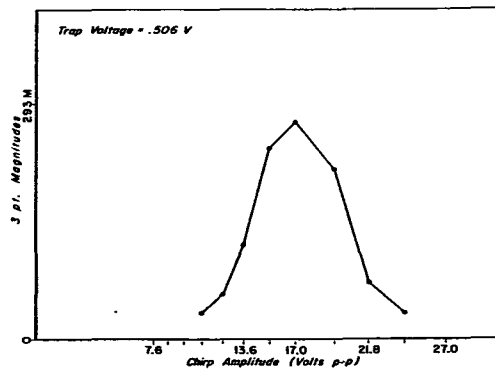


Figure 2. Chirp excitation amplitude scan; 100,000 ions

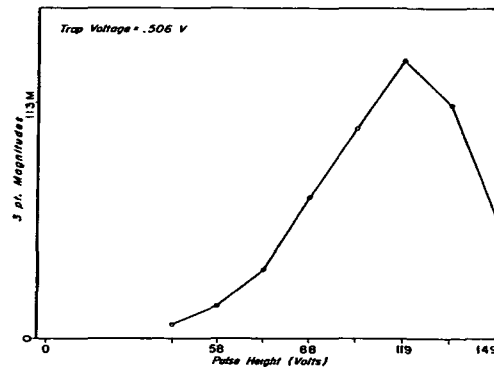


Figure 4. Impulse excitation amplitude scan; 100,000 ions

NUMERICAL ANALYSIS OF ION MOTION IN A TRAPPED ICR CELL

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The motion of ions in an ion cyclotron resonance (ICR) trapped ion analyzer cell can be described by Newton's Second Law, equation 1.

$$\vec{F} = m \frac{d\vec{v}}{dt} \quad (1)$$

The force on the ion in the cell is due to the electric and magnetic fields. The total force can be written as

$$\vec{F} = q (\vec{E} + \vec{v} \times \vec{B})$$

where q = charge of the ion
 $\vec{E}$ = electric field
 $\vec{v}$ = velocity of the ion
 $\vec{B}$ = magnetic field

which when substituted into equation 1 results in the Lorentz equation.

$$m \frac{d\vec{v}}{dt} = q (\vec{E} + \vec{v} \times \vec{B}) \quad (2)$$

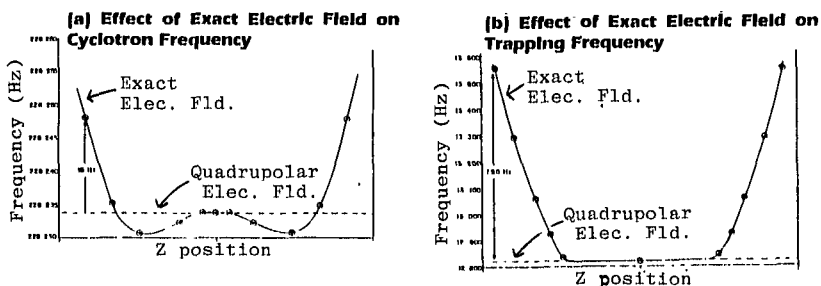
In order to obtain analytical solutions to the equation of motion it is necessary to oversimplify the expressions used to represent the electric and magnetic fields in the cell. The approximations made were to define the magnetic field to be constant in the z direction and, by constraining the ion to the center of the cell, the higher order terms of the expansion of the exact electric field in a Taylor series could be neglected resulting in an approximate quadrupolar electric field. The solutions are only approximate and do not allow one to investigate the effects of magnetic field and electric field inhomogeneities on ion motion.

In ICR and Fourier transform mass spectrometry (FTMS), mass measurement accuracy of sub part per million has been obtained.¹ With this degree of accuracy it is now important to understand the effects due to the inhomogeneities in the electric and magnetic fields in greater detail. Since obtaining an analytical solution to the Lorentz equation using the exact electric field is extremely difficult if at all possible, the approach in this paper is to numerically integrate the Lorentz equation utilizing the expression for the exact electric field. This is accomplished by using a fourth order Runge-Kutta method. A Fourier transform is then applied to the resulting trajectory of the ion to obtain the frequencies of the ion's cycles. These cycles con-

sist of the trapping motion parallel to the magnetic field between the trapping plates of the cell in the z direction and the cyclotron and magnetron motions in the x-y plane of the cell.

One aspect of ion motion to study is the effects of the exact electric field on ions formed at different positions along the electron beam. This was simulated in the trajectory calculations by giving the ion an initial position in the x and y directions near the perimeter of the electron beam, the initial velocity was that of a thermal ion, and the initial z position was varied for each calculation. The results are illustrated in figures (a) and (b). In figure (a), the effective frequency of a cycling ion in a cubic cell is plotted against its initial z position. It can be seen that an ion formed nearer the trapping plate results in a higher frequency than an ion formed at the center of the cell. Whereas for the approximate quadrupolar electric field there is no shift in frequency as a function of the ion's initial z position. This same behavior is found for the trapping frequency of an ion in a conventional rectangular cell as seen in figure (b). Experimental evidence support these observations and will be published in subsequent papers.

The numerical approach to characterizing ion motion in the ICR cell lends itself to a more detailed understanding of the effects influencing ion motion. With this understanding comes the hope of improving the conditions for which ICR and FTMS are applied which might include developing new cell designs or controlling experimental parameters so as to minimize the effects of the magnetic field and electric field inhomogeneities.



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New Mass Calibration Laws for
Fourier Transform Mass Spectrometry

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Recent innovations at the UNL laboratory for FTMS include the use of impulse ("Dirac-delta") excitation of ions, and the successful operation of a rotationally symmetric hyperbolic analyser cell (Penning trap) specifically designed for excitation and detection of ion cyclotron resonance. In addition, improved theories of ion motion and signal generation have led to a new mass calibration law for FTMS instruments employing cubic analyser cells, and to a different calibration law for hyperbolic cells. For a cubic cell with a negligible signal damping and circuit interaction (virtual ground approximation), the relationship between frequency and mass is:

$$m = \left(\frac{qB}{2\pi} \right) \frac{1}{f} - \frac{q}{4\pi^2} \left(\frac{\partial E_r}{\partial r} \right) \frac{1}{f^2} \quad (1)$$

in which m is mass (kg), B is magnetic field (Tesla), f is frequency (Hz), q is ionic charge (Coulomb), E_r is the radial component of electric field (V/m), and r is radial displacement (m). Rearrangement of this formula shows that signal frequency is shifted by the radial electric field gradient (E/r), present in the cell. The radial field gradient has contributions from both the applied electrostatic trapping field, and from ion space charge. For the hyperbolic analyser cell, the mass/frequency relation is:

$$f^2 = \left(\frac{qB}{2\pi} \right)^2 \frac{1}{m^2} - \frac{q}{\pi^2} \left(\frac{\partial E_r}{\partial r} \right) \frac{1}{m} \quad (2)$$

The fact that the mass/frequency relations differ for the two cells involves an analysis (to be published) of the interaction between ion motions and antenna fields of the cells.

The object of the present study was to test these two calibration laws. The major ions in the spectrum of 1,1,1,2-tetrachloroethane were used in preliminary studies. The frequencies of the peaks were measured automatically using a Nicolet Instrument Corporation FTMS 1000 data system, and the best fits to equations (1) or (2) computed by the least squares method. Tests of the software with synthesized spectra verified that mass measurement accuracy was not limited by rounding errors, ill conditioning of matrices, or systematic problems in software. Each spectrum was repeated four times in order to estimate the precision of mass and frequency measurements. Both standard chirp excitation, and the new ion impulse excitation method were employed, with results as follows:

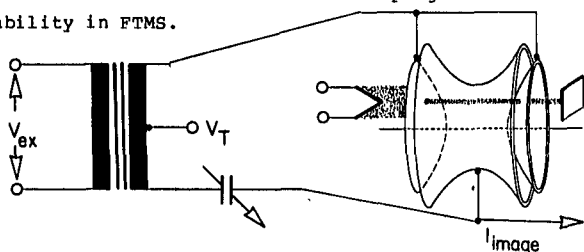
Cubic Cell, Chirp Excitation. A standard 1" cubic cell was operated at 10^{-7} torr (uncorrected ion gauge reading), and 0.34 Volt trap potential. It was found that conformance to the calibration law was sensitive to the number of ions in the cell. With 15,000 ions per decay transient, the rms deviations from the calibration law were 5.1 ppm. With 120,000 ions per transient, the rms deviations were 11.7 ppm. Uncertainties in the measurement were generally smaller than the error displacements, indicating that the errors were systematic.

Cubic Cell, Impulse Excitation. Spectra were run under the same conditions as above, but with impulse excitation of ions. A voltage pulse 1.66 us wide at base and 80 Volt in amplitude was applied differentially to the transmitter plates of the cubic cell. Ion response was monitored differentially with the receiver plates in the usual manner. With impulse excitation the cell showed better conformance to the calibration law, and was much less sensitive to the number of ions. With 15,000 ions per decay transient, the rms mass errors were 1.1 ppm. Measurement precision was typically 3.6 ppm indicating random rather than systematic error. With 120,000 ions per transient, the rms error was 3.6 ppm and the precision 2.7 ppm.

Hyperbolic Cell, Impulse Excitation. The hyperbolic cell was operated at 1 to 3×10^{-7} torr. Ions were created by an off-center electron beam. The cell was incorporated into a capacitance bridge, and the radial antenna field used to excite and sense ion cyclotron motion. Optimal trap voltage was 2.1 Volt. With 40,000 ions in the cell, the rms error was 2.2 ppm, and the measurement precision 3.5 ppm. With 640,000 ions in the cell, the rms error was 2.6 ppm, and the precision 3.5 ppm. This configuration again was far less sensitive to the number ions than the cubic/chirp combination.

These studies indicate that mass calibration of the FTMS is sensitive to space charge effects. Impulse excitation decreases, but does not eliminate, systematic mass measurement errors. Hyperbolic cell geometry may be beneficial in that larger numbers of ions apparently can be stored without perturbing the mass calibration although this should be considered a tentative result. Future work will be directed toward characterizing systematic mass measurement errors with a view toward developing a routine exact mass measurement capability in FTMS.

Figure 1.
Hyperbolic Cell



EI/DIRECT EXPOSURE PROBE ANALYSIS FOR HIGH MOLECULAR WEIGHT PAHS IN DIESEL SOOT EXTRACTS.
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The objective of these studies is to better define the biologically active compounds (PAHs and nitro-PAHs) present in diesel emissions and the influence of aromatic compounds in fuels on emissions. The control of soot production and the associated formation of polycyclic aromatic hydrocarbons are major problems in the reduction of combustion generated pollutants (1). Interactions of PAHs and NO_x during combustion and/or exhaust may form nitro-PAHs, some of which are active mutagens (2). Combustion of aliphatic fuels with addition of chemically pure PAHs is a useful approach to elucidate which PAHs contribute to the formation of active mutagens and soot (2,3). Because of the large number of nitro-PAHs which have been detected in diesel extracts from combustion of commercial fuels, it has not been possible to delineate which are the more significant mutagens.

A second objective is to develop better methods for detecting PAHs with molecular weights greater than m/z 270, which have been detected on diesel particles (4) as well as urban air particulates (5) and oil shale retort ash (6). Because of the low concentrations of high molecular weight PAHs in environmental samples, low volatility and the availability of only a few standards, little is known about this class of compounds. We have found that direct exposure probe analysis (DEP) under electron impact (EI) conditions is a rapid, sensitive and semi-quantitative analysis for detecting high molecular weight PAHs.

METHODS

A single cylinder diesel engine was operated on G. P. hexadecane as described previously (2). Known PAHs were added to fuel at 1% (w/v) final concentration. Particles were collected and filters were ultrasonically extracted first with pentane; secondly with methylene chloride and finally with acetonitrile. The extracts were pooled and the solvents evaporated. Aliquots of 10 to 50 mg of extract were spiked with internal standards (d_{10} -pyrene and d_{12} -benzo(a)pyrene), and fractionated with DMSO to yield an aromatic fraction which was analyzed by GC/MS for low molecular weight PAHs (m/z < 270) or analyzed by DEP/MS for high molecular weight PAHs (m/z > 270). Ames bioassays were carried out with Salmonella typhimurium TA98 as described previously (2,3).

RESULTS AND DISCUSSION

Combustion of aliphatic fuels containing known, single PAHs has been found previously to be useful for estimating the resistance of individual PAHs to combustion (2). This approach also has been found useful for estimating the effects of individual PAHs on emissions of high molecular weight PAHs.

Table 1 shows that individual PAHs exert two types of effects when added to aliphatic fuels and combusted. Certain PAHs (fluoranthene, pyrene) appear to be relatively stable to combustion, are concentrated in soot extracts, and do not appreciably alter emissions of other PAHs, and the corresponding nitro-PAHs were detected in soot extracts by GC/MS. The mutagenic responses of the extracts are shown in Table 1 with and without the addition of liver S-9. PAHs which are known to form highly active nitro-PAHs do not alter the direct mutagenicity over hexadecane fuel alone, and the mutagenicity decreases with the addition of S-9. Methylbiphenyl is sufficiently volatile so that it is not concentrated in soot extracts; also, it is not known to form highly active nitro-PAHs. The addition of methylidiphenyl to fuel was correlated with a 90% reduction in the direct mutagenicity of soot extracts, but no nitro-methylbiphenyl was detectable in extracts.

Certain other PAHs (methylnaphthalene and benzo(a)pyrene) when added to aliphatic fuels increase soot emissions by two to four fold. These soot promoting PAHs reduce the direct mutagenicity compared to the combustion of hexadecane alone, and the mutagenicity increased following the addition of S-9. Combustion of both methylnaphthalene and benzo(a)pyrene resulted in increased concentrations of high molecular weight PAHs (m/z > 270) as shown in Table 1 as well as increased concentrations of low molecular weight PAHs. Since 1-methylnaphthalene is not itself mutagenic, but results in increased indirect mutagenicity in the extracts, this suggests that some of the high molecular weight PAHs may be active indirect mutagens. Since increased emissions of high molecular weight PAHs was associated with increased soot formation, this suggests that some of the high molecular weight PAHs may be intermediates in the processes of soot formation (or radicals/ions thereof).

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ACKNOWLEDGMENTS

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Table 1
EFFECTS OF PAH ADDITION TO DIESEL FUELS ON EMISSIONS

FUELS	Mutagenicity (rev/μg)		M/Z OF PAHS					
	-S-9	+S-9	202*	202**	252***	300	326	350
Hexadecane	26	20	10	1	1	1	1	1
Hexadecane + 1% Fluoranthene	24	20	27	2	0.2	1	0.1	2
Hexadecane + 1% Pyrene	20	14	2	10	0.1	0.8	1	1
Hexadecane + 1% 4-Methylbiphenyl	3	2	0.5	1	0.08	0.5	2	0.2
Hexadecane + 1% 1-Methylnaphthalene	5	12	4	4	1	17	12	6
Hexadecane + 1% Benzo(a)pyrene	1	26	6	7	13	27	24	27

*Fluoranthene

**Pyrene

***Benzo(a)pyrene

All results are averages of duplicate samples. The major component of soot extracts was unburned hexadecane (50-75%).

@The PAH concentrations in soot extracts were normalized to soot extracts from combustion of hexadecane alone to facilitate comparisons.

CHARACTERIZATION OF POLYCYCLIC-HYDROAROMATIC COMPOUNDS IN SYNFUELS

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A potential decrease in worldwide oil supplies has led to the development of alternate fuels made from oil shale and coal. The increased aromatic and heteroatomic character of these fuels has caused concern over the biological activity of the final product. Studies have shown that additional hydrotreatment decreases the mutagenic character of these fuels by reduction of the levels of polycyclic aromatic hydrocarbons (PAH) and heterocyclic compounds (1,2). Several processes (SRC-II, H-Coal, and ITSL) contain varying amounts and compositions of hydroaromatic compounds ranging from 40% to 80% of the fuel. As a class of compounds in synfuels, hydroaromatics have not been well characterized. A procedure was developed to isolate the hydroaromatics from complex fuel mixtures by fractionation on picric acid columns. The procedure allowed for isolation of aliphatics, hydroaromatics, and PAH from various synfuels. The resulting hydroaromatic fraction facilitated characterization by GC/MS and the biological evaluation of these compounds.

Unlike the parent PAH, which exhibit predominantly molecular ions and known retention indices, the hydroaromatic analogs exhibit complex fragmentation patterns and increased numbers of isomers. Although more complex, these mass spectra are often indicative of the isomeric structure of particular hydroaromatics (see Figure 1). Fundamental interpretation of the fragmentation pattern can often yield structural features of the compound, as well as position of alkyl substituents without standards. Careful examination of the mass spectra of the simpler hydroaromatics (tetralin, hydrofluorenes, hydrophenanthrenes) can be applied to larger compounds consisting of the additional aromatic rings (e.g. benzofluorenes relate to fluorenes, etc.) or consisting of subunits of these hydroaromatics.

Generation of standards has been based on a series of model compound hydrogenations of parent PAH. Using conditions similar to actual coal liquefaction processes has allowed for similar isomeric compositions of hydroaromatics. These model compounds are isolated in larger quantities to develop a data base of retention indices and NMR and mass spectra which can be compared with the actual synfuels. This methodology has been applied to the characterization of several synfuels to determine the exact composition of the hydroaromatic fraction.

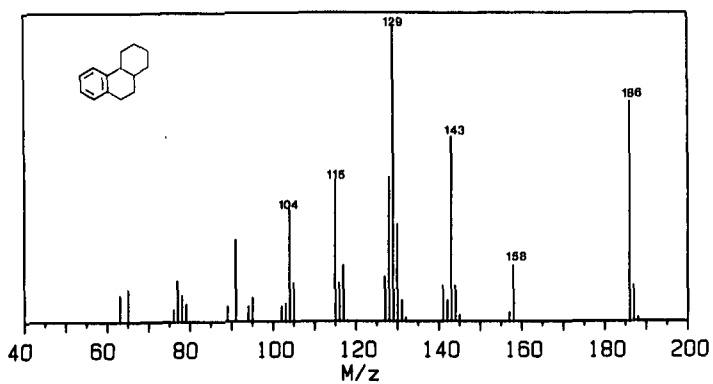
ACKNOWLEDGEMENT

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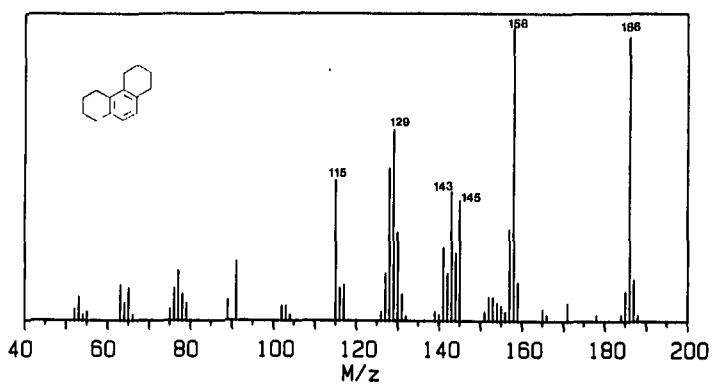
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αs-OCTAHYDROPHENANTHRENE



sym-OCTAHYDROPHENANTHRENE



ANALYSIS OF MIDDLE DISTILLATE FUELS USING HIGH RESOLUTION FIELD IONIZATION MASS SPECTROMETRY

By

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INTRODUCTION

We recently acquired a 1964 vintage MS-9 mass spectrometer and restored it to good working condition. It was then fitted with an SRI field ionization source as reported last year at this conference. Mass resolution exceeds 20,000 when the MS-9 is operated with the field ionization source. This is more than adequate for the analysis of hydrocarbons which normally require a resolution of only 3000-4000 to separate the C-H12 doublet. The MS-9 is now equipped with a Kratos DS-55C data system for the collection and processing of high resolution field ionization mass spectra.

METHOD

For the analysis of distillate fuels, such as jet and diesel fuels, the MS-9 is run at a resolution of at least 4000 to assure that all hydrocarbon doublets are resolved completely. Fuel samples of 2-3 microliters are placed in the sample holder of the heated glass batch inlet. After freezing the sample with liquid nitrogen, the air is pumped away. The sample is then heated and vaporized into the batch inlet expansion volume at a temperature of 225 C. Because the sample is completely vaporized, the gas mixture which bleeds from the batch inlet into the field ionization source is representative of the fuel composition.

Field ionization mass spectra are acquired at a scan speed of 120 seconds per decade. A scan time of 125 seconds plus a magnet settling time of 10 seconds allow one scan to be collected every two and one-quarter minutes. Typically 10-15 scans are collected although the amount of sample used is sufficient to allow two or three times this many to be recorded if desired.

The Kratos DS-55C requires the presence of reference peaks in high resolution mass spectra. These reference peaks are normally produced by introducing perfluorokerosene into the ion source together with the sample. Because of their negative mass defects, the many fragment peaks of PFK are easily resolved from most peaks from the sample. Because field ionization usually produces little or no fragmentation, PFK and the other common reference compounds are unsuitable for use as internal mass references. We have solved this problem by using both added compounds and the common fuel hydrocarbon peaks as references. A mixture of benzene, toluene, and perdeuteromethylcyclohexane is added to each sample when it is placed in the batch inlet. The three molecular ion peaks from these three compounds are used as autostart masses for the time-to-mass conversion software. A combination of saturated hydrocarbon and alkyl benzene peaks are used as reference masses for the rest of the mass range. Several sets of reference peak masses have been tabulated and different ones are used for the different types of fuels.

Time centroid data are usually collected because they can be conveniently reprocessed in the event of an incomplete or unsatisfactory time-to-mass conversion. This can sometimes be a problem because of the highly variable composition of distillate fuel samples. Reprocessing of the data using a different set of reference masses is often necessary in such cases.

The elemental composition of each molecular ion in the field ionization mass spectrum of the fuel sample is calculated from its accurate mass. The data are then sorted into a table of molecular ion peak intensities by both carbon number and z-series. The intensity of each peak belonging to a particular z-series is then divided by a measured field ionization sensitivity factor for that series. After correction for the variation in field ionization efficiency, the data are normalized to a total of 100 mole percent.

RESULTS

The two tables below show the results of the high resolution field ionization analysis of two marine diesel fuels. One of these was typical of such fuels and behaved normally in use and in storage. The other fuel was from a lot which passed all normal tests but began producing large amounts of deposits after only a few weeks of storage. The analysis results are expressed as the mole percent of the total fuel at each value of both carbon number and z-number.

Comparison of the analysis results shows that the unstable fuel has less than 40 percent of the acyclic saturated hydrocarbons of the stable fuel and also has almost twice the level of naphthalenes. The unstable fuel also has significantly higher levels of bicycloalkanes and alkyl benzenes compared to the stable fuel.

Independent analysis of both of these fuels by standard HPLC methods gave results which were in agreement with the sums of the appropriate compound class types within the reproducibility of the HPLC methods used.

The main advantages of the new high resolution field ionization mass spectrometric analysis method are (1) a very detailed analysis is produced in a relatively short time, and (2) the accuracy of the measurements of the amounts of individual compound types is independent of the fuel composition or origin. This second advantage is becoming more important with the increasing variety of processes and feedstocks used to produce distillate fuels.

Stable Fuel Composition by Carbon Number and z-Series

z	2	0	-2	-4	-6	-8	-10	-12	-14	-16	-18
n											
8		0.5			0.7						
9	0.5	1.0			1.5						
10	1.2	1.5	0.5		2.2	0.5		0.4			
11	2.4	2.1	1.2		1.9	1.3		2.1			
12	3.1	2.5	1.6	0.4	1.4	1.5	0.1	3.6	0.2		
13	3.5	2.7	1.6	0.5	1.0	1.3	0.2	2.4	0.4		
14	3.7	2.5	1.7	0.6	0.8	0.9	0.3	1.0	0.6		0.1
15	3.5	2.4	2.0	0.7	0.7	0.5	0.3	0.4	0.4		0.2
16	3.1	2.1	1.3	0.6	0.5	0.3	0.2	0.2	0.2		0.1
17	2.8	1.8	0.7	0.4	0.4	0.2	0.1	0.1			
18	2.2	1.4	0.5	0.3	0.3	0.1		0.1			
19	1.8	1.1	0.4			0.5	0.1				
20	1.1	0.6	0.3	0.2	0.1						
21	0.5	0.3									
Sum	29.4	22.4	12.0	3.6	11.6	7.2	1.3	10.3	1.8	0.0	0.4

Unstable Fuel Composition by Carbon Number and z-Series

z	2	0	-2	-4	-6	-8	-10	-12	-14	-16	-18
n											
8		0.3			1.2						
9		0.8			2.3						
10	0.4	1.2	0.4		3.0	0.9		0.9			
11	0.8	1.9	1.0		2.3	1.7	0.1	4.5			
12	0.9	2.2	1.4		1.7	1.6	0.1	7.2	0.1		
13	1.0	2.3	1.7	0.3	1.0	1.2	0.2	4.3	0.5		
14	1.1	2.1	1.6	0.4	0.7	0.8	0.2	1.3	0.8		0.3
15	1.4	2.1	2.1	0.6	0.7	0.6	0.3	0.7	0.7		0.5
16	1.2	2.0	1.4	0.7	0.5	0.4	0.2	0.3		0.1	0.3
17	1.2	2.2	1.2	0.6	0.5	0.3	0.2	0.1	0.2		
18	1.1	2.3	1.2	0.6	0.4	0.3	0.1				
19	1.1	1.7	1.1			0.8	0.1				
20	0.5	1.2	0.8	0.4	0.2						
21	0.3	0.9	0.4								
Sum	11.0	23.3	14.4	3.7	14.5	8.4	1.6	19.2	2.3	0.1	1.0

QUANTITATION OF 1-NITROPYRENE IN DIESEL

EXHAUST PARTICULATES BY CAPILLARY GC/MS

AND CAPILLARY GC

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The compound 1-nitropyrene (1-NP) accounts for a high percentage of the mutagenicity of the total extract of diesel particulate matter (1), but obtaining accurate quantitative results is difficult because of variable decomposition occurring in the analytical system at the low 10 to 100 ng quantities involved. The procedure described here involves pre-separation of the extract by HPLC and analysis by both capillary GC and capillary GC/MS/SIM. A modified cool on-column injection technique and bonded phase fused silicon capillary column were used to minimize the decomposition of sample and improve the precision for the quantitative analysis. The method is demonstrated using four different diesel particulate extracts containing 1-NP in the 10 to 100 ng/mL range in a complex mixture matrix.

Four CH_2Cl_2 extracts of diesel particulate matter collected from the in-use diesel automobiles were designated as VW-1, VW-2, VW-3 and VW-4. Each sample was fractionated by HPLC using a 10 micron semi-preparation silica column. The moderately polar fraction contained the 1-NP compound. GC and GC/MS/SIM analyses were performed on capillary GC and quadrupole capillary GC/MS with an interface of glass capillary effluent splitter.

The presence of 1-NP in these diesel exhaust samples was confirmed in the GC and GC/MS analyses by comparison of the retention time and mass spectra obtained from the samples with those from the 1-NP standard injected under the same conditions.

In the high resolution GC analysis, the 1-NP peak in the sample chromatogram is reasonably separated from the adjacent GC peaks. Thus, the quantitation of 1-NP in some samples was based on the integrated area of the 1-NP GC peak using an external standard. The calibration curve of 1-NP standard shows good linearity in the analysis range.

The sample giving ambiguous peak area integrated in GC must be cross-checked by GC/MS/SIM. A deuterated 1-nitropyrene (1-NP-d9) was used as an internal standard in this analysis. Three characteristic ions of 1-NP and 1-NP-d9, $(\text{M})^+$, $(\text{M}-\text{NO})^+$ and $(\text{M}-\text{NO}_2)^+$, were selectively monitored. Quantitation was done by comparison of the ion current responses of M^+ of 1-NP with that of 1-NP-d9. Other ions were used for the purpose of confirmation only because their relative abundance changed due to decomposition (2).

The degradation of 1-NP during the GC and GC/MS analyses appears to be a catalyzed thermal decomposition. It has been found that the result of analysis of 1-NP critically depends on the cleanliness of the analytical system. On-column injection at low temperature minimizes the decomposition. However, decomposition is more serious in the GC/MS system because it can occur at both the glass interface and the ionization chamber of the mass analyzer.

The solvent injected on the column at low temperature may strip the stationary phase for the conventional capillary column, leading to an increase in decomposition and a decrease in the reproducibility of both the GC and GC/MS analyses. The use of bonded phase fused silica capillary column minimizes this problem.

For accurate delivery of a small volume of sample (c.a. 1.0 μ l) in cool on-column injection most conventional injection techniques give less reproducible results (3,4). In the modified injection technique, the syring filling sequence is: pure solvent - air space - sample solution - air space. Using this method, a controlled small volume of sample solution can be introduced upon the capillary column at low temperature with high reproducibility.

The recovery of 1-NP in the pre-separation procedure was determined to be 105%. The content of 1-NP determined in the four diesel exhaust sample is tabulated below.

1-Nitropyrene Content in Diesel Particulate Matter

SAMPLE NAME	number of injections	1-NP particulate matter (ng/mg)	Standard Error (ng/mg)
by Capillary GC analysis			
VW-1	2	49.9	2.3
VW-2	3	17.8	1.1
VW-3	2	92.2	2.6
VW-3 (duplicate)	2	102.0	2.1
VW-4	5	67.2	1.9
by capillary GC/MS analysis			
VW-1	2	42.7	0.5
VW-2	2	22.4	0.3
VW-3 (duplicate)	2	103.2	0.6
VW-4	2	64.9	4.5

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MS DETECTION AND CHARACTERIZATION OF NOVEL NITRO-PAH
IN MUTAGENIC FLY ASH EXTRACT

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There has been an increasing interest in the mutagenic activity of extractable organic compounds from fossil fuel combustion products. Much of the attention has centered on rather high concentrations of direct-acting mutagens found in diesel exhaust (1,2) -- especially, 1-nitropyrene (2-5). Mutagenic activity has also been found in conventional coal fly ash, fluidized bed coal fly ash, and particles from gasoline-powered auto engines (6). Presumably as a result of these and other fossil fuel emissions, it is now quite common to find extractable, direct-acting mutagens on the particles from urban air samples (7). The Laboratory for Energy-Related Health Research reported on the mutagenic activity of stack-collected conventional coal fly ash from western low-sulfur, high ash coal burned in a commercial power plant (8). More recent studies on this ash using nitro-reductase deficient strains of *Salmonella typhimurium* have shown that most of the mutagenic activity is due to direct-acting, nitro-organic compounds (9).

In an attempt to identify compounds responsible for the direct acting mutagenicity, samples of fly ash were extracted with 60/40 (w/w) benzene/methanol, the extracts blown to dryness, and the residues separated on a MicroPak CN-10 preparative HPLC column. Twelve cuts were taken and submitted to the Ames assay to determine mutagenicity. Some fractions which were found to be mutagenic were separated further using a MicroPak MCH-10 analytical HPLC column. Selected sub-fractions were submitted for chemical characterization using HPLC, gas chromatography with both universal and nitrogen-specific detection, and mass spectral analysis, including gc/ms, low resolution MS (LRMS), low voltage LRMS (LVLRMS), high resolution MS (HRMS), and metastable ion analysis.

1-Nitropyrene was positively identified in an early-eluting fraction as determined by gc/ms spectra and by gc retention index measurements of nitropyrene and nitrofluoranthenes. A methylated nitropyrene was also identified in the same fraction. The 1-nitropyrene was quantitated at 14 ppb in the fly ash using HPLC measurements, and was calculated to account for .3-1.7% of the total mutagenicity found in the fly ash extract.

Another subfraction was found to contain a homologous series at m/z 223, 237, 251 and 265. On the basis of HRMS measurements (237.0786, $C_{15}H_{11}NO_2$ (.4 mmu) and 751.0945, $C_{16}H_{11}NO_2$ (.3 mmu) and fragmentation patterns found in gc/ms analyses, we have tentatively identified the series as alkylated nitro(phenanthrene/anthracene)s. Nitro-fragmentation patterns (loss of NO , NO_2 , $NO + CO$) were also observed for ions at 236 and 277. The ion at 277 was peak matched at 10,000 resolving power and its mass measured to be 277.0744, which fits $C_{17}H_{11}NO_3$.4 mmu. These data fit for a series of alkylated mono-nitro-fluoranthenes or pyrenes, or perhaps a series of nitrobenzonaphthofurans. The same fraction contains a series tentatively identified as alkylated fluorenones, and a series thought to be alkylated benzantracenones.

A more polar subfraction contained a series of ions at 222, 236, and 250, which are thought to be alkylated (C_1 - C_3) phenanthrenequinones or anthraquinones based on the characteristic losses of H , HCO , $HCO + CO$. A compound with an exact mass of 320.0552, which fits an elemental composition of $C_{16}H_{10}N_4O_4$ to 0.6 mmu, was found in another polar fraction. GC/MS revealed that the compound contained two nitro groups, and it has tentatively been identified as a dinitro-diazachrysene (or chrysene isomer).

Several polar fractions contained compounds with molecular weights of 309, one of which was shown to have an exact mass of 309.0753 by high resolution peak matching. This exact mass fits an elemental composition of $C_{16}H_{11}N_3O_4$ to -0.4 mmu. Other fractions contained compounds with exact masses of 354.0597 and 399.0452, which fit elemental compositions of $C_{16}H_{10}N_4O_6$ and $C_{16}H_9N_5O_8$ to 0.3 mmu and 0.1 mmu, respectively. These molecular formulae correspond to the addition of one and two nitro groups to the $C_{16}H_{11}N_3O_4$ compounds. (It is possible, from the elemental compositions, that the 399, 354, and 309 compounds are tetra-, tri-, and di-nitro substituted, respectively). High resolution mass assignments made on fragment ions in the spectra of the m/z 354 and 399 compounds are consistent with the presence of at least two and three nitro groups, respectively. However, the losses of $CO(NO_2)_2$ from the 399 and one of the 354 compounds suggest that carbonyl groups might be present - presumably in the form of quinones. The

loss of $\text{CO}(\text{NO}_2)_2$ from $\text{C}_{16}\text{H}_9\text{N}_5\text{O}_8$ was confirmed by unimolecular metastable analysis, as were the losses of HNO_2 and HNO_3 . Although several structures can be drawn accounting for the elemental compositions, tentative identification must await further characterization by IR and NMR, if possible.

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SUMMARY

Accurate quantitative data for selected polycyclic aromatic hydrocarbons present on urban dust can be obtained by using a simple procedure consisting of: sonic probe extraction with cyclohexane; clean-up with Florisil®-XAD-2®, and measurement with high resolution gas chromatography and flame ionization detection (HRGC/FID). The analysis can be further simplified by eliminating the clean-up step if HRGC/electron impact mass spectrometry (MS) is available. Both the FID and MS methods give results consistent with those obtained by time-consuming standard procedures. The direct HRGC/MS procedure, combined with chemical ionization, can also be applied to the determination of polycyclic organic materials present in solvent refined coal, shale oil and crude oil.

EXPERIMENTAL

Extraction. An Artex-Sonic Dismembrator equipped with a 3/4" probe and operated at 240 watts was used to extract 0.25 g samples with 30 mL of solvent. Sonication time was 10 minutes after which the solution was filtered and the sample extracted two more times with fresh solvent. The three extracts were combined and reduced to 1 mL.

Clean-up. The extract was transferred to a 0.5 cm I.D. glass column containing 1 g of Florisil®, and then eluted with 15 mL benzene. The collected benzene was concentrated to 0.5 mL and transferred to a 1.4 cm I.D. glass column containing 9 g of XAD-2® wetted with ethanol. A clean PAH fraction was obtained using a modification of the procedure reported by Spitzer¹. Eluates of the XAD-2® column with 25 mL ethanol and 25 mL pentane were discarded. The PAHs were then eluted with 25 mL benzene. The collected benzene was reduced to 1 mL.

Equipment. The HRGC analysis were performed on a Carlo Erba Fractovap equipped with a 30 m DB-5 fused silica capillary column and a FID. The HRGC/MS analyses were performed on a Finnigan 4000 GC/MS equipped with a 30 m DB-5 fused silica capillary column and operated in the multiple-ion-monitoring mode. Ionization was accomplished with electron impact or chemical ionization with isobutane reagent gas and ion source pressure of 0.3 Torr. External standards were used for quantitation. All solvent reductions were performed with a rotary evaporator.

RESULTS AND DISCUSSION

The NBS has issued certified values for the concentrations of five PAHs in SRM 1649, a time-integrated, homogenized sample of Washington DC urban dust. The results of our analyses of this SRM are compared to these NBS certified values in Table 1. The average difference between values is 8%. Comparable reproducibilities were obtained by the two methods. These results are used as validation of the simple and rapid methodology for the determination of selected PAHs in samples as complex as urban dust.

The simplified procedure was applied to other PAHs in SRM 1649 and to PAHs on particles from other sources. The results of these analyses are listed in Table 2. Quantitative values for five additional PAHs in SRM 1649 are given in columns 2 and 3. The PAH content of St. Louis urban dust was also measured and the values are given in column 4. For comparison, the NBS uncertified values for this same dust are listed in column 5. The PAHs measured in a sample of fly ash from a coal-fired power plant and flue tar from a wood burning stove are shown in columns 6 and 7.

If GC/MS instrumentation is available, the analytical procedure for PAHs can be made even simpler. The increased selectivity of the mass spectrometer operated in the multiple-ion-monitoring mode enables the analyst to bypass the Florisil®-XAD-2® clean-up. Direct HRGC/MS analysis, using electron impact (EI), of a cyclohexane sonic probe extract of SRM 1649 was performed and the results agree to within 15% of the results obtained using a clean-up procedure and HRGC/FID.

The direct HRGC/MS procedure, using chemical ionization (CI) and isobutane as the reagent gas, has also been applied to the analyses of polycyclic organic materials (POMs) in other complex mixtures. Samples of solvent refined coal, shale oil and crude oil were diluted 100/1 in hexane and analyzed directly for selected POMs. The results of these analyses listed in Table 3 agree with those obtained in an interlaboratory study.

CONCLUSION

The simplified procedures used to determine twelve PAHs and three POMs on urban dusts and in process streams can certainly be extended to determine other organic compounds. In each case, the additional compounds need only be tested and the procedure finely tuned to obtain quantitative results.

The simple Florisil®-XAD-2® chromatography is not applicable to class separation of all the components present in complex mixtures. The reasons for this conclusion are the known irreversible adsorption of some components on the Florisil® and the insufficient documentation of the separating capability of the XAD-2®.

¹T. Spitzer, J. Chromatogr., 1982, 237, 273.

Table 1. Comparison of the Analysis of Washington Urban Dust SRM 1649.

Compound	Concentration in µg/g	
	NBS Certified	This Study
Fluoranthene	7.1 ± 0.5	7.6 ± 0.5
Benz(a)anthracene	2.6 ± 0.3	2.9 ± 0.4
Benz(a)pyrene	2.9 ± 0.5	2.7 ± 0.6
Indeno(1,2,3-cd)pyrene	3.3 ± 0.5	3.1 ± 0.6
Benz(ghi)perylene	4.5 ± 1.1	5.0 ± 1.1

Table 3. Analyses of Polycyclic Organic Materials Present in Complex Samples by Direct HRGC/CIMS.

Compound	Concentration in µg/g		
	SRG	Shale Oil	Crude Oil
Pyrene	200	161	11
Benz(a)pyrene	250	14	1
Benz(a)pyrene	250	NA ^a	0.5
Dibenzthiophene	1064	NA	41
Acridine	158	NA	13
Carbazole	1913	NA	6
1-Methylnaphthalene	NA	NA	0.6
2-Methylnaphthalene	NA	NA	0.3

^aNA = Not analysed.

Table 2. HRGC/FID Analyses of PAHs in Cleaned-up Extracts from Various Particle Samples.

COMPOUND	Concentration in µg/g					
	Washington Urban Dust - SRM 1649		St. Louis Urban Dust		Fly Ash ^a	Flue Tar ^b
	This Study	NBS Uncertified	This Study	NBS Uncertified		
Phenanthrene	3.3	4.5	4.5	NR ^c	ND ^d	70
Fluoranthene	a	a	7.1	7.9	0.3	143
Pyrene	5.8	7.2	5.9	7.4	0.7	178
Benz(a)anthracene	a	a	2.6	2.8	ND	170
Chrysene	4.3	3.7	6.9	6.6	ND	225
Benz(a)pyrene	a	a	2.4	2.6	0.07	115
Benz(a)pyrene	3.3	3.3	3.6	NR	ND	75
Indeno(1,2,3-cd)pyrene	a	a	3.6	4.8	ND	91
Benz(ghi)perylene	a	a	5.6	5.5	ND	74
Dibenz(ah)anthracene	0.4	0.4	ND	NR	ND	ND

^aElectrostatic precipitator ash from a coal-fired power plant.

^bTaken from the chimney of an air-tight wood burning stove.

^cNR = Not reported.

^dND = Not detected at detection limit of 0.05.

^eCertified values given and compared in Table 1.

Determination of Poly-Aromatic Hydrocarbons in Natural Marine Sediments
by Use of a Simple LC/MS Interface.*
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The use of marine resources is intensifying and therefore accurate analysis of marine materials becomes increasingly important. As a consequence, reference materials are required. The Marine Analytical Chemistry Standards Program (MACSP) was established by the National Research Council of Canada to meet present and future needs for marine based standards and reference materials.

Poly-aromatic hydrocarbons (PAH) and their derivatives occur widely in marine sediments and many of these compounds show carcinogenic / mutagenic characteristics. Many routine analytical procedures developed use high-pressure liquid chromatography. In the majority of samples of interest, PAH are extracted with a complex array of other organic compounds which can physically prevent successful chromatography, cause interfering peaks or excessive shifts in the base line.

The objective of this study was three fold:

- 1) Evaluate the feasibility of using LC/MS with a simple interface to determine PAH in extracts from marine sediments;
- 2) Determine the PAH compounds present and more thoroughly characterise the matrix;
- 3) Compare results with those from other complementary analytical techniques.

The liquid chromatograph employed was a Spectra Physics 8000 equipped with a data system. The extract was chromatographed on a reversed phase column (Brownlee RP-18 5 μ cartridge 2.1mm x 22cm) using as eluant a mixture of methanol/water (3/1) pumped at a flow rate of 100 μ l/min.

Mass spectra were obtained on a Finnigan MAT 4500 instrument interfaced with an INCOS data system. Indicated temperatures for the source, manifold and separator oven were 160°, 125° and 260°C, respectively. Ioniser pressure (indicated) was 0.65 torr.

The interface, similar to some previously reported (1,2,3), consists of a simple nebuliser constructed from glass tubing and 'Swagelok' fittings with a heated spray chamber coupled to a jet separator of the type commonly used as an interface in packed column GC/MS. The total effluent is introduced via the interface into the jet separator.

The general performance of the interface was found to be adequate for the objectives of the study. It could be operated reliably over lengthy periods of time though it was difficult to maintain a constant nebulisation. The nebuliser was critical to the performance of the interface. As reported by others (3) uneven nebulisation gave rise to bursts of solvent which caused strong pressure fluctuations in the source, signal fluctuations and low precision in the measurements. A high background signal extending over a wide mass range

*NRCC No. 21106

was due to ion-molecule reactions involving the eluant. The instrument was usually scanned so these major signals would be avoided.

A limitation of this type of interface is that only chemical ionisation data is obtained and this by use of the eluant as the reagent gas. In the determination of PAH this is not a severe limitation, since the mass spectral information available is that of molecular weight in the form of quasi-molecular ions. This is shown clearly in a spectrum of anthracene studied as an individual component (see Fig. 1). Selected ion monitoring (SIM) is suitable for this type of experiment and allows identification of targeted compounds. Selected mass chromatograms of protonated molecules from samples obtained from the marine sediment extract are given in Fig. 2.

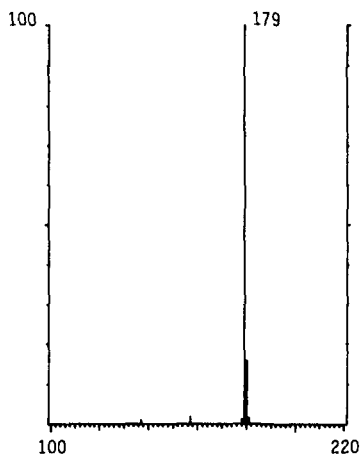


Fig. 1. Anthracene Spectrum

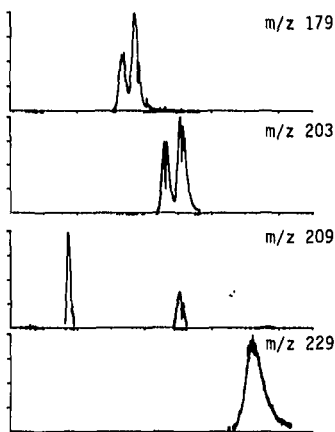


Fig. 2. Selected Mass Chromatograms

Although the LC conditions were inadequate for such an extract the utility of the interface could be evaluated. Many limitations were found, the major one being sensitivity. The simplicity and low cost make the technique attractive.

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APPLICATION OF A SAMPLING PROBE-MASS SPECTROMETRIC TECHNIQUE FOR MEASUREMENT OF THE CONCENTRATIONS OF GASES IN RAMJET COMBUSTOR FLOWFIELDS*

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For several years, the Ramjet Technology Branch, Air Force Wright Aeronautical Laboratories has supported research on combustion phenomena, with the primary objective of developing a superior combustor design for volume-limited ramjet applications. The principal data which are necessary for evaluating the performance of a ramjet combustor are the detailed concentration profiles of the major compounds and species present in the combustor flowfield. In this presentation, the development and application of a sampling probe-mass spectrometer system for monitoring the concentrations of various gases in ramjet combustor flowfields will be described.

In order to minimize potential sampling errors which are inherent in the use of a sampling probe, and which can arise from various effects, such as aerodynamic and thermal disturbances, inadequate quenching, surface interactions, mass discrimination, and soot formation, a radically new type of probe was designed and constructed. This probe (Fig. 1) consists of a quartz microprobe tip, coupled with a water cooled stainless steel body, in order to provide the required physical support and protection. Fig. 2 shows the results of a theoretical analysis of the gas flowfield within such a probe under sampling conditions which are characterized by high temperature, high gas velocity and high pressure, as is typically the case in a ramjet combustor flowfield. A quantitative estimate of the capabilities of the new probe for sampling a typical gas, such as CO, was made by using the calculated gas flowfield temperature, velocity, density, and pressure profiles along with the known oxidation rate of CO as a function of temperature and pressure. The results of this analysis demonstrate that the new probe system can provide more valid sampling data than can be obtained with conventional probes.

Following several laboratory heating tests of initially-constructed probes, which resulted in various design changes, a prototype of the quartz-stainless steel probe was fabricated, and field tested by inserting it into a ramjet combustor flowfield (Fig. 3). The combustor test facility employed is essentially a coaxial dump combustor which consists of a 6 in. I.D. x 18 in. long stainless-steel combustor chamber, an inlet section of 3 in. I.D., and a nozzle of 4.24 in. I.D. Liquid fuel (JP-4) is injected into the inlet section through a set of 8 injection ports upstream of the inlet duct exit. Six combustion sampling tests were conducted, each one lasting for 2 minutes. The experimental parameters varied during these tests included fuel/air ratio (0.027 and 0.046), inlet air temperature, and air mass flow rate. The combustor temperature ranged from 2360 to 3408°R, while the combustion efficiency was found to vary between 79.9% and 94.4%. A batch combustor sampling stand was employed to collect the sampled gas in several reservoir bottles. The collected sample was then analyzed using a quadrupole mass spectrometer system. The results of this preliminary combustor probe sampling experiment, which will be presented, were quite promising, although it was found that some additional changes in the probe design are desirable. A second prototype probe is currently being constructed.

The analytical instrumentation used in these studies and the application thereof for combustor sampling will be described. A gas chromatograph has recently been interfaced to the quadrupole mass spectrometer used in this system in order to enhance the analytical capabilities. The mass spectrometer system incorporates a 16-channel digital mass programmer and 16-channel demultiplexer which are employed for operation of the mass spectrometer in the selected-ion monitoring mode. Ion signals are displayed on a 20-channel oscillograph recorder, and are also acquired by a computer system. Still further improvements to the mass spectrometric sampling system are in progress. Fig. 4 shows a new molecular beam mass spectrometer system currently being constructed. This system will be directly interfaced to the ramjet combustor for in-situ measurements of the concentration of gaseous species present in the combustor flowfield, including transient species.

* This work was supported under AFWAL Contract No. F33615-81-C-2086.

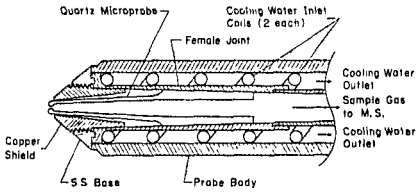


Fig. 1. First Prototype of the Quartz-Stainless Steel Probe.

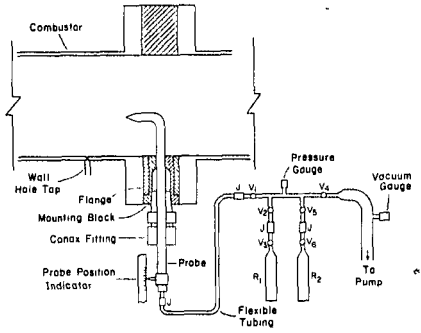


Fig. 3. Schematic Illustration of a Portable Batch Sampling System.

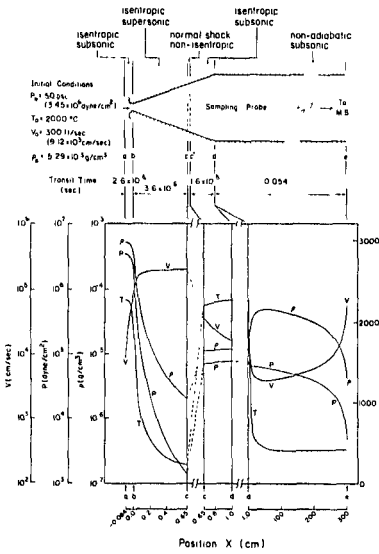


Fig. 2. Schematic Diagram of the Gas Probe Sampling System and Plot of the Flowfield Profiles.

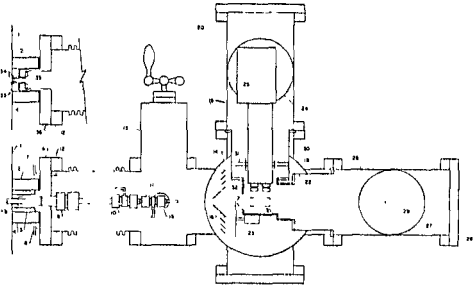


Fig. 4. Schematic Diagram of a Molecular Beam Mass Spectrometric Gas Monitoring System Under Construction.

IDENTIFICATION OF THE COMPONENTS OF FOSSIL FUELS COMBUSTION
PRODUCTS BY A COMBINATION OF GC/MS AND GC/FTIR DATA

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Although GC/MS is a very powerful tool for mixture analysis, its application to the identification of polycyclic aromatic compounds (PAC) is hampered by the similarity in the mass spectra of many of the isomeric and isobaric PAC. In order to positively differentiate these compounds, one then has to depend on the gas chromatographic retention behavior. For this to be reliably used, authentic samples or their retention data must be available for all possible isomers and they must differ sufficiently to be clearly differentiated. This is, however, not often feasible when one considers that the larger the molecules become and the more substituents they have, the more isomers are possible, and this soon reaches the point where it is not feasible to acquire all the necessary reference compounds.

In order to achieve a more definitive identification of these compounds, ancillary GC/FTIR data are acquired to complement the GC/MS experiments. The complementarity of these spectral data was evaluated by the analysis of two relatively complex samples including an extract of the combustion products of coal and a solvent refined coal liquid. Specific examples are presented in which the combination of the two techniques resulted in identifications which could not have been possible using either technique alone.

Thus, in the coal combustion products, the combined spectral data demonstrated that many mass spectrometric equivalent pairs, eg: 1- and 2- substituted naphthalenes including the methyl, ethyl, cyano and phenyl homologs and phenanthrene/anthracene can be easily differentiated by the arC-H bending frequencies from 900 to 700 cm^{-1} (Fig. 1). The same signals can also be used to interpret the substitution patterns in more condensed ring systems.

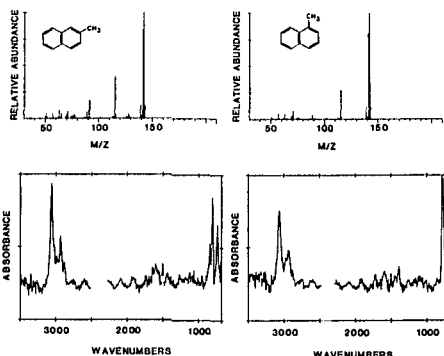


Fig. 1. Mass spectra and infrared spectra of 1- and 2- methylnaphthalene. The spectral region from 2450-2300 cm^{-1} in the IR was not reported due to CO_2 background.

The presence of a carbonyl band can be used to recognize aromatic ketones, and the frequency further distinguishes the ring size, eg: 9-fluorenone (1736 cm^{-1}) and 1-phenalenone (1662 cm^{-1}). The C-O-C stretching frequency (near 1200 cm^{-1}) identifies aromatic ethers and differentiates dibenzofuran and its methyl and benzo homologs from other isomeric or isobaric PAC. The values of the C-O-C stretching frequencies together with the corresponding arC-H bending frequencies further aid in distinguishing the methyl dibenzofuran isomers. The acetylenic and allenic stretching frequencies also confirm the presence or absence of compounds containing these functional groups, eg: phenylacetylene, indene and p-methylphenyl propadiene (Fig. 2).

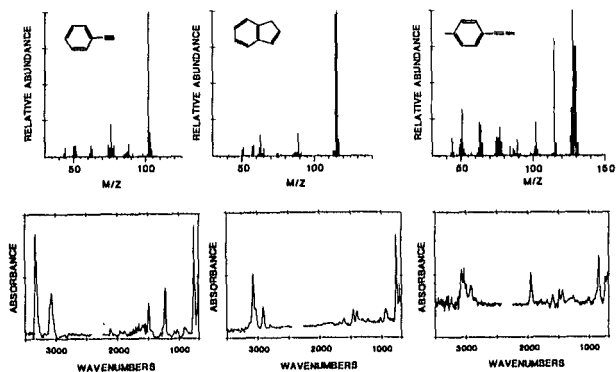


Fig. 2. Mass spectra and infrared spectra of phenylacetylene, indene and p-methylphenyl propadiene.

The combined spectral data again provide valuable information in the identification of nitrogen-containing PAC in solvent refined coal liquid. Although the mass spectral data could not differentiate the amino-substituted PAC from the isomeric azaarenes, the infrared data unequivocally distinguish them from their N-H stretching (3500 to 3400 cm^{-1}) and bending frequencies (near 1600 cm^{-1}). Thus, in the coal liquid, one of the major components is identified as carbazole and differentiated from isomeric amino-acenaphthylene (Fig. 3). Similarly, alkyl homologs of carbazole are identified and distinguished from the corresponding alkyl substituted amino-acenaphthylenes.

Since the IR spectra are indicative of the presence or absence of certain structural features in isomeric and isobaric PAC and the mass spectra reveal their molecular weight, the combination of the two sets of data logically complements each other, and when used together for GC analyses, will greatly improve the conclusive characterization of most of the components.

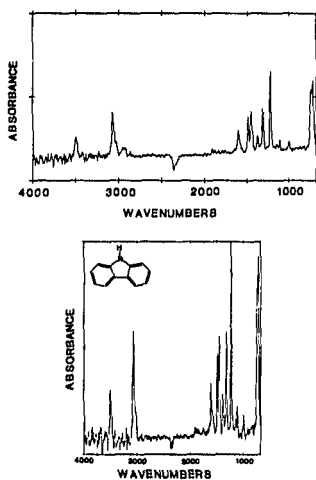


Fig. 3. Infrared spectra of carbazole in solvent refined coal liquid (top) and reference carbazole standard (bottom).

In the process of identifying combustion products from home heating fuel, we have become particularly interested in the heteroatom-containing polycyclic aromatic compounds. Following collection of the combustion effluent from a domestic burner, the XAD-2 extract was first fractionated on an alumina column (1), and subsequently analyzed by several complementary techniques. One of these techniques, gas chromatography-high resolution mass spectrometry (GC-HRMS), makes it possible to differentiate compounds with identical nominal mass, similar mass spectra, but different elemental composition. For example, Fraction X-2 (2nd fraction, eluted with benzene) was found to contain alkyl-naphthalenes (C-1 to C-5) and alkyl-dibenzothiophenes (C-1 to C-3), some of which have the same nominal mass. Fraction X-3 (3rd fraction, eluted with chloroform) was found to contain aromatic ketones but no nitrogen heterocycles which, when present, elute in this fraction.

In the work presented here, a five component standard mixture and two fractions were analyzed by GC-HRMS. Figure 1 is the Total Ion Plot and selected mass chromatograms of the GC-HRMS acquisition of Fraction X-2. Table 1 is a partial listing of the elemental compositions representative of that fraction. Conditions for this analysis included scanning a mass range of 100-250 amu, 2.2 sec/decade scanrate, 0.8 sec interscan time, 20 kHz digitizing frequency and a dynamic resolution of 3500. The instrument used was a Finnigan MAT 212 with an SS200 data system.

Also shown is the GC-HRMS of an X-3 fraction shown to contain two $C_{13}H_{10}O$ isomers (Figure 2). One isomer, phenalene, has the same nominal mass, the same mass spectra and a retention index very close to that of benzo(c)cinnoline ($C_{12}H_8N_2$) (2).

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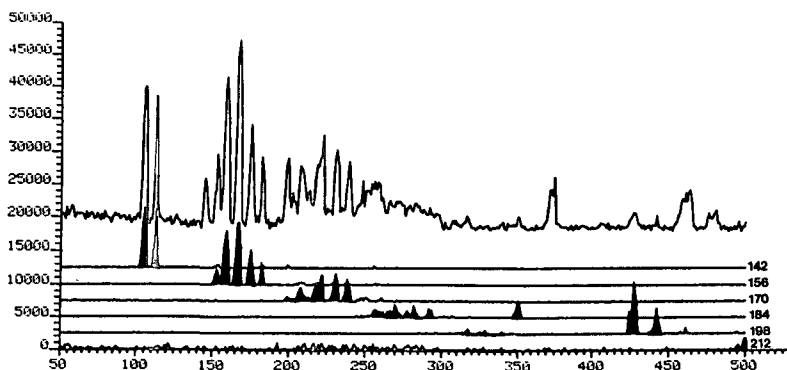
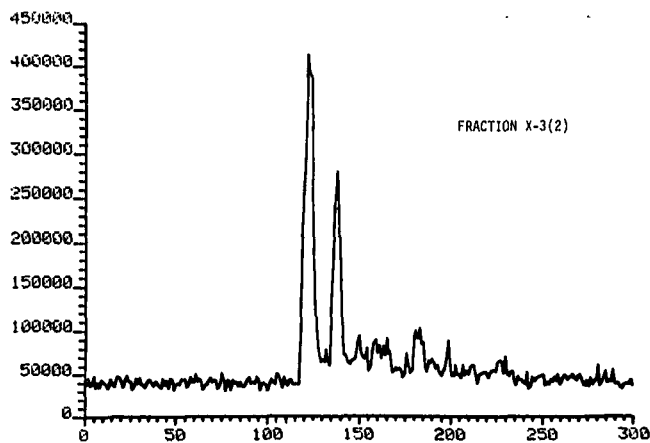


Figure 1. Total Ion Plot.

TABLE 1. FRACTION X-2

SCAN #	CALCULATED MASS	EXACT MASS	DIFFERENCE (mmu)	ELEMENTAL COMPOSITION	COMPOUND
103	142.0780	142.0782	0.2	C ₁₁ H ₁₀	2-METHYL NAPHTHALENE
113	142.0777	142.0782	0.5	C ₁₁ H ₁₀	1-METHYL NAPHTHALENE
151	156.0964	156.0939	2.5	C ₁₂ H ₁₂	C-2 NAPHTHALENE
175	156.0967	156.0939	2.8	C ₁₂ H ₁₂	C-2 NAPHTHALENE
208	170.1098	170.1095	0.3	C ₁₃ H ₁₄	C-3 NAPHTHALENE
218	170.1077	170.1095	1.8	C ₁₃ H ₁₄	C-3 NAPHTHALENE
240	170.1104	170.1095	0.9	C ₁₃ H ₁₄	C-3 NAPHTHALENE
255	184.1243	184.1252	0.9	C ₁₄ H ₁₆	C-4 NAPHTHALENE
282	184.1255	184.1252	0.3	C ₁₄ H ₁₆	C-4 NAPHTHALENE
317	198.1427	198.1408	1.9	C ₁₅ H ₁₈	C-5 NAPHTHALENE
352	184.0309	198.0347	3.8	C ₁₂ H ₈ S	DIBENZOTHIOPHENE
427	198.0477	198.0503	2.6	C ₁₃ H ₁₀ S	C-1 DIBENZOTHIOPHENE
442	198.0500	198.0503	0.3	C ₁₃ H ₁₀ S	C-1 DIBENZOTHIOPHENE
496	212.0660	212.0659	0.0	C ₁₄ H ₁₂ S	C-2 DIBENZOTHIOPHENE
500	212.0619	212.0659	4.0	C ₁₄ H ₁₂ S	C-2 DIBENZOTHIOPHENE



SCAN #	CALCULATED MASS	EXACT MASS	DIFFERENCE (mmu)	ELEMENTAL COMPOSITION	COMPOUND
161	180.0565	180.0575	1.1	C ₁₃ H ₈ O	FLUORENONE
197	180.0575	180.0575	0.0	C ₁₃ H ₈ O	PHENALENONE

Figure 2.

MASS SPECTRA OF AROMATIC HYDROCARBON METABOLITES FOUND IN BILE OF FISH FROM THE DUWAMISH ESTUARY.

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Aromatic hydrocarbons (AHs) are routinely reported in environmental samples, particularly sediments [1]. However, when tissues from fish that live in contact with highly contaminated sediments are analyzed, the AH concentrations found in them are quite low. Several laboratory exposure studies show that AHs are readily metabolized by fish [2,3]. Moreover, there is evidence that metabolites may be more toxic to organisms than the parent hydrocarbons [4,5]. A method has been devised to isolate and identify AH metabolites formed during laboratory exposure studies [6], although little has been done to analyze for them in environmentally exposed biota. Accordingly, we have developed a method to isolate AH metabolites from the bile of English sole (*Parophrys vetulus*). The bile of a fish caught in the Duwamish estuary (Seattle, WA.), an area polluted with many different AH compounds, was analyzed to identify AH metabolites by their mass spectra.

EXPERIMENTAL

A fraction of bile containing conjugates of the AH metabolites was isolated using HPLC/fluorescence. Following hydrolysis, a Du Pont Prep I extractor/concentrator was used to isolate all liberated AH nonconjugates. Another portion of the extract was treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) to form the trimethylsilyl (TMS) ethers of the hydroxylated compounds. Both portions were analyzed by GC/MS using a HP 5840A GC interfaced to a Finnigan 3200 MS/INCOS 2300 data system. A 30m x 0.25mm fused silica WCOT capillary column coated with DB-5 was used in the GC, and the temperature was programmed from 80° to 300°C at 4°/min. The MS was run in EI mode with a one second scan time from 45 to 545 m/z at 70 eV ionization energy. All identifications and quantitations were done by GC/MS.

RESULTS

Metabolites of several AHs in the hydrolyzed bile of a fish from the Duwamish Waterway were identified by GC/MS analysis (Table I). However, the primary metabolite of phenanthrene in flounder is reported to be 1,2-dihydro-1,2-hydroxyphenanthrene [7], rather than the phenanthrols. To determine if dihydrodihydroxyphenanthrene had been dehydrated in the GC, the hydrolyzed sample was derivatized. A peak with a mass spectrum similar to that reported by Solbakken et al. [8] for the diTMS ether of 1,2-dihydro-1,2-dihydroxyphenanthrene was identified, but there were no detectable TMS ethers of phenanthrol. The TMS ethers of biphenylol and hydroxyfluorene were identified in the derivatized sample. No trace of identifiable xenobiotic compounds were found in a fish from Meadow Point (reference area). Some of the mass spectra of the metabolites were presented in the session.

CONCLUSIONS

Metabolites of phenanthrene, anthracene, fluorene, biphenyl and dimethylnaphthalene have been detected, without derivatization, by GC/MS. However, because of the apparent thermal instability of AH dihydrodiols, it is necessary to derivatize them for identification by GC/MS. Unfortunately derivatization reduces the sensitivity for detection ca. 10 times. Therefore GC/MS analysis of the extract before and after derivatization is necessary both to detect the dihydrodiol metabolites and to obtain maximum sensitivity for the other metabolites.

TABLE I. GC/MS quantitation of polar xenobiotic compounds in hydrolyzed bile of an English sole from the Duwamish Waterway. Three replicate samples were analyzed.

Scan No.	Compound*	Amount (ng/g wet wt)	R.S.D. (n=3)	Quantitation method**
1645	Dimethylnaphthol	410	51	B
1692	Methylnaphthalenemethanol	87	26	B
1745	9-Hydroxyfluorene	890	52	A
1851	Biphenylol	1370	26	B
2074	Hydroxyfluorene	3500	37	B
2137	9,10-Anthraquinone	4800	31	A
2323	Phenanthrenecarboxaldehyde	560	38	B
2400	Phenanthrol***	19000	39	B
2416	Phenanthrol***	16000	42	B
2587	Anthracenecarboxaldehyde	2700	44	B

* The internal standard, hexamethylbenzene, elutes at scan no. 1185. Other compounds tentatively identified, but not quantitated: phenanthrene, dibenzofuranol, dibenzodioxin, and benzonaphthofuran. Several peaks remained unidentified; some appeared to be N- or S-containing compounds. Xenobiotic compounds were not found in bile of a fish from Meadow Point (reference area).

** GC/MS estimation of amounts using response factor of (A) the reference compound; (B) an isomer of the compound.

*** The phenanthrol isomers may result from dehydration of 1,2-dihydro-1,2-dihydroxyphenanthrene in the gas chromatograph.

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MULTITECHNIQUE APPROACH TO THE ANALYSIS OF SHALE OIL

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Introduction

Liquids derived from oil shale constitute an important resource for both synfuel and specialty chemicals production. The presence of olefins and high levels of polar compounds makes shale oils much more difficult to characterize than petroleum or coal liquids. An in-depth characterization requires the use of several analytical techniques.

Experimental

Approximately 10-15 gram samples of various shale oils were fractionated using preparative liquid chromatography. A Waters Prep 500A liquid chromatograph was used to separate each deasphalted sample into a saturate + nonaromatic olefin fraction, four aromatic fractions, and a polar fraction, using a Prep PAK NH_2 cartridge and a Prep PAK silica cartridge in series. Pentane was used to elute the aliphatic and aromatic fractions; polars were recovered by backflushing the columns with methylene chloride. The nonaromatic olefins were separated from the saturates on a 20% AgNO_3 on silica column, using pentane to elute the saturates and methylene chloride to elute the olefins.

Mass spectrometry data were obtained using the following instruments: a Kratos MS50 high resolution mass spectrometer, a CEC 103, and a Finnigan 4023 GC/MS instrument. NMR data were obtained with a Varian XL-200 instrument.

Results

A typical prep LC chromatogram for a shale oil is shown in Figure 1. Fraction "A" contains paraffins, naphthenes, and nonaromatic olefins. Aromatic hydrocarbons in the -6, -8, and -10 series and thiophenes are the major components in Fraction "B". Both NMR and GC/MS show the presence of significant amounts of aromatic olefins, e.g. styrenes, in that fraction. The major components in Fraction "C" are aromatics in the -10 (including indenenes), -12, and -14 series, benzothiophenes, and benzofurans. Fraction "D" contains mostly 2-3 ring aromatics including biphenyls, fluorenes, phenanthrenes, dibenzothiophenes, and dibenzofurans. Fraction "E" contains mostly 3+ ring aromatics including phenanthrenes, pyrenes, benzofluorenes, and terphenyls. The polar fraction contains large amounts of 1-4 ring phenols, 1-2 ring dioxygen compounds, 1-4 ring nitrogen compounds, and aliphatic ketones and nitriles.

The Prep LC separation of the shale oils simplified the analyses by all of the techniques used. An example of how the separation simplified the GC/MS analysis is shown in Figure 2. The top part of the Figure shows a small portion of the GC/MS reconstructed ion chromatogram for a total shale oil (material boiling between 395 and 415 degrees F). The bottom part of the figure shows the corresponding RIC for the 1-2 ring aromatic fraction from the LC separation. Components in the separated fraction are much more easily identified than in the total oil. The LC separation makes it possible to obtain a more detailed characterization of shale oils than can be obtained on unseparated samples. For example, sulfur compounds are difficult to characterize in shale oil by high resolution mass spectrometry. With most commercially available high resolution mass spectrometers, the molecular ions from sulfur compounds are difficult to separate from the corresponding molecular ions from larger-ring aromatic hydrocarbons, e.g. the $\text{C}_{17}\text{H}_{20-16}$ series compounds interfere with the $\text{C}_{17}\text{H}_{20-6}\text{S}$ compounds. In the LC fractionation, the larger-ring compounds are separated from the lower-ring sulfur compounds, and thus the interference is minimized.

Both proton and C_{13} NMR were used to provide detailed information on the different olefinic types in shale oil. In addition, the use of "2D" NMR, a relatively new technique, made it possible to obtain useful information about the olefins even in very complex samples. The

C13 and proton spectra are obtained together in the "2D" technique, making it possible to directly correlate the proton signals with the C13 signals. GC/MS can also provide information about the olefins, especially the 1- and 2- olefins; but many of the other olefins are difficult to distinguish from naphthenes. Low resolution mass spectrometry was very useful in analyzing the fractions from the 20% AgNO₃ on silica separation. Both GC/MS and NMR showed that the separation of olefins from saturates was quite good, although the total recoveries of the fractions from the separations were low (<60%). The AgNO₃ did not appear to change the distribution of the olefins, however.

Using the multitechnique approach, we were able to determine differences as well as similarities in shale oils from various sources. Each technique has its limitations, but the combined data from the different analyses gave a more reliable characterization of the shale oils.

Figure 1.

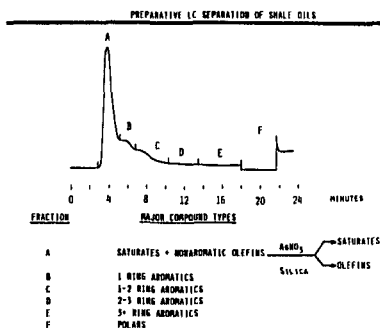
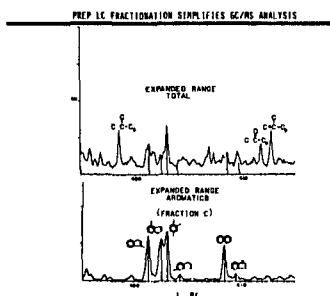


Figure 2.



RAPID ANALYSIS OF TRACE AROMATICS AND POLAR COMPOUNDS IN FOSSIL FUEL DISTILLATES

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This presentation deals with the rapid, accurate and inexpensive determination of trace saturate, aromatic and polar components in fossil fuel distillates. The methodology selected consists of combining the separation capabilities of prep scale high pressure liquid chromatography with the identification and quantitative determination capabilities of high and low resolution mass spectrometry and GC/MS.

Examples of the calculations used to determine total aromatics in saturates from the 50 to 10,000 ppm level and of detecting individual aromatic homologs of the 2 to 60 ppm level are given in Tables 1 and 2. Table 1 includes typical data on the good agreement found between this and other methods on total aromatics; the prep HPLC + MS or GC/MS method is preferred because it is the only one that gives information on individual components. The excellent reproducibility of the overall method and of the MS step alone are illustrated in Tables 3 and 4.

Determination of hydroxy-aromatics at the parts per million to parts per billion level is illustrated in Table 5. Particularly noteworthy is the close agreement between high resolution MS and GC/MS data (Table 6).

Determination of a few parts per billion anilines is illustrated in Table 7. As in the case of the hydroxy-aromatics, determinations at these very low concentration levels were made possible mainly because of the very high sensitivity to sample amount required ratio of the high resolution mass spectrometer used. This table also shows that separation plus MS data on total N are compatible with direct determination, although the latter has a much higher detectability limit.

Table 1			
TRACE AROMATICS IN PETROLEUM DISTILLATES			
A. PREP HPLC + MS LUNA	WEIGHT PERCENT		
	DISTILLATE A	DISTILLATE B	
AROMATICS IN TOTAL SOLVENT (MS)	---	1.15	
AMOUNT OF SAMPLE COLLECTED AS "AROMATIC" FRACTION BY PREP HPLC	0.80	2.25	
AROMATICS IN "AROMATIC" FRACTION (MS)	0.65	54-70	
AROMATICS IN TOTAL SOLVENT	0.0053**	1.22**	
B. UV CROSS-CHECK			
AROMATICS IN TOTAL SOLVENT	0.0220	1.14	
C. ANALYTICAL HPLC CROSS-CHECK	0.0075	---	
* BELOW LOW RES. MS DETECTABILITY LIMIT OF ABOUT 0.1 PPM.			
** BEST VALUE			

Table 2			
DISTRIBUTION OF AROMATICS BY LOW RES. MS (PARTIAL DATA)			
COMPOUND	CONCENTRATION, PPM		
	DISTILLATE A	DISTILLATE C	DISTILLATE B
20 H/MS			
C ₉	4	2	21-4
C ₁₀	23	5	24-5
C ₁₁	14	---	55-2
C ₁₂	7	---	44-3

Table 3

REPRODUCIBILITY OF OVERALL DETERMINATION OF TRACE AROMATICS

COMPONENT/TYPE	CONCENTRATION, PPB			
	DISTILLATE 1		DISTILLATE 2	
	81	82	81	82
TOTAL AROMATICS	5800	5900	5800	5900
BENZENES*	1245	1128	2205	2216
C10	81	90	176	162
C12	412	418	268	276
C14	27	49	392	391
C16			313	154
C18			79	120
C20			82	62
INDANS & TETRALINS	1747	9128	3207	1195
NAPHTHALENES	811	541	634	310

* DATA ON INDIVIDUAL HOMOLOGS ARE ONLY PARTIAL; ODD C NUMBERS SHOW SIMILAR TRENDS.

Table 4

REPRODUCIBILITY OF MS ANALYSIS FOR TRACE AROMATICS

COMPONENT/TYPE	DISTILLATE 6	
	CONCENTRATION, PPB	
	81	82
BENZENES*	3601	3606
C10	58	58
C12	210	210
C14	1300	1300
C16	110	110
C18	1000	1000
C20	58	60
INDANS & TETRALINS*	580	588
NAPHTHALENES*	85	97

* DATA ON INDIVIDUAL HOMOLOGS ARE ONLY PARTIAL; ODD C NUMBERS SHOW SIMILAR TRENDS.

Table 5

DETERMINATION OF TRACE AMOUNTS OF OXYGEN COMPOUNDS

a "ACTIV" FRACTION	240 PPB (0.5 mg)				
b TOTAL IONIZATION BY HIGH RES MS (RP = ~20 B)	2769249				
c IONIZATION OF SMALLEST COMPONENTS	100-150 (3-5 PPB)				
d DYNAMIC RANGE	~18,000				
e OXYGEN COMPOUNDS BY HIGH RES MS (PPB)					
C NO.	PHENOLS	INDANOLS	HYDROXY-TETRALINS	NAPHTHOLS	HYDROXY-ACETNAPHTHOLS
6	4589	---	---	---	---
7	20400	---	---	---	---
8	18675	---	---	---	---
9	1715	301	62	---	---
10	450	162	43	---	---
11	787	58	35	15	---
12	21	68	5	5	---
13	8	49	14	---	25
14	---	36	---	---	---
15	35	18	3	---	---
TOTALS	52179	648	160	20	25

GRAND TOTAL: 52872 PPB

Table 6

COMPARISON OF HIGH RES MS AND GC/MS DATA ON PHENOLS

	CONCENTRATION, PPB	
	HIGH RES MS	GC/MS
PHENOL	8.1	8
CRESOLS	28.8	26
ORTHO META & PARA	---	12.6 13.4
C ₈ PHENOLS	16.3	17.8
2,6 - DiHa	---	2.4
2,5 + 2,6 - DiHa	---	2.5
O-H-methyl	---	4.7
M-H-methyl	---	1.1
P-H-methyl	---	2.3

Table 7

DETERMINATION OF TRACE AMOUNTS OF NITROGEN COMPOUNDS

a "BASE" FRACTION	50 PPB (2.5 Mg)
b TOTAL IONIZATION BY HIGH RES MS (RP = ~20 B)	290615
c IONIZATION OF SMALLEST COMPONENTS	100-160 (2-3 PPB)
d DYNAMIC RANGE	~2200
e NITROGEN COMPOUNDS BY HIGH RES MS	308 PPB
PARIS PER BILLION	
C NO.	PIPERIDES ANILINES
5	4 ---
6	--- 52
7	--- 185
8	--- 80
9	--- 7
TOTAL	4 308

f ELEMENTAL NITROGEN DETERMINATION, PPB

MS	CHEMICAL
51	< 1000 (DETECTABILITY LIMIT IS ~1000 PPB)

NON-RANDOM DECOMPOSITIONS OF $C_3H_6O^+$, $C_4H_8O^+$ and $C_4H_8O_2^+$;
THE ROLE OF EXCITED ELECTRONIC STATES

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MIKE spectra, kinetic energy release distributions (KERDs) and appearance energies were determined for metastable enol ions of acetone and butanone, and for metastable propylene oxide and butanoic acid cations.

Bimodal KERDs have been obtained for $C_2H_3O^+$ formation from $C_3H_6O^+$ and $C_4H_8O^+$ cations^{1,2}, although only a single product channel — acetyl cation — is open energetically. The bimodality is due to a low-energy relaxed part and a high-energy part, which has escaped internal energy relaxation, due to incomplete intramolecular energy redistribution in the short-lived intermediate keto ions, $CH_3COCH_3^+$ and $CH_3CH_2COCH_3^+$, respectively. Figure 1 shows the high-energy and low-energy components of the synthesized translational distributions for the indicated enol ion reactions.

The dissociation of CF_3Cl^+ has previously³ been shown to demonstrate a bimodal KERD due to dissociation from the ground $\tilde{X}$ state, following autoionization, and from the excited $\tilde{A}$ state, following direct ionization. The $\tilde{A}$ state of acetone cation lies within reach of the enol-keto barrier and has been invoked recently.⁴ Internal conversion from excited electronic states can lead to non-random dissociation paths by channeling vibrational energy into a limited number of degrees of freedom.⁵

Metastable propylene oxide ions dissociate to $C_2H_3O^+ + CH_3\cdot$ demonstrating a bimodal distribution as well. We suggest that propylene oxide isomerizes to acetone prior to dissociation, on a somewhat higher point on the potential energy surface, than the metastable enol $C_3H_6O^+$ ion does.

Finally, data on butanoic acid reactions ($CH_3\cdot$ and C_2H_4 elimination) have been obtained. KERDs and AE values for electron impact metastable ions, do not contradict PI results⁶ and are discussed in accordance with previously suggested^{6,7} dissociations from isomeric ions.

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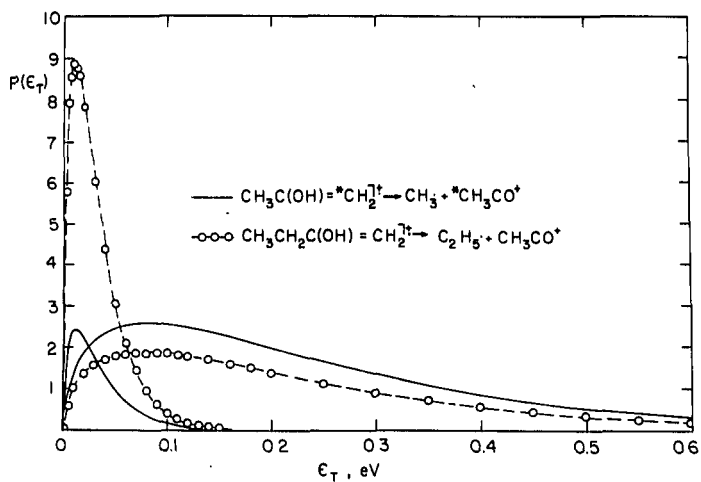
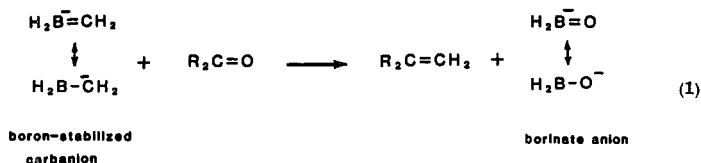


Figure 1. Overall kinetic energy release distributions are synthesized by using two three-dimensional Maxwell-Boltzmann distributions (reference 1). The high-energy and low-energy components of the synthesized distributions are represented here for the indicated enol ion dissociations.

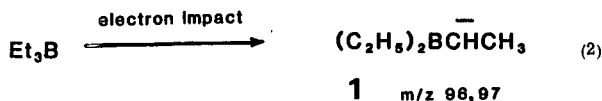
WITTIG REACTIONS OF BORON-STABILIZED CARBANIONS OBSERVED BY FTMS

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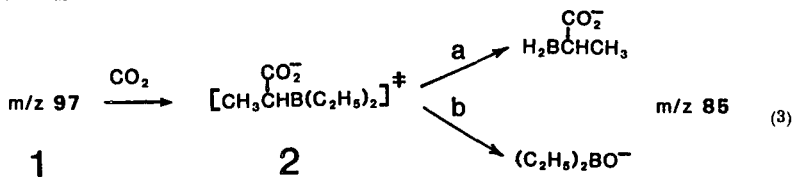
The boron Wittig reaction, known for more than a decade, has been a subject of recent interest. From reported *ab initio* calculations [Hinde, Pross, and Radom, *J. Comput. Chem.* 1, 118 (1980)], reaction 1 can be estimated to be exothermic by 34 kcal/mol. The boron-stabilized carbanion is converted to a borinate anion, and the strength of the boron-oxygen bond drives the reaction to the right.



We report evidence that carbon dioxide undergoes the boron Wittig reaction in the gas phase. Using FTMS, we observe that boron-stabilized carbanions react with CO_2 at pressures on the order of 10^{-7} Torr to yield borinate anions. Our reagent ion, 1, is derived from 0.1 sec bombardment of triethylboron with 6-7 eV electrons. In the absence of other reagents, 1 slowly transfers hydride to neutral triethylboron to produce triethylborohydride. Ion 1 reacts with a variety of oxygen-containing molecules to yield diethylborinate anions, $(\text{C}_2\text{H}_5)_2\text{BO}^-$, which are observed at m/z 84 and m/z 85 (corresponding to the two naturally occurring boron isotopes). Reaction with water or with molecular oxygen is rapid; reaction between 1 and N,N-dimethylformamide is also observed, but is much slower. The corresponding neutral products are, presumably, ethane (from H_2O), acetaldehyde (from O_2), and dimethylaminopropene (from DMF), and all of these reactions are estimated to be exothermic. Reaction of 1 with carbonyl compounds that have α -hydrogens proceeds via proton abstraction.



Reaction between 1 and CO_2 also leads to product ions at m/z 84 and m/z 85. We have taken some care to ensure that this is not due to trace impurities of air or water: the CO_2 sample was frozen in Dry Ice to remove water, and when the sample bulb was cooled with liquid nitrogen, the product ions evanesced. Two different molecular formulas could correspond to the nominal masses of the products, as shown below in reaction 3. In either case, the reaction can be viewed as proceeding via initial nucleophilic attack of carbon to form vibrationally excited intermediate 2, which can then fragment by two conceivable pathways: a via loss of the elements of C_4H_8 or b via loss of methylketene. Thermochemical estimates indicate that neither pathway is exothermic, although b is nearly thermoneutral.



High resolution mass measurements can distinguish between the alternative fragmentation pathways of 2. The isobaric m/z 85 ions differ in exact mass by 0.036 amu, and baseline resolution of this difference is well within the capability of FTMS. Exact mass measurements (calibrated with CF_3O^- from perfluorokerosene) show that the m/z 84 and m/z 85 products are, indeed, $(\text{C}_2\text{H}_5)_2\text{BO}^-$.

ENERGY DEPENDENCE OF THE FRAGMENTATION OF ALKYL BENZENE MOLECULAR IONS

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Both photo-dissociation (1) and variable-energy collision-induced dissociation (2,3) studies of the n-butylbenzene molecular ion have shown that reactions (1) and (2) occur with the ratio $C_7H_7^+/C_7H_8^+$ increasing with increasing energy input to the molecular ion. The present work reports a comparison of the $C_7H_7^+/C_7H_8^+$ ratios observed in tandem-quadrupole CID



studies of n-alkylbenzenes with the ratios observed in charge exchange mass spectrometric studies where the internal energy of the fragmenting ion is known; this comparison permits an estimation of the average energy converted into internal energy in the CID process.

Figure 1 shows the $C_7H_7^+/C_7H_8^+$ ratios observed for n-butyl to n-octyl benzene upon collision of the molecular ions with N_2 in a triple quadrupole mass spectrometer, plotted as a function of nominal ion kinetic energy. Following an initial increase with ion energy the ratios reach plateau values which decrease with increasing size of the alkyl chain. The results for n-butylbenzene are in essential agreement with earlier work (2).

Figure 2 shows the $C_7H_7^+/C_7H_8^+$ ratios observed on charge exchange ionization (4) of the same alkyl benzenes plotted as a function of the internal energy of the molecular ion, taken as the recombination energy of the charge exchange reactant ion minus the ionization energy of the alkyl benzene. The ratio increases with increasing internal energy; this is expected since although formation of $C_7H_8^+$ has the lower critical reaction energy it also has a lower frequency factor. The variation of the $C_7H_7^+/C_7H_8^+$ ratio with energy clearly diminishes with increasing size of the alkyl chain.

From a comparison of the results of Figure 1 and 2 the average energy converted into internal energy in the collision process can be evaluated. For the plateau regions of Figure 1 the average energies in eV are: R(Eint (eV)): C_7H_7 (5.1), C_7H_8 (5.6), C_8H_9 (5.8), C_8H_{10} (6.3), C_9H_{11} (6.8). The plateau region of Fig. 1 is reached for ions of ~100 eV (lab) energy corresponding to centre-of-mass collision energies of 13 (C_7H_7) to 17 eV (C_7H_8); thus the fraction of the collision energy converted into internal energy is in the range 30 to 50% and increases with increasing size of the impacting ion.

We are indebted to Sciex Division of MDS for providing access to the TAGA 6000 triple quadrupole mass spectrometer.

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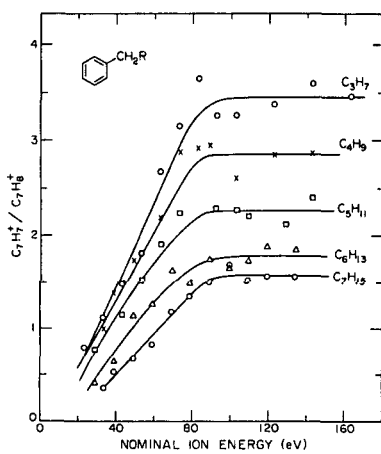


Figure 1

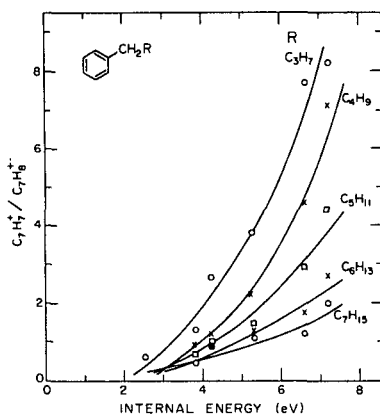


Figure 2

NON-COMPETING ISOMERIZATION UPON IONIC DISSOCIATION OF NITROBENZENE

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The Quasi-Equilibrium Theory (QET) successfully describes large gaseous ions because an increase in state density leads to facile radiationless transitions to the ground electronic state, which is a fundamental hypothesis of this theory. Non-interconverting states, however, have been found for fragmentation of some molecules containing a heteroatom (1). We are investigating the role of the heteroatom in possible competition between statistical and state-specific reaction, and have chosen nitrobenzene as the next compound because its fragmentation is characterized by rearrangement followed by the formation of $C_6H_5O^+$ ion, and because its HeI photoelectron spectrum (PES) shows deep valleys around 12.0 and 14.3 eV.

We report here the detailed study of the unimolecular decomposition of nitrobenzene ion by threshold photoelectron-photoion coincidence spectroscopy. The breakdown graph (BDG), which represents the relative abundance of product ions as a function of molecular ion internal energy, was obtained over the range covered by the HeI PES. Major fragment ions at photon energies less than 18 eV are $C_6H_5O^+$ (m/z 93), $C_6H_5^+$ (m/z 77), $C_5H_5^+$ (m/z 65), NO^+ (m/z 30), $C_4H_3^+$ (m/z 51), $C_3H_3^+$ (m/z 39), $C_6H_5NO^+$ (m/z 107) and $C_6H_2^+$ (m/z 74), respectively. Relative abundances were obtained by measuring peak areas in the coincidence TOF spectra and subtracting a background contribution. The overlapping peaks at m/z 77 and 74 were deconvoluted assuming a Gaussian peak shape for each individual peak.

The BDG obtained experimentally (Fig. 1) shows complicated features especially for the m/z 123 and 30. These are attributed to transmission of energetic electrons through a threshold electron detector (2). The BDG corrected for the hot electron effect is shown in Fig. 2; curves A, B, and C in the figure represent sums of ion abundances: $A = [93] + [65] + [39] + [30]$, $B = [65] + [39]$, and $C = [77] + [74] + [51]$, where $[m]$ denotes the relative abundance of fragment m . The curve A corresponds to the all processes requiring rearrangement. The long tail of this curve suggests that isomerization is in competition with direct cleavage at these energies. The possible fragmentation pathways for the production of major ions are elucidated in Scheme 1. Thus the BDG can be explained by QET. The variation of kinetic energy release (KER) observed for the m/z 77 ion, which were measured from the TOF peak width, were consistent with that calculated by an empirical formula based on statistical energy partitioning.

Asymmetric TOF peaks due to slow decomposition were observed for m/z 30, 51, 65 and 93 (3). The decay rates measured for NO -loss and NO^+ formation appear to be about the same. The latter process, however, shows a two-component structure. Fig. 3 shows the TOF distributions of NO^+ measured for several ionization energies. The figure has revealed that the one component disappears with increasing energy and the other becomes dominant without competition.

It has been reported on the basis of a metastable peak analysis that the loss of NO from nitrobenzene molecular ion occurs via two reaction channels which have similar activation energies yet lead to products of very different stabilities (4). One was proposed to involve a three-centered cyclic transition state with the formation of the aryloxy cation, and the other oxygen rearrangement to the ortho position which gives nitro-nitrite isomerization. The smooth behavior of the m/z 30 breakdown curve and the identity of the decay rates for the NO^+ formation and NO -loss processes seem to suggest that NO^+ production results from the same precursor as NO -loss. The reappearance of a slow process for NO^+ formation at higher energies suggests a second mechanism for NO^+ formation possibly requiring first isomerization to the nitrite structure (Fig. 4). That is, we believe that isomerization is a most attractive interpretation for our results. We plan to publish a full account of this investigation in the Henry Rosenstock memorial issue of IJMSIP.

Acknowledgements: We wish to thank the Department of Energy for support under contract number DE-AC02-76ERO2567

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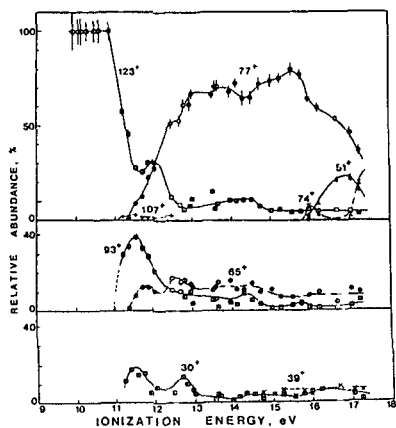


Fig. 1

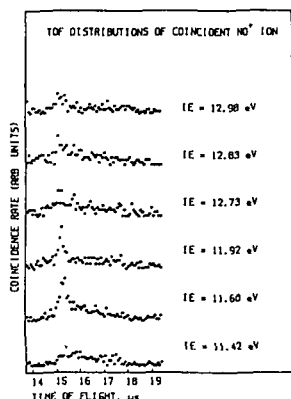


Fig. 3

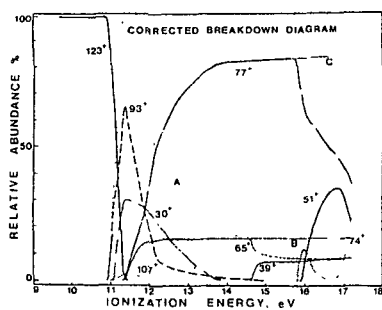
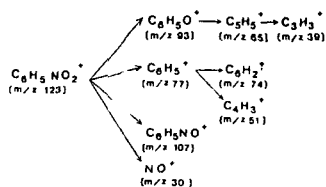


Fig. 2



POSSIBLE MECHANISM FOR THE FORMATION OF MAJOR IONS
IN THE IONIC DISSOCIATION OF NITROBENZENE

Scheme 1

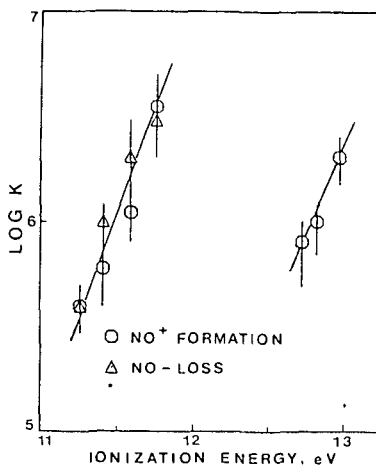


Fig. 4

SCALING OF KINETIC ENERGY RELEASE DISTRIBUTIONS

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The PEPICO technique for preparing ions with a well-defined internal energy, and for studying the dynamics of their subsequent fragmentation, is well established.¹ In particular, one can measure the kinetic-energy release distribution following dissociation. We are concerned with the use of this information to maximum advantage.

2-iodopropane ions yield $C_3H_7^+$ at the thermodynamic threshold for a fragment with the isopropyl structure. Fragmentation of 1-iodopropane ions occurs only at a higher energy, yet we find that the average energy release is equal to that from 2-iodopropane at the same global energy. This suggests, not only that the final structures are the same,² but that the implied isomerization of 1-iodopropane ion occurs prior to dissociation. A more detailed scrutiny confirms this, as follows:

Powis³ has suggested that kinetic-energy release distributions might be scaled so as to facilitate intercomparison. His choice of the total available energy as the appropriate scaling parameter, however, has several defects: it presupposes that the initial energy and the structure of the final products are known; and the constraint imposed by the total available energy is pertinent only to the uninformative high-energy tail of the distribution. Our choice of the average energy-release as the optimum scaling parameter avoids these difficulties. If a distribution can be measured, its average is also measurable; it emphasizes the most pertinent part of the distribution; and its use can be justified via quasi-equilibrium theory.

Kinetic-energy release distributions from 1- and 2-iodopropane fragmentation at arbitrary energy, when scaled as we suggest, are coincident. They indicate a dissociation along an attractive asymptote, moderated only by an angular momentum barrier. Isomerization of 1-iodopropane ion clearly precedes dissociation.

A previously-noted² coincidence between the threshold for fragmentation of 1-iodopropane ion and its first electronically excited state can now be reformulated. There is remarkable coincidence between the barrier for isomerization and the $^2E_{1/2}$ state. This coincidence, which occurs elsewhere as well, may not be accidental. Reverse internal conversion to this state may be a prerequisite to isomerization.

Figure 1 summarizes our understanding of the energetics of this system; Fig. 2 illustrates the scaling properties of the measured kinetic-energy release distributions.

*Operated by Union Carbide Corporation with the U.S. Department of Energy.

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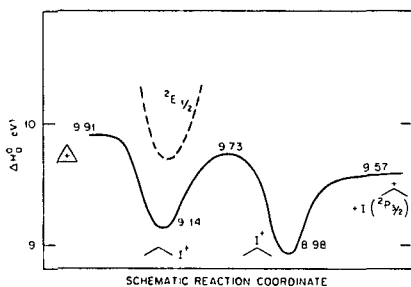


Fig. 1. Energetics of formation, isomerization, and dissociation of 1- and 2-iodopropane ions.

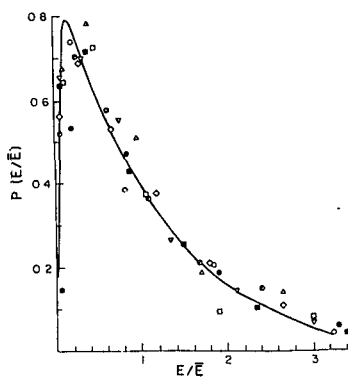


Fig. 2. Scaled kinetic energy distributions from 1- and 2-iodopropane.

ROTATIONAL PREDISSOCIATION OF H_2^+ IONS OF DIFFERENT PRECURSOR ORIGINS

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The spectroscopy of H_2^+ has been extensively studied over the last half century. In this investigation we were concerned with examining, by translational spectroscopy, the highly excited ro-vibronic states of $(1s\sigma_g)H_2^+$ which lie above the first dissociation limit that result in fragmentation by the mechanism of rotational predissociation, i.e. tunnelling. We have used a novel method for changing the ro-vibronic population of H_2^+ by forming it as a fragmentation product in an electron impact source, using a variety of hydrogen containing precursor molecules. This method not only gives information on these excited states but also reflects the transition state phenomena occurring in the precursor. We have recalculated the complete manifold of ro-vibronic states, including their lifetimes and widths, with a new programme¹ which pays particular attention to those states above the dissociation limit and to the modelling of the rotational barrier, i.e. the region where spectroscopic information is sparse.

A mass spectrometric method has been employed in which the states are characterised by measuring the translational energy release (ΔE) during the unimolecular (i.e. spontaneous) fragmentation $H_2^+ \rightarrow H + H$, where $\Delta E = (16 m_2 m_3 W / m_1^2)^{1/2}$ and m_1 , m_2 and m_3 are the masses of H_2^+ , the proton and neutral respectively, W the kinetic energy of m_1 and W the excess internal energy release which is related to the energy level of the quasi-bound state. In a previous study² six states were identified where H_2^+ was formed from hydrogen gas in a monoplasmatron source.

Translational energy spectra of $H_2^+ \rightarrow H + H$ were obtained on a modified double-focusing mass spectrometer which has three sectors (magnet/electric sector/electric sector). Ions extracted from the ion source pass into a large magnetic sector which is set to transmit only H_2^+ . Unimolecular fragmentation of H_2^+ occurs continuously along the flight path of the ion beam. Fragment ions formed after the magnetic sector but before the first electric sector were energy analysed by scanning the voltages on the electric sector plates. An initial survey of the thirteen different samples was carried out at a moderate setting of energy resolution and fine structure was observed corresponding to predissociation from different quasi-bound states. The relative pattern of intensities of this structure changed upon the choice of sample. Four main states were identified clearly. The energy resolution was then increased to resolve states of smaller energy separation. Methane gas was found to give the most intense spectra from which at least nine states were assigned. There are a total of 56 ro-vibronic energy levels above the dissociation limit. However, because the time scale for observation is very limited (ca. 1 to 10 μ .sec.) only states with lifetimes in the range 10^{-7} to 10^{-3} sec. are potentially observable and these total 14. Table 1 lists these states together with their lifetimes (τ) calculated energy release (W_c) and measured energy release (W_p). The relative population of states ($I_{v,j}$), see Table 1, as formed in the ion source was calculated using the lifetime data and from a knowledge of the apparatus collection efficiency.

Confirmation of our assignment of states was made by varying the time scale of observation (i.e. by changing the kinetic energy of H_2^+ and by selecting the next adjacent field-free region in our 3-sector instrument). Here we noted the relative changes in the pattern of peak intensities and found it to agree in the manner predicted from their lifetimes.

An interesting result of this work has been that highly rotationally excited states were not observed when hydrogen gas was used and also that we were able to vary the ro-vibrational population of states by choosing various precursors. Certain groups of samples were found to produce similar spectra. We have made qualitative arguments to account for the above behaviour in terms of i) the formation of a highly excited complex of H_2^+ ; and ii) as a transition state phenomena of the precursor.

TABLE 1

v, J	τ (u. sec.)	W_c (meV)	W_E (meV)	$I_{v, J}$
0, 39	3.4	233.5	220	1
1, 37	46.5	177.7	171	.3
2, 35	3960	127.8	123	<.05
3, 34	.48	143.4	139	.25
4, 32	36.1	102.2	104	.15
5, 30	18400	66.2	-	<.05
6, 29	.21	83.8	-	<.05
7, 27	21.8	55.6	51	.9
8, 25	17900	32.4	-	<.05
10, 22	.14	33	33	.4
11, 20	2.6	20.6	18	2.9
12, 18	34.4	12.3	12	.5
13, 16	83.6	7.5	~7	<.2
14, 14	3.4	5.5	5	2.7

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THE KINETIC SHIFT IN IODOBENZENE; A TIME-RESOLVED ELECTRON IMPACT STUDY

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Kinetic shifts¹ have prevented accurate determination of thermochemical data from appearance energy measurements in many instances, since the early days of mass spectrometry. The unimolecular fragmentation rate, $C_6H_5I^+ \rightarrow C_6H_5^+ + I\cdot$, which demonstrates a kinetic shift, was a benchmark measurement by Henry Rosenstock and coworkers, shortly before his death.² That study employed the variable time photoelectron-photoion coincidence technique. Yet many measurements to date have employed electron impact. We are using Trapped Ion Mass Spectrometry (TIMS)¹ to determine appearance energies for normal fragment ions as a function of storage time under conditions of constant sensitivity. This instrumentation has been employed in an electron impact study of the iodobenzene reaction. The appearance energy is seen to decrease with storage time (Fig. 1) and level-off at long times. The experimental data are reproduced by a model QET calculation (Fig. 1) based on the rate energy dependence of Dannacher et al.² In this calculation the $k(E)$ was convoluted with $P(E)$ due to the thermal energy distribution of the neutral. The kinetic shift of 0.45 eV is approximately compensated by the thermal energy effect at 200°C.

Time-resolved metastable peak shapes were obtained up to a storage time of 100 μ sec. The time dependence of the kinetic energy releases reproduces the kinetic shifts.

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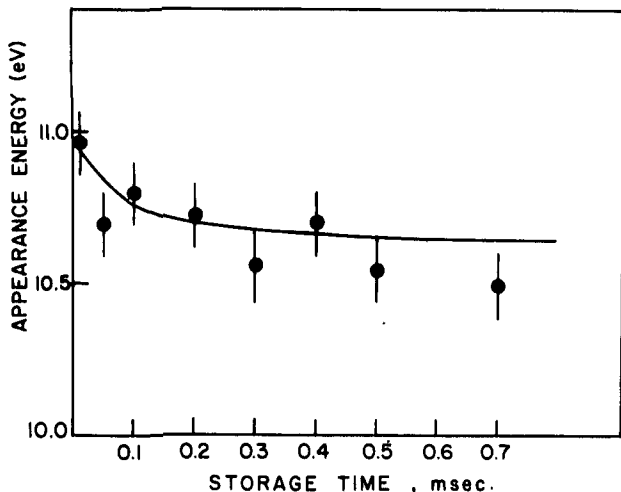


Figure 1: Time-resolved appearance energies for $C_6H_5^+$ from iodobenzene, points are experimental (with error bars); curve is calculated based on the $k(E)$ dependence of reference 2.

THE MASS SPECTROMETRIC DETECTION OF LARGE VAN DER WAALS CLUSTERS

by

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In an earlier study, Milne, Beachey, Bennett, and Greene¹ obtained anomalous velocity distributions for a number of gases following their expansion in transition flow. It was observed that when measurements were made on the nominal parent ion for the particular gas, two partially overlapping velocity distributions were obtained. In the current study some of the experiments were repeated using higher resolution (4000-5000). It was found that the widths of the nominal molecular ion peaks were greater than predicted, and that there were diffuse satellite peaks on the low mass side of the molecular ion peaks. The diffuse character of the satellite peaks and their dependence on ion source conditions indicate that they are produced by metastable ions. The variation of both the molecular ion peak widths and the satellite peak intensities with expansion conditions was found to be consistent with the presence of large numbers of neutral clusters in the molecular beam. Measurements of the velocity distributions of the neutral precursors of the nominal molecular ions suggest that the clusters may be very large.

These results indicate that the extent of clustering may have been underestimated in previous mass spectrometric studies. The presence of large undetected clusters could also cause major errors in the calibration of mass spectrometers using total mass flux as in the procedure of Lee and Fenn² for dimers.

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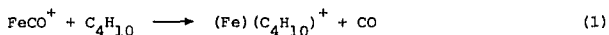
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STRUCTURAL CHARACTERIZATION OF THE GAS PHASE IRON-ALKANE IONIC COMPLEXES

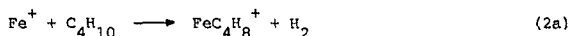
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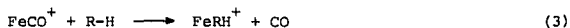
The clusters of atomic iron atoms with hydrocarbons are formed by ion-molecule reactions in mixtures of iron carbonyls and hydrocarbons. Electron impact on $\text{Fe}(\text{CO})_5$ produces a number of fragments, particularly Fe^+ and FeCO^+ which react with the neutral hydrocarbons. Reactions of $\text{Fe}(\text{CO})^+$ and the butanes produces $\text{Fe}(\text{C}_4\text{H}_{10})^+$ as indicated in reaction (1).



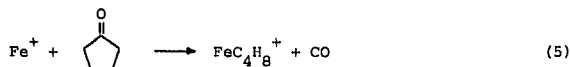
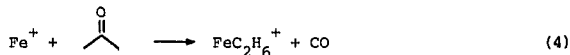
The present collision-induced decomposition (CID) results indicate that the structure of the $(\text{Fe})(\text{C}_4\text{H}_{10})^+$ ion produced in reaction (1) with n-butane differs from the ion produced in reaction (1) with i-butane. In each case, the hydrocarbon seems to fall apart on the metal. The reaction of Fe^+ with the butanes produces the products indicated in reaction 2a-2d.



The CID products of reaction (2) were compared with the CID spectra of the products of reaction (3) where R-H is 1-butene, 2-butene, butadiene, propene, and ethylene.



The CID spectra were obtained on the MS50 TA at the MCMS. On this instrument it was possible to obtain CID results on each of the following: $\text{Fe}(\text{CO})_2^+$, $\text{Fe}(\text{CO})\text{C}_2\text{H}_4^+$, and FeC_4H_8^+ , all of which have nominal mass 112. The CID results on the product of reaction 2a with i-butane indicates that the structure for the ion is $(\text{Fe})(\text{i-C}_4\text{H}_8)^+$. The similar reaction with n-butane results in a mixture of structures which are $(\text{H}_2)(\text{Fe}^+)(1-3 \text{ C}_4\text{H}_6)$ and $(\text{Fe}^+)(\text{C}_2\text{H}_4)_2$ ions. The products of reactions 2c and 2d, $(\text{Fe})(\text{C}_3\text{H}_6)^+$ and $(\text{Fe})(\text{C}_2\text{H}_4)^{+2}$, each have only one structure regardless of the reacting C_4H_{10} isomers. The CID spectra of $\text{Fe}(\text{C}_3\text{H}_8)^+$, $\text{Fe}(\text{C}_2\text{H}_6)^+$ and $\text{Fe}(\text{CH}_4)^+$ were obtained and interpreted. The CID results on the products of reactions (4) and (5) were also obtained and interpreted in terms of related results mentioned above.



The results suggest that the mechanism for reaction (2) involves the oxidative addition to either the C-C or C-H bonds. Furthermore, reaction (1) appears to occur through the oxidative addition of the metal to the hydrocarbon.

An approximate value for the ionization potential of the FeCH_3^+ species can be determined from the CID spectra in which that ion appears as a fragment. The CID spectra of the $\text{Fe}(\text{C}_4\text{H}_{10})^+$ complex formed in reaction (1) between FeCO^+ and isobutane has an FeCH_3^+ peak, but no $\text{i-C}_3\text{H}_7^+$ peak. This indicates that the $\text{I.P.}(\text{FeCH}_3) < \text{I.P.}(\text{i-C}_3\text{H}_7) \approx 7.36 \text{ eV}^1$. In the CID spectra of the $\text{Fe}(\text{C}_3\text{H}_6\text{O})^+$ species formed in the reaction between FeCO^+ and acetone, both FeCH_3^+ and CH_3CO^+ fragments are observed. Formation of the FeCH_3^+ and CH_3CO^+ probably involves a competition for charge between FeCH_3 and CH_3CO . This suggests that the $\text{I.P.}(\text{FeCH}_3) \approx \text{I.P.}(\text{CH}_3\text{CO}) = 6.78 \text{ eV}^2$. Furthermore, this implies that $\text{D}(\text{Fe}^+-\text{CH}_3) - \text{D}(\text{Fe}-\text{CH}_3) = 27.2 \text{ kcal/mole}$. The reported value for $\text{D}(\text{Fe}^+-\text{CH}_3) = 69 \pm 5 \text{ kcal/mole}^3$ which indicates that the $\text{D}(\text{Fe}-\text{CH}_3) \approx 42 \text{ kcal/mole}$.

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WOA 11 | STRUCTURAL STUDIES ON METAL ION COMPLEXES
USING FOURIER TRANSFORM MASS SPECTROMETRY: D.B. Jacobson and
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The reactions of gas phase group 8 transition metal ions (Fe, Co, Ni) with organics are studied using a prototype Nicolet FTMS-1000 mass spectrometer. Metal ions are generated by focusing the beam (frequency doubled to 530 nm) of a Quanta Ray Nd:YAG laser onto metal foils of high purity attached to the rear receiver plate of a 1 in. cubic trapping cell. Structures of product ions are determined by both collision-induced dissociation (CID) and specific ion-molecule reactions.

All Three metal ions are observed to dehydrogenate n-butane by a 1,4- elimination process generating a bisolefin complex. In addition, Co^+ and Fe^+ also dehydrogenate n-butane by a 1,2- elimination process producing a butene-metal ion complex. Stable metallocycles are also generated by decarbonylation of cyclopentanone and cyclobutanone to produce metallocyclopentane and metallocyclobutane, respectively. The gas phase chemistry of these metallocycles will be discussed.

QUANTITATIVE ANALYSIS IN THE PETROLEUM INDUSTRY

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Quantitative mass spectral analysis began in the petroleum industry over forty years ago. It was originally used for gas analysis in refineries. This use was aimed at process optimization and feedstock characterization. It was also used for product sales and trade agreements. Quantitative mass spectrometry is now used for these purposes, as well as, environmental impact studies and geochemical applications.

In the early days of quantitative analysis, different labs often calculated different analyses. This was either because they were using different instrumental conditions, calibrations, or calculation procedures. The original ASMS meetings were Consolidated Electrodynamics Corporation (CEC) users meetings. These meetings, where the state-of-the-science of mass spectrometry were discussed, formed the origins of the American Society for Testing and Materials (ASTM) Committee E-14 on Mass Spectrometry. Until 1969, ASTM E-14 organized the Annual Conference on Mass Spectrometry and Allied Topics.

Concurrently with the Annual Conference, another ASTM Committee on Petroleum Mass Spectrometry would, and still does, meet and develop methodology for the analysis of various petroleum streams. This committee, D-2, Section M, has written several consensus agreement methods for petroleum. Today, this committee is working not only in the area of petroleum but also coal and shale liquid characterization.

In petrochemicals, there are basically two types of quantitative analysis; static and dynamic. The static analysis is typically characterized by the use of a batch inlet system, either heated or unheated. The dynamic methods include GC/MS, probe micro-distillation, and field ionization and desorption techniques.

The static, or batch inlet, analyses were first performed in the 1940's on gas streams. Essentially, batch inlets consist of an evacuated reservoir (2-3 liters) into which the sample (ca 0.25 microliters of liquid) is introduced. The expanded sample is then leaked into the ion source by means of a molecular leak, either gold or vitreous glass. Typically, a single composite spectrum is then acquired, or several spectra may be averaged together. The analysis of heavier streams did not come about until the late 1950's when heated all glass inlet systems were developed. The bulk of the static analyses are still primarily low resolution methods yielding information on types, or averages, rather than specific materials.

Gas analyses are still being routinely performed today. Mass spectrometry has an advantage over gas chromatography in both the precision and speed of analysis. Furthermore, mass spectrometry is able to deal with samples containing fixed gases and at a wide variety of pressures.

Gas analysis requires good instrumental stability and spectral repeatability. Typically, a calibration set is developed by analyzing pure components, picking non-interfering calibrant mass peaks and constructing a matrix to solve the set of simultaneous equations which are generated. To insure accuracy, blends approximating the composition of the sample should be analyzed. Typical repeatability and reproducibility standard deviations for gas analyses are found in ASTM D-2650, or ASTM D-1137. The methodology of calibration, analysis, and calculation are explained in these two methods.

As one goes to increasingly higher boiling ranges, the resolution of the analysis decreases. This is because of the increasing complexity of the isomeric distribution of samples and also because type analyses have never been considered as absolute measurements but rather as relative measurements. Type analyses are best used for comparative or correlative purposes.

In gas analysis, it is not uncommon to report the amount of specific compounds or isomers. When one gets to the gasoline and higher boiling ranges, specific compound data is lost. Here one deals with mass spectral methods aimed at the measurement of specific types or classes of materials present. There are many different type analyses in the literature. The majority of them share several common factors:

low resolution mass spectral methods
require a sample separation into saturate and aromatic portions
specific limitations on boiling range, heteroatom, and olefin content
require a mathematical treatment of the raw mass spectral data

Type analyses are based primarily on fragment ion techniques and are mostly 70 eV techniques. Typically, fragment ions for specific classes of materials are summed together, e.g.:

Paraffins:	71+85+99+113+...
Cycloparaffins:	69+83+97+111+...
Dicycloparaffins:	109+123+137+151+...
Alkylbenzenes:	91+105+119+133+...
Alkyl naphthalenes:	141+155+169+183+...

These ion summations are then multiplied by the coefficients of the inverted sensitivity matrix which includes corrections for interferences.

The calibration set for type analyses is an approximation. Due to the complexity of petroleum and the large number of possible isomers, only a small number of pure materials have been run to generate the sensitivity coefficients. Aside from extrapolation of literature values, calibration sets can be generated by the analysis of very narrow well-defined distillate cuts. These cuts are then blended together and re-analyzed. This blending process is continued until the original material is again being analyzed. The intermediate sensitivity coefficients are re-evaluated at each step and a composite sensitivity matrix is generated for the final analyses.

Once a method has been developed and cross-checked by other laboratories, repeatability and reproducibility limits calculated, only then does the method get considered for adoption by ASTM. There are several methods which have never been adopted by ASTM either because they are too limited in scope of application or because they require too severe a sample pre-treatment.

After a method is adopted, it often undergoes changes, both in scope of analysis and methodology over the years. During the past year, the analysis of gas oil saturate fractions by high ionizing voltage had its statistical section revalidated by round-robin in which 11 laboratories participated. The same sample is being sent to mass spectrometer manufacturers by ASTM D-2 to measure the repeatability of the modern quantitative mass spectrometers.

Quality control charts are kept by many laboratories to track the day-to-day performance of their instruments and to provide quality assurance data. Since the published methods of analysis require a mathematical treatment of the data, when the amount of one component goes up, it is not surprising that the amount of another goes down.

High resolution mass spectral batch analysis is also a relative technique. The major method in high resolution requires separation of the sample into saturate, aromatic and polar fractions and then the aromatic and polar fractions are analyzed by low voltage high resolution mass spectrometry. The advantage of high resolution is that it provides a more exact split between various overlapping series.

Quantitative analysis by batch inlet mass spectrometry in the petrochemical industry has been an extremely useful technique since the 1940's. More than 100 methods of analyses have been published in that time applying to more than 30 different complex mixtures. Batch inlet analyses are limited to materials which boil below 600°C and each method has limitations imposed on it concerning the heteroatom and olefin content.

CONSIDERATIONS ON THE QUANTITATIVE
ANALYSES OF COMPLEX PETROLEUM AND SYNFUEL MIXTURES

By

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This paper deals with concerns related to the quantitative analysis of complex petroleum and synfuels mixtures. Issues examined include analytical options, such as electron impact and field ionization, operations at low and high voltages, at low and high resolution, as well as inter-laboratory and inter-technique comparisons.

The overall quality and reliability of the MS analyses is established through calculations of physical properties from MS determined composition data and by comparing these values with conventional measurements.

Preferred modes of analysis at Exxon Research and at DOE, BETC are low voltage, high resolution, field ionization, and probe distillation. Advantages of these techniques include rational and predictable correlations between chemical structure and gram sensitivities. Calibration sets can be calculated from a theoretical basis, making feasible the analyses of mixtures containing thousands of components that are mostly not available as pure compounds. For example, low voltage, high resolution sets developed at Exxon contain more than 10,000 sensitivity coefficients arrived at on this basis and that have been proven quite reliable in the analysis of thousands of samples. Work by DOE and Exxon have shown that FI calibration sets are essentially independent of structure and instrument geometry; that EI low voltage sets are predictable, with relative sensitivities independent of ionizing voltages in the 10-12 eV range; that accurate standardization leads to very reproducible data in the same laboratory and among different laboratories, and, finally, that EI and FI analyses are compatible.

Probe distillation, that gives an additional dimension of information, can be also made quantitative through accurate temperature control and computerized data acquisition systems, such as developed by DOE at BETC, and illustrated by the data shown in Table I.

Physical properties calculated from detailed composition data, as calculated by high resolution, low voltage mass spectrometry, include elemental analysis for C, H, S, O, N; distillation characteristics; density; refractive index; MW distribution; and the calculation of averages for chemically different C and H types as generally determined by NMR. These calculations serve a two-fold purpose: quality assurance for the MS analyses of very complex mixtures and actual determination of these physical properties in the many cases when the sample available is sufficient only for MS analyses. As strange as it might seem, this is often the case even in the petroleum and synfuels industry in case of exploratory research. This important function of MS analyses is made possible through the excellent agreement of the values calculated by MS and measured directly, as illustrated in Tables 2-5.

Table 1 TYPICAL DATA ON 19-COMPONENT MIXTURE				
COMPOUND	WEIGHT PERCENT			
	DEMO	BATCH (WELL)	6/7/70	7/7/70
2,5-DIHYDRO-1H-INDOLE	8.47	8.24	7.85	7.40
6-METHYL-2-METHYL-1H-INDOLE	4.87	5.06	5.50	5.07
4,10-DIHYDROPHENANTHRENE	9.50	9.88	9.55	9.40
FLUORANTHENE	4.58	4.56	5.17	4.58
CHRYSENE	1.35	1.40	1.45	0.96
AVERAGE PERCENT ERRORS (ALL 19 COMPONENTS): $\pm 0.2 \pm 0.58$ $\pm 0.8 \pm 10.12$ $\pm 0.4 \pm 10.8$				

Table 2 ELEMENTAL ANALYSIS BY MS COMPARED WITH CHEMICAL DETERMINATIONS			
ATOMIC N/C		ELEMENTAL N	
MS	CHEM	MS	CHEM
0.45	0.46	0.10	0.12
0.49	1.00	0.40	0.70
1.12	1.14	5.92	6.00
1.28	1.32	ELEMENTAL O	
1.70	1.69	MS	NEUTRON ACT
1.90	1.91	0.71	0.76
2.17	2.17	2.80	2.63
2.55	2.52	5.68	5.77

Table 3 ELEMENTAL S BY MS AGREES VERY WELL WITH XRD SOLUTION		
SAMPLE	WEIGHT PERCENT S	
	SA	ED
1	0.01	0.02
2	0.06	0.10
3	0.67	0.77
4	1.55	1.70
5	1.69	1.78
6	2.00	1.90
7	2.04	2.25
8	2.88	3.15
9	2.81	4.13
10	2.91	3.07
11	4.62	4.76
12	6.05	5.76

Table 4 MS DISTILLATION AGREES WITH GC DISTILLATION		
TEMPERATURE, °	MS	GC
500	1.4	1.2
600	8.1	7.5
700	77.7	76.4
800	50.7	49.8
900	71.7	76.0
1000	87.0	91.2
1100	96.7	98.7
1200	100.0	100.0

Table 5 COMPARISONS OF MS AND NMR DATA ON DISTILLATE ASPHALTHINES		
PARAMETER	VALUE	
	NMR	MS
AVERAGE NO. OF RINGS	4.5	5.7
FRACTION OF AROMATIC C	0.77	40.82
AROMATIC/PERIPHERAL C	1.48	1.58
AVG. HYDROGEN DEFICIENCY	---	22.42
C ATOMS IN SIDECAINS	---	3.52
NO. OF ATOMS PER SIDECAIN	1.42	---
COMBINING MS, NMR DATA GIVES:		
AVG. NO. OF UNIT STRUCTURES:	1.0	
AVG. NO. OF SIDECAINS:	2.5	

The State of the Art of High-Precision Stable Isotopic Analysis in Organic Materials

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The measurement at high precision in organic compounds of abundances of the stable isotopes of C, H, N, and O is of intrinsic interest in the natural sciences and of practical importance in isotope-dilution analyses. While a remarkable variety of techniques has been developed, the technique of differential isotope ratio mass spectrometry lies at the heart of all of them. This method requires that samples be presented in the form of gases producing simple mass spectra free of hydrogen-transfer phenomena ($M + 1$ or $M - 1$ peaks) that might otherwise obscure small shifts in isotopic abundances. In practice, the precision and sample requirements of isotopic measurements in organic compounds are limited primarily by the techniques used to prepare those sample gases from organic precursors, and it is on those sample-preparation techniques that this review will focus.

This discussion is restricted also to techniques capable of measuring incremental variations in the natural abundances of the stable isotopes noted above; that is, procedures with a precision of approximately 0.01% or better. Such small isotopic variations are discussed by isotopic analysts in terms of "per mil" units (for an extended discussion of isotopic calculations, see ref. 1). Two samples differing by one per mil unit have isotope ratios differing by one part per thousand. Isotopic abundances are customarily referred to arbitrary standards (1); the values tabulated in this abstract, for example, represent differences between the materials analyzed and carbon in the "Pee Dee Belemnite," the primary isotopic standard for carbon. The ratio of carbon-13 to carbon-12 in the PDB standard is 0.0112372. The ratio in the free hexanoic acid sample noted in Table 2 is, thus, 0.0108803 ± 3 .

Combustion of Organic Molecules

The combustion of organic material in the presence of excess oxygen yields carbon dioxide and nitrogen, which can be analyzed directly, together with water, which can be analyzed for its deuterium content after reduction to hydrogen gas. The use of large vacuum lines designed for open-circuit combustion and immediate collection and purification of the combustion products has given way to the more convenient alternatives described below.

Sealed-tube procedure. The use of sealed quartz bombs was pioneered for elemental analysis by Frazer and his coworkers at Lawrence Livermore Laboratories (1). This highly successful procedure has been widely adapted for isotopic analysis. A description of the technique has been given by Wedeking *et al.* (3). A variant employing a pyrex bomb has been described by Sofer (4).

In all cases, the organic matter is placed in the bomb together with cupric oxide and some scraps of elemental silver. After sealing under vacuum, the bomb is heated either to 850°C (quartz) or 550°C (pyrex) for about two hours; pyrolytic decomposition of the cupric oxide furnishes the oxygen required for combustion. Regulation of the cooling pattern assures reduction of all nitrogen oxides and complete trapping of all sulfur (1). Products can be isolated and quantified by standard vacuum-line techniques (3).

The lower temperature employed in the pyrex-bomb procedure often leads to incomplete combustion (4), the residue appearing as a carbonaceous film on the inner surface of the bomb. While such nonquantitative yields commonly cause inaccuracies in isotopic analyses, no such problems have been reported with this procedure. The precision of the quartz-bomb procedure is extremely high -- at least 0.02 per mil. That of the pyrex-tube procedure appears to be significantly lower (4). In both cases, submicromole samples can be analyzed.

In this -- or any other -- combustion procedure, care must be taken that materials yielding a basic ash (*i. e.*, alkali metal or alkaline earth oxides) are mixed with an acidic flux (5) to insure complete recovery of carbon dioxide.

Adaptation of elemental analyzer. Automated elemental analyzers that yield separated products of combustion can be easily adapted to the preparation of gases for isotopic analysis. In our laboratories we have had particular success with the unit produced by Carlo-Erba. A precision of carbon isotopic measurement of 0.05 per mil is obtained for samples as small as ten micromoles. A single operator can prepare carbon dioxide from as many as 50 samples per day -- elemental analyses of all materials are, of course, obtained as a byproduct of the procedure.

Methods for Analysis of Oxygen-Isotopic Abundances

Before development of the Unterzaucher technique for direct analysis of oxygen in organic material, the abundance of oxygen was commonly determined by difference. That expedient, certainly not optimal even for elemental analyses, is completely inapplicable for isotopic measurements. Until recently, there has been no technique applicable to measurement of natural variations of oxygen isotopic abundances in organic material, though several approaches adequate for the detection of labelled substances have been described (6,7). The impetus recently provided by research problems in paleoclimatology and biogeochemistry has been strong, however, and two alternative approaches to precise measurements can now be described.

Adaptation of elemental analyzer. Adaptations of the Unterzaucher technique applicable to the detection of labelled materials have been described by many authors, most recently by Taylor and Chen (7). Among the numerous problems encountered, a high blank and persistent memory effects have been most difficult. We have recently shown, however, (see abstract by Santrock and Hayes elsewhere in this volume) that utilization of a microprocessor-controlled instrument stabilizes those effects to the extent that they can be accurately modelled. Corrections of adequate precision result, and oxygen isotopic abundances can be determined with a precision of 0.2 per mil on samples containing as little as one micromole O.

Pyrolysis in sealed nickel tube. The technique of pyrolysis of organic material in sealed nickel tubes was developed in the laboratories of Prof. S. Epstein at CalTech and first described in detail by Thompson and Gray (8). That method, and a more recent improvement described by Brenninkmeijer and Mook (9), have been applied with success to the measurement of variations in the natural abundance of oxygen-18 in milligram quantities of pure cellulose prepared from tree rings. The sample is heated to 900°C together with excess carbon. The diffusion of hydrogen gas through the walls of the tube removes all hydrogen from the system and, in the absence of sulfur, oxygen is recovered as a mixture of carbon monoxide and carbon dioxide (the latter product strongly predominating in the procedure of ref. 9). The recovery of oxygen is nearly quantitative (but see below).

Attempts to miniaturize the procedure and to apply it not only to the analysis of pure cellulose, but to mixtures of sedimentary organic materials and biological specimens, reveal (i) that the recovery of micromole quantities of oxygen is frequently very incomplete when small samples are employed and (ii) that the procedures employed by Brenninkmeijer and Mook in order to maximize conversion of all oxygen to the form of carbon dioxide are not generally applicable. By exploring reaction conditions and plausible side reactions, Wedeking and Hayes (10) have shown that pyrolysis times as short as a few hundred seconds are frequently adequate (this appears to minimize reactions between pyrolysis products and the walls of the tube, thus improving recovery values dramatically) and that conditions employed during the tube-opening and product-recovery steps are critical. Using this newly developed variant of the procedure, samples containing as little as 0.3 micromole O can be analyzed with a precision of 0.3 per mil.

Post-Column Combustion of Chromatographic Effluents

The carbon isotopic analysis of pure organic compounds can be facilitated by combining the processes of sample purification and combustion. The resulting procedures should not only be more efficient, but also offer improved performance by reducing sample requirements and eliminating some steps that might otherwise contribute to the analytical blank. Two approaches to this procedure can be described. In the first, the process of integration is carried to its logical conclusion and isotope-ratio measurement is also carried out on line. A reduced precision barely applicable to measurement of natural-abundance variations is obtained. Very high precision is obtained in the second alternative, in which the carbon dioxide is separately trapped and submitted to conventional isotopic mass spectrometry.

Isotope-ratio monitoring GCMS. Matthews and Hayes (11) have described in detail a technique applicable to the on-line measurement of carbon and nitrogen isotopic abundances in gas-chromatographic effluents. The carrier gas stream is led directly to a post-column combustion furnace and, by way of a conventional GCMS interface, to the ion source of a mass spectrometer. High-speed beam switching techniques are employed to measure the ion-current ratio of interest (45/44 or 29/28). A precision of 3 per mil is obtained on samples as small as 100 nanomoles carbon.

Trapping of CO₂ for high-precision measurements. Seeking to couple the precision characteristic of isotope ratio mass spectrometers with the advantages of on-line combustion of chromatographic effluents, Monson, Qualls, and Hayes (unpublished results) have explored procedures in which on-line combustion is followed by trapping of the carbon dioxide, with purification and packaging of the carbon dioxide taking place off line.

Obtaining perfect trapping of the carbon dioxide from the carrier gas stream is not trivial. We have found, however, that traps formed from ultra-thin-wall stainless steel tubing work well. Tubing with an outer diameter of 3 mm is employed and is formed into six loops approximately 2 x 8 cm. With the long axis of the loops oriented vertically, the trap is immersed only halfway in liquid nitrogen. Micromole quantities of carbon dioxide can be trapped with perfect efficiency from helium flow rates as high as 1 L/min.

Propane is used as a test sample allowing convenient measurement of the completeness of combustion. As shown in Table 1, this hydrocarbon happens to have the same molecular weight as carbon dioxide and to share its low vapor pressure at liquid nitrogen temperature. Accordingly, any uncombusted propane is also trapped from the furnace effluent and

Use of Propane to Detect Incomplete Combustion

	MW	45/44	"δ"	P ₋₁₉₆ , torr
CO ₂	44	0.01	0	10 ⁻⁸
C ₃ H ₈	44	0.03	2,000	2 x 10 ⁻⁶
1 C ₃ H ₈ + 2,997 CO ₂	44	0.01	2	

In general:

$$X_{\text{combusted}} = 1 - 0.0005\Delta\delta$$

$$\Delta\delta \approx 1 \rightarrow X = 0.9995$$

packaged together with the carbon dioxide. Because it contains three carbon atoms (as opposed to one in carbon dioxide), however, the propane makes a disproportionate contribution to the mass-45 ion current (as noted in the Table, if pure propane were measured as though it were carbon dioxide, it would appear at +2000 per mil on the delta scale) and is very easily detected (note the result for a 1:2997 propane:carbon dioxide mixture). Using this procedure, it can be shown that complete combustion is obtained over cupric oxide at 850°C under normal operating conditions.

Successful chromatographic separation often depends on derivatization of polar or reactive samples. The carbon added by the derivatizing reagent adds to that from the initial sample and will, in most cases, seriously distort the isotopic result. It is shown in Table 2, however, that this effect can be very conveniently taken into account by mixing a carefully chosen (i. e., one with similar functional groups, reactivity, and chromatographic characteristics) internal standard with the materials of interest. In this case, hexanoic acid was chosen as an internal standard for use in the analysis of nonanoic acid. The isotopic composition of the hexanoic acid was known from prior, sealed-tube combustion measurements (see Table 2). Combining that information with the observed isotopic composition of the methyl hexanoate processed by the post-column combustion technique allowed calculation (by difference) of the composition of the methyl carbon added during the derivatization procedure. The calculation is carried out using the equation shown at the top of Table 2, in which the n terms designate molar quantities of carbon and the F terms designate fractional abundances of carbon-13 (see ref 1). The subscript T designates the total carbon pool resulting from combination of the parent compound and the derivatizing reagent. As noted, the observed isotopic composition of the methyl nonanoate can be corrected in turn, the result agreeing well with previously known values for this material.

$$n_T^F = n_{\text{parent}}^F + n_{\text{derivative}}^F$$

$\delta^{13}\text{C}_{\text{PDB}}, \%$

Hexanoic Acid (free)	-31.75 ± 0.09	from static combustion
as Methyl Hexanoate	-35.42 ± 0.06	from post-column combustion
Methyl Group	-57.44 ± 0.47	calculated by difference
Methyl Nonanoate	-23.01 ± 0.12	from post-column combustion
Nonanoic Acid (free)	-19.18 ± 0.15	calculated, cf. -19.34 ± 0.06

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HIGH SENSITIVITY QUANTITATIVE ANALYSIS OF DRUGS & METABOLITES BY GC/MS

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The intent of this paper is to discuss some of the factors which affect precision and accuracy in high sensitivity measurement of drugs and metabolites in physiological specimens. For this purpose I define "high sensitivity quantitative analysis" as measurement of drug concentrations below 10 ppb (i.e. 10 ng/ml), since in my experience the technical problems associated with accurate measurement often become enormously greater as the drug concentration falls below this level.

The degree to which various techniques and operations affect sensitivity and quantitative reproducibility is highly dependent on the specific compound being measured. Consequently, one should be very cautious about stating generalizations regarding specific techniques to be used in quantitative GC/MS analysis. My comments are based primarily upon experience in our laboratory using a basic procedure consisting of the following steps:

1. Addition of a stable isotope labeled analog as internal standard to 1 ml of specimen.
2. Equilibration, after vortexing, at 4°C overnight.
3. Conventional solvent extraction.
4. Derivatization, if beneficial.
5. Selected ion monitoring using capillary gas chromatography/chemical ionization mass spectrometry performed on a quadrupole instrument.

In virtually all of our GC/MS assays we use deuterium labeled analogs as internal standards. We would prefer to use analogs labeled with ^{13}C , ^{15}N , or ^{18}O because there would be a reduced likelihood of showing isotope effects, but these compounds are seldom available and are prohibitively expensive to prepare. The advantages of stable isotope labeled analogs as internal standards are well known, although there is not universal agreement that they are always a better choice for internal standard than a well-chosen homolog or structural isomer. Our own experience strongly favors the stable isotope labeled internal standard. For example, we recently compared the precision obtained in the measurement of different concentrations of methaqualone using two internal standards, methaqualone- ^3H , and mecloqualone (an analog of methaqualone in which a methyl group is replaced by a chlorine.) The precision obtained with the deuterated internal standard was slightly, but consistently, better than that obtained with the chloro analog internal standard.

Undoubtedly the most perplexing problem encountered in high sensitivity GC/MS analysis is the adsorption of the analyte onto surfaces. This results in loss of analyte during the sample preparation procedures and/or during the chromatographic separation. The loss of analyte due to adsorption always reduces the sensitivity and precision of the assay and can even result in complete disappearance of the analyte. Many different techniques have been described for minimizing adsorptive losses. We routinely silylate all of our glassware even though not all assays appear to require or even benefit from this procedure. Adsorption of basic drugs is often reduced by prewashing glassware with a solution containing a strong base, such as triethylamine. Addition of a "carrier" compound to the specimen before extraction has proven to be one of the most effective methods of reducing adsorptive losses. The carrier is normally a compound having a similar structure to that of the analyte. The internal standard can act as a carrier, but it is preferable to add larger quantities of the carrier ($>1\text{ }\mu\text{g}$) than are optimum for the internal standard.

Other ways of reducing losses due to adsorption include the practice of keeping sample and extract volumes and the number of transfers to a minimum, and the addition of a small amount ($\sim 20\text{ }\mu\text{l}$) of a high boiling "keeper solvent", such as isoamyl acetate, to

extracts before they are concentrated by evaporation. Adsorption losses are usually greatest when extract residues are allowed to evaporate to dryness. We have found that use of a keeper solvent significantly improves the reproducibility of recovery of nanogram quantities of drugs such as amphetamine and tetrahydrocannabinol. Unfortunately, the use of a keeper solvent makes it difficult to totally remove derivatizing reagents such as trifluoroacetic anhydride. Since trifluoroacetic anhydride can be deleterious to the chromatographic column, we do not use a keeper solvent when trifluoroacetylation is required.

All of our GC/MS quantitative analyses are now performed on fused silica capillary columns. Typically we use a 15 meter, 0.32 mm I.D. column with a thick film (1.0 microns), bonded phase, such as DB-1 or DB-5 (J & W Scientific, Inc., Rancho Cordova, CA). The extract (1-2 μ l) is injected in the splitless mode at a column temperature approximately 60° below the temperature which results in the analyte eluting in 2 to 4 minutes. The column temperature is then rapidly increased. Hydrogen is used as the carrier gas at flow velocities of 50 to 100 cm/sec.

The improved sensitivity and resolution achievable with capillary columns is well recognized. Of equal importance to trace analysis is the generally lower adsorptivity of a well-prepared fused silica column. Our greatest difficulty with capillary columns has been encountered in the chromatography of strongly basic drugs such as flurazepam and 1-acetylmethadol, both of which contain tertiary amine groups. Fused silica is inherently slightly acidic so that it is extremely difficult to prepare a fused silica column that shows no adsorptive loss of strongly basic compounds.

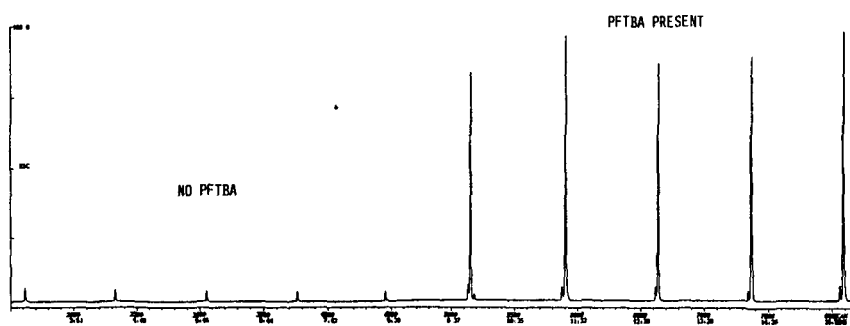
The choice of method of ionization can be a major factor in determining the sensitivity and precision of an assay. Ideally the ionization process should be highly efficient for the analyte but inefficient for the co-extractants. It should also result in concentration of the analyte's ion current at only one or two high mass m/z values. All of our drug assays use either positive ion chemical ionization with ammonia as the reagent gas, or electron-capture negative ion chemical ionization. For most drugs these two methods of ionization afford greater sensitivity and selectivity than electron impact ionization. However, chemical ionization does tend to be more sensitive to experimental parameters than electron impact ionization. For example, most of the benzodiazepine drugs show intense molecular anion peaks when the negative ion chemical ionization is conducted at low (< 100°C) ion source temperatures. However, at ion source temperatures above about 180°C, the only abundant ion in the EC-NCI mass spectra of chlorine-containing benzodiazepines is usually the Cl^- ion.

Another experimental factor which can strongly affect the appearance of EC-NCI spectra is the presence of small quantities of oxygen in the ion source; this can result from minute air leaks, as recently demonstrated by Garland and Miwa (Biomedical Mass Spectr., 1983, 10, 126). Thus, contaminants in the reagent gas can significantly affect the chemical ionization process, even though the presence of the minor component may be difficult to detect. This effect of minor reagent gas components upon the ionization process can make it difficult to achieve high precision, particularly if something other than a stable isotope labeled analog is used as the internal standard. In some circumstances, however, minor reagent gas components can be used to advantage. At last year's ASMS conference, Feng, Rudewicz, and Munson reported that addition of strong electron capturing species into the ion source under ammonia chemical ionization conditions can significantly increase the abundance of positive ions generated from various analyte molecules. We also had observed this effect, and have now shown that the intensity of the MH^+ ion in the ammonia CI mass spectrum of a drug such as cocaine can be increased by a factor of more than 20 by introducing into the ion source a small quantity of perfluorotributylamine (PFTBA). Figure 1 shows the MH^+ ion current profile resulting from successive injections of 1 ng of cocaine into the GC/MS; first in the absence of any PFTBA, and then after introduction of PFTBA. Under the ammonia chemical ionization conditions used no abundant PFTBA positive ions are observed. The generality of this effect and its possible application to high sensitivity analysis of drugs is currently being explored.

Figure 1

EFFECT OF PERFLUOROTRIBUTYLAMINE (PFTBA) ON SIM RESPONSE FOR COCAINE

AMMONIA PCI-MS



WHAT IS ADEQUATE QUALITY ASSURANCE
IN ANALYSIS OF ENVIRONMENTAL
POLLUTANTS: Denis C.K. Lin; Environmental Testing
and Certification Corporation, Edison, New Jersey
08837.

The analysis of environmental pollutants has become increasingly important in terms of impact on quality of life as well as economic issues. The more severe the impact the more emphasis has been placed on the quality of the data. While non-chemists may not appreciate the need for precise, accurate and sensitive data, it is helpful that competent analytical chemists have a consensus on what an individual technique can achieve in environmental analysis. In this presentation various mass spectrometric methods will be evaluated and their applicability to appropriate situations will be discussed.

THE DEVELOPMENT AND ROLE OF DEFINITIVE METHODS IN CLINICAL CHEMISTRY

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Definitive methods (DM's) in clinical chemistry have been defined as analytical methods which have been subjected to in-depth investigation and evaluation for sources of inaccuracy, including nonspecificity. The mean value determined by a DM is taken as the "true" value [1]. For the past several years, NBS researchers have been involved in the development of isotope dilution/mass spectrometric (ID/MS) methods as DM's for serum analytes. These methods are not intended for routine or even occasional use by clinical laboratories, but are intended primarily for the purpose of obtaining highly accurate values for constituents of serum pools. Pools with accurately known analyte concentrations are useful for evaluating potential reference methods and allow clinical laboratories to monitor their own performance and to calibrate their instruments. DM's are proving valuable for survey programs, such as those of the College of American Pathologists, that seek to determine how well individual laboratories perform routine methods and whether overall mean values of specific methods show evidence of bias.

To achieve the accuracy and precision which are demanded for a definitive method in clinical chemistry [1], we find that careful and exacting chemical and measurement protocols must be followed. In our methods, serum aliquots are accurately weighed and all further aliquoting and additions are on a weight basis to eliminate the imprecision associated with volumetric determinations and additions. To each serum sample we add a weighed amount of a stable isotope labeled analog of the compound to be determined (e.g., cholesterol-25,26,26,26,27,27,27-d₇ for the determination of cholesterol). The sample is thoroughly mixed and is allowed to equilibrate to ensure homogeneity. (Studies are performed to determine the time needed for equilibration.) The sample is lyophilized and/or other separation steps are carried out to isolate the mixture of labeled and unlabeled compound from the serum matrix. A chemical derivative is prepared to give a compound suitable for GC/MS analysis. Considerations included in the choice of a derivative are: volatility, presence of a few, but intense, ions for isotope ratio measurements, and occurrence of "high mass" and "unique" ions in the spectra to enhance the specificity of the analysis. The gas chromatograph provides for reproducible sample introduction and for final separation of the compound from impurities.

For the isotope dilution measurements, the magnetic field of a single-focusing mass spectrometer is switched between the m/z values of the ion of interest for the unlabeled and labeled analyte. The ion abundances generally are measured for a one-second time period for each selected mass throughout the elution of the GC peak and converted to digital counts. The data system accumulates the one-second counts separately for each ion, corrects for the background measured at each mass just prior to the elution of the GC peak, and calculates a ratio between the total counts for each ion.

Simply measuring the samples in this manner is not, however, sufficient to generate the accurate and precise results required for a DM. Calibration standards made from mixtures of known amounts of high purity unlabeled material (NBS Standard Reference Materials are used) and the same labeled material that is added to the serum samples are run frequently. The protocol used involves bracketing each sample both in time and ratio with calibration standards. That is, immediately preceding the measurement of a sample, a calibration standard, with a ratio slightly lower (or higher) than the sample, is measured and immediately following measurement of the sample, another calibration standard, whose ratio is slightly higher (or lower) than the sample, is measured. The weight ratio of labeled to unlabeled analyte in the sample is found by linear interpolation between the two standards. To ensure that bias is not introduced by the order of analysis, the order is reversed on a second day. The two days' values are averaged to produce the reported result. On the same occasion, when the two days' values differ by more than a pre-set limit (e.g., 0.5 percent for urea), a third determination is made and the three results are averaged. Finally, to ensure that no method-dependent bias exists, confirmatory measurements are made on different ions and using alternate modes of ionization.

Although the results from this procedure have proven to be excellent, there are pitfalls. Each analyte has unique problems associated with its isolation from potential interferences, derivatization for gas chromatography, and mass spectrometric behavior. The mass spectrometer itself presents problems for this type of analysis. Small aberrations which would not affect normal uses of a mass spectrometer can noticeably worsen precision and result in costly and time-consuming shutdowns of the instrument.

To date, we have developed ID/MS methods for total cholesterol, glucose, urea, and uric acid in serum. As an example of the manner in which data is obtained, Figure 1 contains single ion chromatograms for the labeled and unlabeled glucose derivative, D-glucosylboronate cyclic 1,2:3,5-bis(butylboronate)-6-acetate (glucose BBA). With the capillary columns required to separate this glucose derivative from the derivatives of other hexoses that may be present in serum, the bracketing procedure involved injecting one standard, followed seven minutes later by a sample, and fourteen minutes later by the other standard. This allowed us to maintain high precision, while saving time. Perhaps the best data obtained to date have been for serum urea. Table 1 summarizes the results obtained on replicate analyses of a human serum sample measured using three different pairs of ions. Urea-¹⁸O was the labeled material and the trimethylsilyl ether of 6-methyluracil was the derivative measured.

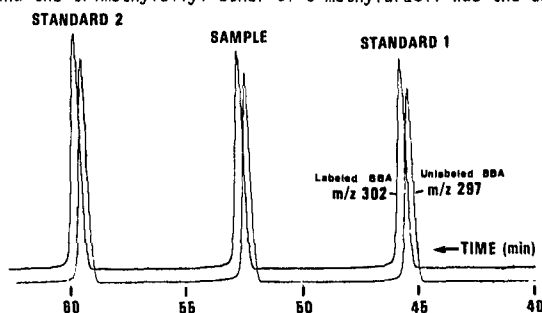


Figure 1. Single ion chromatograms showing the responses of labeled and unlabeled glucose BBA for a serum sample bracketed by calibration standards.

Table 1

Replicate Results on a Human Serum Sample Measured Using Three Different Pairs of Ions
(mg urea/g freeze-dried serum)

	m/z <u>255, 257</u>	m/z^* <u>271, 273</u>	m/z <u>120, 121[#]</u>
Mean of six samples	7.242	7.240	7.239
CV	0.12%	0.12%	0.12%
Difference from m/z 255, 257	--	0.03%	0.05%

*isobutane CI

[#]doubly-charged ions

Reference

- [1] "Guidelines for the Development of Definitive Methods for Use in Clinical Chemistry for the National Reference System in Clinical Chemistry," NRSCC 1, National Committee for Clinical Laboratory Standards, Villanova, PA 19085.

WOC 1 } ANALYSIS OF ORGANIC MATERIAL ACCUMULATED
ON GRANULAR ACTIVATED CARBON FILTERS USED IN DRINKING WATER
TREATMENT; D. S. Millington, T. Kamei, D. Bertino and D.
Maltby; Department of Envr. Sci. & Engr., U.N.C., Chapel
Hill, NC 27514.

Granular activated carbon (GAC) filter beds are employed in about 40 locations in the U.S. to help eliminate organic pollutants having malodorous or distasteful properties. We have developed a method for analysis of the organic material accumulating on the GAC based on sequential solvent extraction using ultrasonic agitation and capillary column GC/MS analysis of the extracts. EI with low resolution accurate mass measurements, isobutane CI and library search techniques were used to identify specific components. Quantitative analysis of selected pollutants--a difficult problem owing to an inability to reproduce the sample matrix--was accomplished by standard addition of the selected pollutants to a portion of the spent carbon and comparing signals derived from reconstructed ion current profiles for the spiked and unspiked carbon extracts. Results have shown that this method is a useful approach to the qualitative and quantitative analysis of a broad spectrum of organic pollutants accumulated by these filters.

_____ } CAPILLARY GC/MS PROFILING OF XENOBIOTIC
CHEMICALS IN HUMAN TISSUE USING EI AND PNCIMS. Bertha L.
Proctor, Graduate Program in Environmental Sciences, University
of Texas at Dallas, Richardson, TX 75080

Erie and Niagara Counties in New York State have over 150 dumpsites which contain large quantities of toxic chemicals. The spread of pollution from these sites into the Niagara River and Lake Ontario has been documented. Environmental toxicants are in many instances greatly concentrated in biological compartments such as adipose tissue.

I will present results from the GC/MS profiling of xenobiotic chemicals in human adipose tissue collected from long time area residents. Donors died from "spontaneous" causes (i.e. fractured skull) and were western New York State residents for 20-75 years.

Positive-negative chemical ionization mass spectrometry effectively highlighted the presence of several halogenated organic compounds. Of particular interest are several chlorinated trifluoromethylbenzene derivatives whose origin may be the Hyde Park Disposal Site.

The presence of specific compounds was confirmed by comparison of EI, PNCI mass spectra and relative GC retention time with those of standards.

ACCURACY AND PRECISION IN THE GC/MS DETERMINATION OF TOXIC ORGANIC COMPOUNDS IN HAZARDOUS WASTES

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The Resource Conservation and Recovery Act of 1976 (RCRA) established a Federal program to provide comprehensive regulation of hazardous wastes. Accurate quantitative data is vital to any program for the proper management of hazardous waste. The use of Standard Reference Materials (SRM's), where available, or the use of well-characterized materials as standards and methods whose accuracy and precision are known can help ensure the accuracy of analyses. Unfortunately, SRM's for hazardous waste analysis are not available. Analytical methods necessary for the certification of such materials for organic compound content must be developed, and the long-term stability of the materials must be assured before such standards can be made available.

In order to determine the accuracy and precision achievable in the analysis of a hazardous waste, we have characterized and analyzed a liquid sludge material by combined high resolution capillary column gas chromatography/mass spectrometry.

Sample Preparation

A sample of an industrial organic sludge from a solvent recovery/hazardous waste storage facility was diluted with a high-boiling ligroin (bp 90-110 °C) and fortified with known amounts of eleven priority organic pollutants so that the resulting concentrations were in the 10-50 ug/g (ppm) range. Fifty liters of the material were prepared, and 100 mL aliquots were packaged in 500 clean, amber sample vials and sealed with Teflon-lined caps.

Sample Analysis

A stratified random sampling scheme was used to select fifteen samples from the total batch of material for quantitative analysis. Fifteen separate strata were used, and a random sample within each stratum was chosen. This insured full examination of the sample set and at the same time insured a degree of random choice of samples. Duplicate analyses of the fifteen samples were made over three days. The order of the individual analyses was randomized.

Figure 1 shows a full scan GC/MS run of the quality assurance sludge material. A 1.0 µL aliquot of the sample was injected in the split mode into a nonpolar fused silica capillary column. The major peaks in the chromatogram are normal aliphatic hydrocarbons; the broad unresolved envelope of peaks eluting between C9 and C11 are aliphatic hydrocarbons (predominantly C3-C5 substituted cyclohexanes). The organic compounds added to the sludge material are the lettered peaks in Figure 1.

Quantitative determinations for the eleven analytes were made by GC/MS with selected ion monitoring using p,p'-DDE as an internal standard. The molecular ions for the analytes and the p,p'-DDE were monitored with dwell times of 100 ms, yielding approximately 25 data points per gas chromatographic peak. Response factors for the analytes relative to the p,p'-DDE internal standard were determined from a standard solution of the analytes and internal standard, prepared to closely resemble the concentrations of the analytes in the sample. Analyte concentrations were calculated from the peak areas of the analytes, the peak area of the internal standard, the response factors of the analytes, and the internal standard concentration. Table 1 shows the difference between the gravimetric concentration and the average experimentally determined concentration and the percent CV. Actual analyte concentrations are not given because samples of this material are currently being used in quality assurance monitoring programs by the EPA.

For each of the eleven analytes, a within- and between-bottle analysis of variance (ANOVA) was performed [1,2]. The results showed no statistically significant ($\alpha = 0.05$) between-bottle variability. Furthermore, approximately one-half the calculated between-bottle components of variance were slightly negative, and the other half were small and slightly positive, a behavior characteristic of a homogeneous material.

The GC/MS data was also used to characterize the GC/MS measurement variability. A within- and between-day ANOVA was made for the eleven analytes to characterize the overall variability of the GC/MS measurements. This yielded statistically significant ($\alpha = 0.05$) between-day components of variance for almost all analytes. The components of variance were used in the calculation of weights for the weighted averages of the analyte concentrations, however the straight averages were practically identical to the weighted averages.

The standard error (standard deviation of the weighted average) for each analyte was calculated from the within- and between-day components of variance. The coefficient of variation (Table 1), which is an expression of uncertainty, was about 3 percent for all analytes. These data indicate that accurate quantitative results in the determination of toxic organic compounds in a complex matrix can be obtained using high resolution capillary column GC/MS with selected ion monitoring.

References

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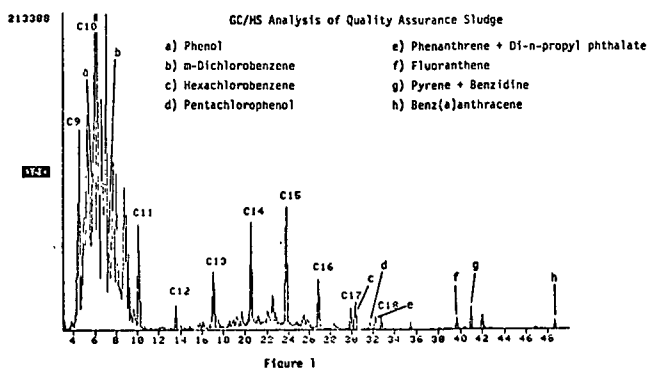
Table 1

Analytes in Quality Assurance Sludge at the 10-50 ppm Level Determined by GC/MS

Analyte	Difference Between Gravimetric Concentration and Experimental Concentration ^a (µg/g)	Percent CV ^b
Phenol	- 0.61	5.7
m-Dichlorobenzene	- 0.82	4.7
Hexachlorobenzene	0.39	2.1
Pentachlorophenol	0.75	2.5
Phenanthrene	- 0.13	3.4
Fluoranthene	0.44	1.5
Pyrene	1.21	1.6
Benzidine	3.36	3.3
Di-n-propyl phthalate	0.00	2.4
Di-2-ethylhexyl phthalate	0.56	1.1
Benz[a]anthracene	0.97	1.4

^aAverage of 29 measurements (1 analysis was discarded due to faulty injection). Values represent gravimetric concentration - experimental concentration.

^bPercent CV = (standard error/ $\bar{X}$) x 100



REACTIONS OF POLYCYCLIC AROMATIC HYDROCARBONS
IN NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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Analysis of polycyclic aromatic hydrocarbons (PAH) has annoyed researchers ever since the discovery of these carcinogens in the environment. The similarities in the structures of the isomers make separation difficult, even with the advances that have been made with capillary column gas chromatography. Identification by mass spectrometry is also hampered since the electron impact and most chemical ionization mass spectra of the isomers are often indistinguishable.

The 13 PAH listed in Table I were studied by GC/NCI MS with different moderating gases. Separation was done using an SE-54 (25 m X 0.32 mm ID) fused silica capillary column which was inserted directly into the ion source of an HP-5985B GC/Mass Spectrometer. The instrument was tuned under normal conditions with methane as the moderating gas.

Table I-Compounds Studied (Molecular Weight)

<u>Group I</u>	<u>Group II</u>	<u>Group III</u>
Anthracene (178)	Phenanthrene (178)	Benzo(b)fluoranthene (252)
Fluoranthene (202)	Pyrene (202)	Benzo(k)fluoranthene (252)
Benz(a)anthracene (228)	Chrysene (228)	Benzo(a)pyrene (252)
Tetracene (228)	Triphenylene (228)	
Perylene (252)	Benzo(e)pyrene (252)	

A trace amount of an ion identified as (M-H+O) had been identified in the NCI(CH₄) mass spectra of the higher molecular weight compounds, apparently from reaction of residual atmospheric oxygen in the source. Use of isobutane moderating gas containing 1.4% oxygen increased the relative amount of oxygen addition. The Group I mass spectra, except for fluoranthene, contained a molecular ion base peak, small amounts of hydride abstraction and addition, and addition of oxygen. Fluoranthene and the Group II compounds gave mass spectra which contained an (M-H) base peak with varying amounts of molecular ion and hydride addition. There was more (M-H+O) observed in these spectra than in the spectra of the other compounds.

A 9:1 mixture of nitrogen and nitrous oxide is widely used as a source of O⁻. Under these conditions, the Group I mass spectra included the molecular ion base peak with some (M-H+O). In the Group II spectra, the (M-H+O) ion is usually the base peak. Ions from the abstraction of one or two hydrogens are also present and the abundance of the molecular ion is low or absent.

In a 5:1 mixture of nitrogen and carbonyl sulfide, S₂⁻ through S₆⁻ ions were produced in great abundance. The mass spectra gathered with this moderating gas were dominated by these ions (especially Group II), and no addition of any sulfur species was observed.

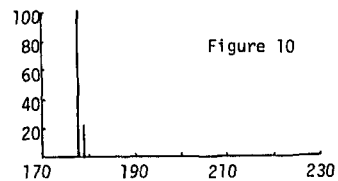
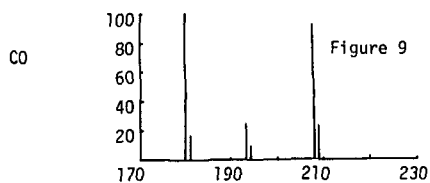
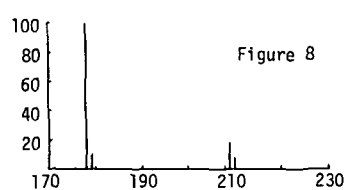
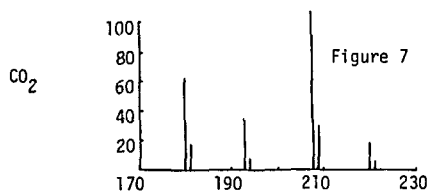
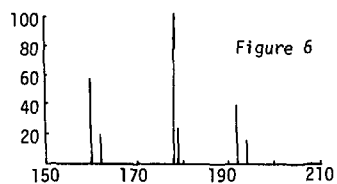
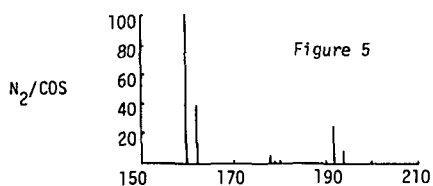
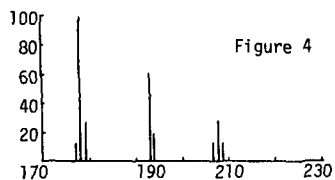
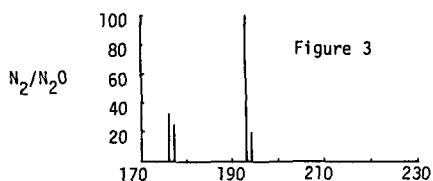
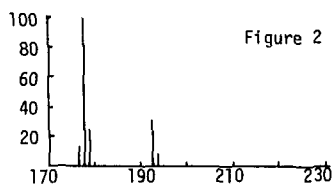
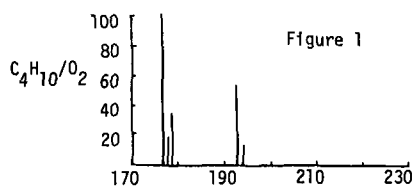
Spectra obtained using CO₂ and CO as moderating gases are similar and distinct from those obtained by other methods. The mass spectra of the Group I compounds contained the molecular ion as the base peak and varying amounts of (M-2H+O). Spectra of Group II were different. The molecular ion was absent (except for pyrene) and ions at (M+2H), (M-H+O), (M-2H+2O) and (M-2H+CO₂) (except in CO) were observed.

The spectra of the benzofluoranthenes and benzo(a)pyrene remained identical under all conditions tried. The molecular ion base peak and the C-13 peak were present and there was no sign of any addition ions.

The mass spectra of phenanthrene and anthracene are representative of the compounds in their groups. The molecular ion regions of the spectra obtained with the various moderating gases are illustrated in Figures 1-10.

PHENANTHRENE

ANTHRACENE



HIGH RESOLUTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY METHOD FOR
QUANTITATION OF POLYNUCLEAR AROMATIC HYDROCARBONS IN GROUNDWATER SAMPLES

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The ubiquitous existence of polycyclic aromatic compounds (PAH) has been well documented with recent advances in analytical techniques. This knowledge has created concern because of the toxicity, mutagenicity, and carcinogenicity associated with this class of compounds. These pollutants are often present at very low levels (parts-per-trillion) in a water matrix that may often contain much higher levels of organics and/or inorganic particulates. Analysis of the PAH compounds at trace levels can be highly variable, subject to interferences, and difficult to identify.

An improved method has been developed for the quantitative analysis of polynuclear aromatic hydrocarbons at trace levels in water to overcome these problems. Inherent in environmental monitoring of this type is some form of organic enrichment procedure. Several articles have been written on the extraction of organics from water with different types of macroreticular resins.¹⁻⁵ Wolkoff and Creed validated the resin efficiency of C-18 cartridges for the collection and concentration of organics in environmental samples.⁶ For this study a resin concentration system was developed incorporating an all teflon manifold that permits two C-18 resin bed cartridges in tandem to be connected to a water source (Fig. 1). The water sample is passed through the resin beds in tandem to trap and concentrate pollutants that may be present. To monitor for losses, three deuterated analogs, D₁₀ phenanthrene, D₁₀ pyrene and D₁₂ chrysene were spiked onto the head of the resin bed prior to sampling. The levels placed on the resin bed were chosen to correspond to a concentration of 25 parts-per-trillion in a 50 liter water sample.

Data on the three deuterated analogs indicate recoveries better than 90% are achieved. A breakthrough study was conducted and showed that for some compounds a significant portion was trapped on the second resin bed. The percentage of material found on the second bed was D₁₀ phenanthrene 52%; D₁₀ pyrene 1.3%; D₁₂ chrysene 0.3%. The organics are stripped off the resins with THF, dried on a sodium sulfate microcolumn, and concentrated under a stream of dry nitrogen to a volume of 0.1 ml. The concentrated extract was spiked with a deuterated internal standard D₁₀ anthracene, and immediately analyzed by GC/MS.

Fused silica capillary column (30 m x 0.24 mm SE 54) gas chromatography directly coupled to an HP 5985B Mass Spectrometer operated in the selected ion mode provided the required sensitivity, selectivity, and specificity for the analysis. Quantitation was based on the ratio of the detector response for each analyte compared to the response of the labelled internal standard. Instrument detection limits were determined from 11 replicate analysis of standard solutions. Based on this data the PAH's could be quantitated in water concentrations as low as 0.84 ppt (phenanthrene). The average level of detectability is 4.1 ppt for all analytes. The lower and upper confidence limits were 0.699 and 1.755 x analyte D.L. respectively or for the average (4.1 ppt) the range was 2.86 to 7.19 ppt. The total ion chromatogram shown in Fig. 2 was typical of a contaminated water sample encountered in the study.

The results of this preliminary set of experiments demonstrates the method is simple,

fast, and yet both sensitive and accurate in measuring pollutants at trace levels.

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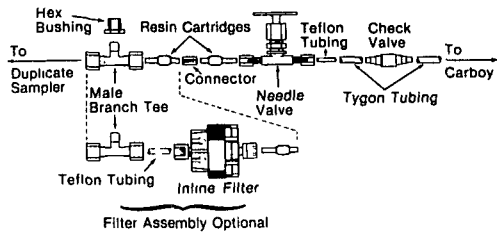


Fig. 1 Tandem Resin Bed Sampler

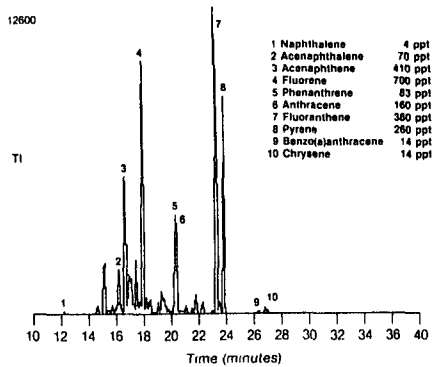


Fig. 2 Total Ion Chromatogram of Contaminated Water Sample

OPTIMIZING PRODUCTIVITY FOR THE TRACE ANALYSIS OF REAL-LIFE SAMPLES USING FLASH GAS CHROMATOGRAPHY MS/MS. Scott Tanner, Angie Ngo and William R. Davidson, Sciex®, 55 Glen Cameron Road, Thornhill, Ontario, Canada L3T 1P2

INTRODUCTION

Recent methodology developments in GC/MS have provided for the reasonably sensitive and specific analysis of trace contaminants in real-life samples. However, for complex or "dirty" samples, such as dioxin in fish, the clean-up and extraction procedures are laborious and can require several days. Combined with the long chromatographic runs that are required, this leads to poor laboratory productivity.

The types of matrix interference which are responsible for this labor-intensive analysis can fall into either of two general categories. Qualitative interference is encountered when a component of the matrix gives rise to a response at the same m/z ratio as the analyte; ie. isobaric interference. Quantitative results will be in error if the reagent ion concentration is depressed due to the presence of a high concentrations of concomitant compounds in the mixture. In the clean-up procedures for dioxin, the effort is largely directed at obviating the quantitative interference since the qualitative interferents (such as PCB's) are generally transparent to chemical separation. Long chromatographic separations are then required to gain the required specificity.

We report here the development of "flash" GC/MS/MS procedures which greatly improve laboratory productivity while retaining high specificity and sensitivity of analysis.

EXPERIMENTAL

After various stages of clean-up, samples were splitless injected onto a short (12 metre) capillary GC column. Although little dependence on the column coating and diameter were observed under the temperature programming used, the best results were obtained on a narrow bore DB-5 column. N_2 at 1.8 ml/min was used as the carrier gas, and the oven temperature was ramped from 170°C to 220°C at 30°C/minute and then held. (For the higher congeners of dioxin the final temperature was held at 280°C.) The GC effluent was passed through a transfer line at the final oven temperature to the mass spectrometer ion source. A chemical ionization source utilizing a Townsend discharge at 1 milliamp was operated at ca. 4 torr and 195°C. The reagent gas used was 20% O_2 in N_2 (synthetic air). Mass analysis was performed with a TAGA® 6000 tandem mass spectrometer. Mass programming was used in concert with the GC run time to adjust the masses monitored as each congener of dioxin and furan was eluted. In all cases, the first quadrupole was set to transmit the molecular ion (M^+) and the third quadrupole monitored the loss of $COCl$ from the parent ion. Collision Induced Dissociation (CID) conditions were selected to optimize the yield of the $m/z=257$ daughter ion for 2,3,7,8-TCDD.

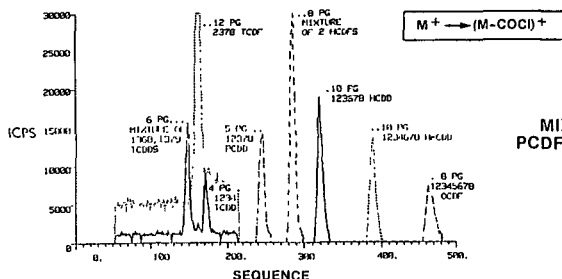
RESULTS AND DISCUSSION

The retention time for 2,3,7,8-TCDD on the column used is approximately 3 minutes; OCDD elutes in under 12 minutes. Therefore, each sample could be analyzed for all eight congeners of dibenzodioxin and eight congeners of dibenzofuran in approximately 15 minutes, including GC recycle time. As opposed to the normal use of the GC to obtain compound specificity on the basis of elution time (which requires along GC run), the primary function of the GC separation in this operating mode is to reduce matrix interference (reagent ion depression) at the retention time of the target contaminant. The large reagent ion concentration in the "bright" Townsend discharge also helps to alleviate reagent ion depression. In the single MS mode of operation the most sensitive response (approximately 2×10^{-7} Coulombs/microgram) is observed in the negative mode. However, under CID conditions the negative parent ions give rise primarily to the non-specific Cl^- daughter ions. As this was considered to provide unsatisfactory specificity, the loss of $COCl$ from the M^+ parent ions was employed (with a tolerable loss of sensitivity to 5×10^{-9} Coulombs/microgram). It can be seen, then, that in this approach the specificity of analysis (elimination of isobaric interference) is provided by the MS/MS detector rather than the GC retention time. Also, it is not insignificant to the improvement of laboratory productivity that under the operating conditions the CI source is self-cleaning. The use of oxygen as a major component in the discharge gas ensures that the source is continually cleaned. As well, the quadrupole analyzers are relatively tolerant of contamination, so that the system can be operated for months, if not years, without a thorough cleaning.

As an example of the data obtained with this technique, Figure 1 shows a chromatogram of a standard mixture of PCDD's (polychlorodibenzodioxins) and PCDF's (polychlorodibenzofurans). The entire chromatogram was obtained in approximately 8 minutes. It will be seen that under these conditions complete isomer specificity is not obtained; if this information is required the experiment could be repeated in the negative mode (for example, 1234-TCDD and 2378-TCDD give rise to parent negative ions of different mass) or the temperature programming could be adjusted (1234-TCDD and 2378-TCDD can be separated by approximately doubling the elution time).

Figure 2 demonstrates the ability of the MS/MS detector to mitigate isobaric interference in the analysis of a real-life fish sample for TCDD. In the GC/MS run (upper figure) a strong response for the $m/z=320$ ion is observed within the TCDD elution window. However, the lack of a concomitant increase of the $m/z=322$ isotope indicates that the positive response was due to an isobaric contaminant. As a result of the incorrect isotope ratio one could not determine the presence of TCDD in this sample. However, the correct isotope response (100:98.8) in the GC/MS/MS chromatogram (lower figure) allows the quantitation of the dioxin contamination at 20 ppt.

An attempt will be made to compare the laboratory productivity, including both sample preparation and analysis, of four detector configurations: (1) "Flash" GC/MS/MS, (2) "Flash" GC/MS, (3) High Resolution GC/MS, and (4) DIP/MS/MS.

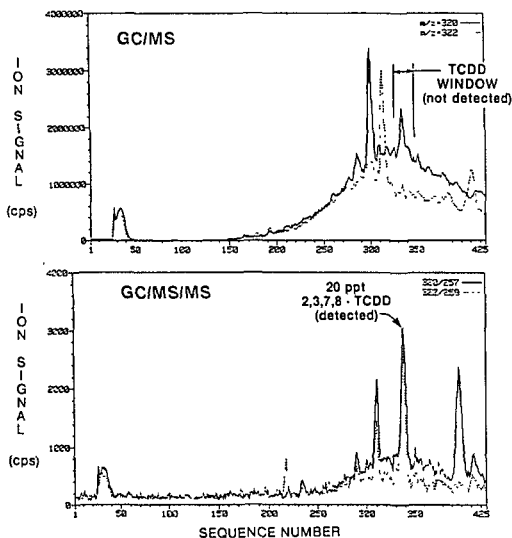


MIXTURE OF PCDD'S (DIOXINS) & PCDF'S (DIBENZOFURANS) STANDARDS BY GC/MS/MS

FIGURE 1

DETECTION AND QUANTITATION OF TCDD IN SALMON

FIGURE 2



APPLICATIONS OF A COMPREHENSIVE SCHEME FOR THE DIRECT ANALYSIS OF ORGANICS IN ENVIRONMENTAL MATRICES BY MS/MS

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A comprehensive analytical scheme for the direct analysis of organics in environmental matrices by MS/MS on a triple quadrupole mass spectrometer was presented by the authors at the 1982 ASMS meeting. The actual procedure involved and the application of this methodology to real world samples, with comparison to GC/MS results, will be presented this year.

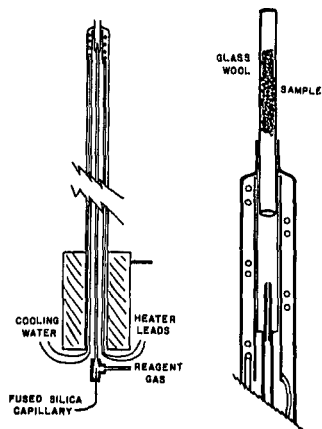
Solid matrices (1-10mg) and residues from lyophilized aqueous samples are placed on or between glass-wool plugs in a glass tube. If necessary, 10-20uL of CH_2Cl_2 could be added to the glass tube to facilitate extraction of the organics out of the matrix on to the glass wool. Amines and phenols in the matrix are converted into ureas and carbamates, respectively, by saturating the sample with 10uL of ethyl acetate-(freshly distilled) methyl isocyanate (4:1) containing 100ppm triethylamine. After 5min at room temperature, excess reagents are removed by purging with N_2 . PAH's are then converted into nitroaromatic compounds by adding 1uL of trifluoroacetic acid and then exposing the sample matrix to gaseous N_2O for 3-5 seconds. A stream of N_2 is again used to remove excess reagent and the sample tube is then placed on the end of a specially constructed heated solids probe, evacuated, and inserted into the removable ion source volume of the Finnigan TSQ. CH_4 and N_2O , at a pressure of 0.5 torr in the ion source, is employed both as the carrier gas and to generate the CI reactants, CH_5^+ and OH^+ . Gas flows through the sample tube while it is heated from 25° to 350°C over a period of 5-10 minutes facilitating volatilization of organics from the matrix. During this period the instrument, under computer control, is cycled repetitively through a series of eight 0.5 sec experiments (PIS 149 for phthalates, NLS 17,30,46,47 for PAH's, NLS 35 and 36 for chlorocarbons and NLS 57 for amines and phenols). At the end of the heating period all data acquired from each type of scan is summed together and printed out in a conventional bar graph format. The result is a plot of molecular weight vs. relative abundance for all mixture components containing the particular function group being detected. Total time for sample derivatization, data acquisition and data processing is typically 25 minutes.

Tabulated below are the kind of results readily attainable by the above methodology. A standard unknown (spiked sand) used by the USEPA to evaluate all contract GC/MS labs was analyzed. All of the components were detected and semiquantitated to within a factor of five, with the exception of the bromo compounds, which would have been detected had a NLS 80 (HBr) been used. The low values for the phenols were rectified by using freshly distilled MeNCO. The results on the "rag oil" (a by-product of petroleum distillation) sample compared favorably with the GC/MS data, however it illustrates the lack isomeric specificity that is obtainable by GC/MS. The sample from a dump site illustrates the wider dynamic range and the additional information available on "real world" samples when the MS/MS methodology is used.

Summary of Results Obtained on EPA Cl.B/C3 Sample

Specially Constructed Heated Solids Probe and Sample Tube

compound	scan type	MS/MS value (ppm)	EPA value (ppm)
Priority Pollutant			
diethyl phthalate	PIS 149	1	-
dibutyl phthalate	PIS 149	48	33
dioctyl phthalate	PIS 149	12	-
dichlorobenzene	NLS 36	16	50
chloronaphthalene	NLS 36	13	33
trichlorophenol	NLS 35	36	67
4,4'-DDD	NLS 36	1	5
Heptachlor epoxide	NLS 36	8	7
fluorene	NLS 17	40	67
nitrobenzene	NLS 17	13	67
phenol	NLS 57	12	80
dimethylphenol	NLS 57	9	67
2-nitrophenol	NLS 46	-	33
Non-Priority Pollutants			
bromotoluene	-	-	111
ethylnitrobenzene	NLS 17	40	111
chloronitrotoluene	NLS 46	80	111
bromopentafluorotoluene	-	-	111
bromophenol	NLS 57	2	111



Summary of Results Obtained on a "Rag Oil" Sample

compound	compound group	GC/MS (ppm)	GC/MS (ppm)	MS/MS (ppm)
ethylbenzene	C2-benzenes	6800	14000	12400
m/p-xylene		5600		
o-xylene		1600		
toluene		6000	6000	3700
4-methyldibenzothiophene	C1-dibenzothiophenes	1800	3400	8000
3-methyldibenzothiophene		1600		
9-methylphenanthrene	C1-phenanthrenes	1700	3900	900
1-methylphenanthrene		1400		
2-methylphenanthrene		1000		
7-methylphenanthrene		800		
1,2,4-trimethylbenzene	C3-benzenes	1800	3100	8600
1-isopropylbenzene		1300		
phenanthrene		2000	2000	1000
dibenzothiophene		1500	1500	4000
1-methylnaphthalene	C1-naphthalenes	780	1510	500
2-methylnaphthalene		730		
p-methyl-n-propylbenzene	C4-benzenes	610	1210	4300
m-methyl-n-propylbenzene		600		
2,7-dimethylphenanthrene	C2-phenanthrenes	1100	1100	300
dimethyldibenzothiophene	C2-dibenzothiophenes	1100	1100	4800
benzene		1000	1000	2000
1,5-dimethylnaphthalene	C2-naphthalenes	850	850	200
Other Compounds Found by MS/MS				
	C5-benzenes			1500
	C6-benzenes			600
	C7-benzenes			400
	C3-naphthalenes			100
	tetrahydronaphthalenes			100
	C1-tetrahydronaphthalenes			200
	C2-tetrahydronaphthalenes			200
	tetrahydrophenanthrenes			1300
	C1-tetrahydrophenanthrenes			2300
	C2-tetrahydrophenanthrenes			1300
	C3-tetrahydrophenanthrenes			500

Summary of Results Obtained on a Black Solid Sample
from a Drum at a Chemical Dump Site

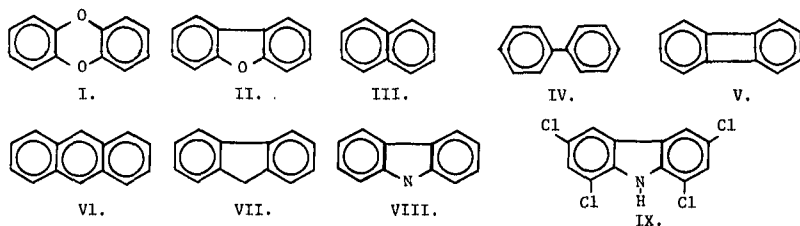
compound	GC/MS value (ppm)	MS/MS value (ppm)
<u>Priority Pollutants</u>		
<u>Volatiles</u>		
toluene	110	6300
ethylbenzene	93	33300
m-xylene	130	
o/p-xylene	160	
dichloromethane	7	-
<u>Base/Neutrals</u>		
naphthalene	10000	40000
phenanthrene	-	120
anthracene	-	
<u>Acids</u>		
phenol	-	280
dimethylphenol	-	120
<u>Non-Priority Pollutants</u>		
C3-benzenes	150	102300
C4-benzenes	-	37000
C5-benzenes	-	11400
C6-benzenes	-	4000
methylnaphthalene	+	90000
C2-naphthalenes	-	14700
C3-naphthalenes	-	5000
C4-naphthalenes	-	750
C3-naphthenes	-	3600
C4-naphthenes	-	5500
C5-naphthenes	-	750
indan	-	3000
methylin dan	-	6800
C2-indan	-	10600
C3-indan	-	10600
C4-indan	-	5000
C5-indan	-	3000
methyphenanthrene/anthracene	-	100
C2-phenanthrene/anthracene	-	100
C3-phenanthrene/anthracene	-	250
C4-phenanthrene/anthracene	-	425
C5-phenanthrene/anthracene	-	230
C1-phenol	-	200
C3-phenol	-	47

ISOLATION, IDENTIFICATION AND QUANTIFICATION OF TETRACHLORO-9H-CARBAZOLE
IN SEDIMENT SAMPLES FROM THE BUFFALO RIVER, NEW YORK.

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The Buffalo River in New York which receives both industrial and municipal sewage discharges is recognized as one of the most heavily polluted river systems in the United States. Fishes in the Buffalo River were found to exhibit a high incidence of tissue lesions, which lead investigators to correlate high levels of mutagenic (Ames Test) poly-nuclear aromatic hydrocarbons with proximity to a dye manufacturing plant.¹ Other studies showed that sediments from this section of the river also contained high levels of aromatic amines.^{2,3} Many amino containing textile dyes have been shown to be mutagenic by the Ames Test. As a result of a continued concern over high levels of potentially hazardous chemicals in the sediments and fish from this river, GC/MS analysis for planar-polychlorinated aromatics in sediments from three sites near the mouth of Lake Erie was initiated. Planar-polychlorinated aromatic chemicals are of particular interest because this class of compounds contains the very toxic 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD).⁴

Although no TCDD was found in any of the sediment samples, many chemicals of analogous structure were identified. These include dibenzofurans, ^{II} naphthalenes, ^{III} biphenyls, ^{VIII} biphenylenes, anthracenes (phenanthrenes), ^I fluorenes, ^{VI} and 9H-Carbazoles. A tetrachloro-9H-carbazole was identified as the planar organic residue at the highest level. This compound was therefore synthesized to confirm its structure by mass spec and NMR as 1,3,6,8-Tetrachloro-9H-Carbazole. ^{IX}



IX was therefore quantified in each of the three samples along with 1,2,3,4-TCDD a surrogate spike (100 ppt). The quantitative results are shown in Table I.

Sample Identification	% Recovery 1,2,3,4-TCDD	IX, ppb*
Cazenovia Creek	77	0.30
Black Rock Canal	82	0.60
Dye Plant Site	ND**	25, 22

* PPB = parts-per-billion wet weight.

** ND = not determined.

Table I.
Compound IX Quantitation Data

The Buffalo River sediments were found to contain many planar-polychlorinated chemical residues of similar instructure to TCDD. Also, in agreement with other published data, levels of chemical residues found in the river sediment appear to be highest near an area where a dye manufacturing plant has previously discharged into the river.

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CHEMICAL IONIZATION MASS SPECTRAL IDENTIFICATION OF THE WATER PHOTOLYSIS PRODUCTS OF AMDRO® FIRE ANT INSECTICIDE (AC 217,300)

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Introduction

The imported fire ant, *solenopsis invicta*, infests approximately 230 million acres in nine southern states of U.S.A. The imported fire ant is a problem principally because the sting of the ant is painful, causing serious reactions in hypersensitive persons, and killing livestock, and because the ant mounds blemish the landscape and may interfere with normal agricultural operations such as mowing and harvesting. AMDRO fire ant insecticide (the active ingredient of which is AC 217,300) has been developed by American Cyanamid Company and is conditionally registered in the U.S. as an insecticide for the control of fire ants, harvester ants, and big headed ants. This compound belongs to a new class of insecticides called "the amidinohydrazones". In order to assess the impact of a new pesticide on the environment, it is essential to determine its photostability.

Experimental

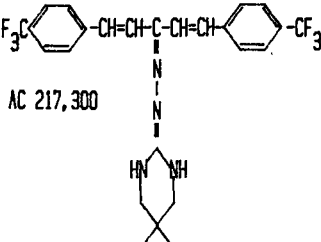
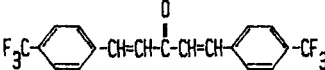
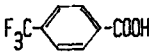
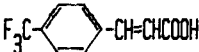
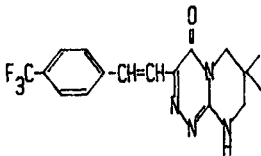
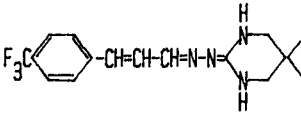
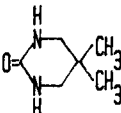
For identification of the photoproducts of AC 217,300, both carbon-14 labeled and carbon-13 labeled AC 217,300 were employed. Experiments were performed using a mixture of one part carbon-13 labeled AC 217,300 and one part unlabeled AC 217,300 containing sufficient carbon-14 radiolabeled AC 217,300 to allow ready detection and measurement of AC 217,300 and its photodegradation products by conventional radiotracer techniques. By virtue of the carbon-12/carbon-13 ratio, the mixture provided doublet ion peaks in the mass spectra of isolated AC 217,300 and its photodegradation products. Separate photolysis studies were performed for benzylic labeled AC 217,300 and pyrimidine labeled AC 217,300.

Photolysis experiments were conducted on a 20 ppm concentration of AC 217,300 in distilled water using a borosilicate-filtered Xenon Arc light in a Mallory environmental chamber at 27°C for various time intervals. At the end of each exposure period, the distilled water was extracted with ethyl acetate:diethyl ether (1:1, v/v) at various pH's (neutral, pH 2, pH 8). Following removal of the solvent, the radioactive residue was redissolved in hexane:2-propanol:methanol (85:5:10) for analysis by high pressure liquid chromatography (HPLC). The column effluent was monitored by UV^{254nm} and carbon-14 radiotracer detectors and was collected in 2-minute fractions for more precise quantitation of carbon-14 radioactivity by conventional liquid scintillation counting techniques. Following separation and quantitation, identifications of the photolytic products were accomplished using chemical ionization mass spectrometry (CIMS) in both the positive ion (PI) and negative ion (NI) modes coupled with cochromatography of the photoproduct with a standard reference compound.

Results and Discussion

The mass spectral data and photoproduct identifications are summarized in Table I. Photoproducts retaining the conjugated system gave tremendous NICI (CH₄) response and poor PICI (CH₄) response while compounds containing only the pyrimidine ring behaved conversely. Since parent AC 217,300 was labeled at 50 mole % at only one benzylic carbon atom, the number of benzylic carbon atoms retained in the benzylic labeled photoproducts could be determined from the carbon-12/carbon-13 ratios in their mass spectra. Retention of both benzylic carbons gave the expected carbon-12/carbon-13 ratio of 1/1 while retention of only one benzylic carbon resulted in a 3/1 ratio.

Table I. Photoproducts of AC 217,300

<u>No.</u>	<u>Pyr.</u> <u>Label</u>	<u>Ben.</u> <u>C's</u>	<u>Ms Data</u>	<u>Structure</u>
0	Yes	2	m/z 494 ⁻ , 495 ⁻	 AC 217,300
1	No	2	m/z 370 ⁻ , 371 ⁻	
2	No	1	m/z 190 ⁻ , 191 ⁻ m/z 191 ⁺ , 192 ⁺	
3	No	1	m/z 216 ⁻ , 217 ⁻	
4	Yes	1	m/z 350 ⁻ , 351 ⁻	
5	Yes	1	m/z 326 ⁻ , 327 ⁻	
6	Yes	0	m/z 129 ⁺ , 130 ⁺	

STUDIES ON THE PROTEIN BINDING OF HEXABROMOBIPHENYL (HxBB)
IN ^{14}C -HxBB SPIKED AND ENVIRONMENTALLY CONTAMINATED SERUM.

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In 1973, polybrominated biphenyls (PBB) were accidentally introduced into the food chain in the State of Michigan when Firemaster BP-6, an industrial flame retardant, was inadvertently used as a cattle feed supplement in place of magnesium oxide. Field studies revealed (1) that more than 90% of the Michigan population still carries a body burden of PBB. Following a report of altered lymphocyte behavior in exposed subjects (2), PBB were identified by positive CI mass spectrometry in separated blood compartments and the β -lipoprotein fraction was recognized as a major carrier (3). The objectives of the present work were to investigate the binding of 2,2',4,4',5,5' hexabromobiphenyl (HxBB, the most abundant constituent of Firemaster BP-6) to individual serum proteins, particularly β -lipoproteins, in *in vitro* model systems and to compare this with the PBB binding in environmentally contaminated subjects. HxBB, universally labeled with ^{14}C (10.2 mCi/mmol), was utilized for spiking serum (incubation: 1 hr at 37°C). Radioactivity was determined by conventional scintillation counting. Unlabeled HxBB and other PBBs in the environmentally contaminated samples were quantified by a recently developed negative CI technique (4), based on the monitoring of bromine anions at $m/z=79$. This method is based upon our studies of the nature of the negative CI spectra of several polybrominated compounds which established that bromine anions form in great abundance by dissociative electron capture with nitrogen as the moderator gas (5). The quantification technique has detection limits at the pg/ml serum level (4).

HxBB was found in serum in a free (approx. 20%) and protein bound (approx. 80%) form. A 2:1 excess of methanol was required to sufficiently weaken the PBB-to-protein binding so that PBB could be quantitatively solvent extracted (hexane-ether, 1:1). Extraction of the methanol precipitated protein fraction showed 80% protein binding in environmentally contaminated samples and in the model system. In *in vitro* studies binding of HxBB to 3 individual proteins was determined by incubating (in air, 37°C, 1 hr) to 2, 10, 20, 40 mg of protein with 40 ng HxBB. HxBB binding was: β -lipoprotein 79-88%, γ -globulin 13-37%, albumin 7-19%, standard deviation 1-13, independent of the amount of protein.

When antisera to β - and α -lipoproteins were used to precipitate individual lipoproteins (3 hr incubation) from environmentally contaminated and model samples, β - and α -lipoprotein bound HxBB in a 3:1 ratio and accounted for almost all the protein binding of PBB. In the environmentally contaminated samples no selected protein binding of the other congeners of Firemaster BP-6 was found. A second approach that we have been applying to obtain protein fractions from serum is the use of the semirigid gel columns (Model I-250, Waters Associates) that have been developed for the separation of proteins by HPLC. Using spiked serum, fractions were collected every 2 min and the radioactivity determined. The 6-8 min fraction contained 48% of the radioactivity, and the next 2 fractions contained 31% of the radioactivity, showing good agreement with the 75-80% protein binding range found by GC/MS in samples from Michigan subjects. The free PBB, determined by solvent extraction was 22%, again in good agreement with the GC/MS runs. HPLC thus offers a simple and practical means of obtaining protein fractions from environmentally contaminated human samples for the study of the extent of the binding of PBB to various serum proteins.

In conclusion, we have determined that PBB in serum is 80% protein bound. We have confirmed our initial observation that β -lipoproteins are the major carrier

proteins, and determined that approximately 75% of the protein bound PBB is carried by these proteins. We have established that most of the remaining protein bound PBB is carried by the α -lipoproteins. We have shown that albumin and other serum proteins are of little significance as carriers. Finally, we have shown that ^{14}C -labeled HxBB in serum is a good model for the investigation on environmentally contaminated subjects. These techniques are now being utilized for comparative studies involving Michigan chemical workers who were directly exposed to PBB and subjects from the general population who were exposed by consuming PBB contaminated dairy and meat products.

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STUDIES OF ENVIRONMENTAL TEST WELL SAMPLES
USING GC/MS AND LC/MS

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The analysis of environmental samples for trace levels of organics is a complex problem. We have investigated an approach which involves sample profiling by a variety of methods prior to the use of complementary chromatographic techniques coupled to mass spectrometry.

Traditionally, Gas Chromatography/Mass Spectrometry (GC/MS) has been applied to the analysis of environmental samples. The use of fused silica columns has improved detectability by improving peak shape and allowing compounds of a wider range of polarities to be observed without derivatisation.

In the field of High Performance Liquid Chromatography/Mass Spectrometry (LC/MS) the advent of microbore columns has improved the detection limits by allowing the major portion of column effluents to be introduced into the Mass Spectrometer. Used in tandem GC/MS and LC/MS can cover a wide range of organic compounds. It is possible, however, that some organics may not be observed even by a combination of chromatographic techniques.

A profiling technique would provide useful information on the range of compounds to be expected in a sample and their approximate concentration ranges. The approaches used are:

- Desorption Chemical Ionisation (DCI)
- Field Desorption (FD)
- Spotting on an LC/MS Interface Belt

This technique has indicated the presence of compounds not apparent by GC/EIMS or LC/EIMS and has demonstrated the need for a variety of Mass Spectrometric techniques to be used for the full characterisation of a complex sample. These profiling techniques can also be used to indicate the presence of target compounds.

This analytical approach can be demonstrated by considering acidic and basic fractions of an environmental test well sample. DCI and FD were applied to the acid fraction. DCI showed a range of prominent ions, many of these could be related to compounds seen by GC/MS and LC/MS. Several ions at masses greater than 310 daltons were unique to the profiling techniques. FD showed a similar profile and did not indicate any additional compounds at higher molecular weight.

GC/MS on the same acid fraction showed the presence of four compounds not seen by LC/MS. Three of these were benzothiazole derivatives and all were minor components and the lesser sensitivity of LC/MS may be responsible for their lack of identification. The LC/MS analysis indicated several compounds which were not observed by GC/MS. These included phenyl phenylacetate which was a major component, and 2-phenyl benzimidazole which was a smaller component. In addition, several free carboxylic acids were seen as minor components by LC/MS. Derivatising the sample would have allowed their detection by GC/MS.

The basic fraction of the sample was subjected to DCI and FD. Both techniques indicated a prominent ion at M/Z 288 daltons. This compound was not observed in Electron impact (EI) studies of GC/MS or LC/MS.

A microbore LC/MS run using chemical ionisation with ammonia as reagent gas did indicate the compound of interest to be the major component. Its molecular weight appears to be 287 daltons and contains an anilino group, but its structure has not been fully characterised.

The LC/MS analysis under EI conditions shows the presence of two peaks which correspond to unresolved mixtures. One peak corresponded to aniline and 2-hydroxy benzothiazole while the second contained a mixture of methyl benzothiazole and methylmercaptobenzothiazole. The GC/MS analysis demonstrated the resolution of both pairs of compounds which coeluted under the LC/MS conditions used. In addition, both chromatographic techniques were able to indicate the presence of two compounds not seen by the other technique.

In conclusion, the use of GC/MS or LC/MS in isolation can often not fully characterise a complex sample. When used in tandem in a single ionisation mode, the techniques are complementary and additional information can be obtained, but some compounds may still be missed. The use of profiling techniques such as FD, DCI or introduction directly into an LC/MS interface, can add an extra dimension to the analysis and alert the analyst to the presence of additional components.

WOD 1 | MULTIPHOTON IONIZATION DETECTED BY FOURIER
TRANSFORM MASS SPECTROMETRY M. P. Irion, W. D. Bowers, R.
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Ions produced by multiphoton ionization (MPI) of various polycyclic aromatic hydrocarbons and oligopeptides have been detected by Fourier transform mass spectrometry (FT-MS). Parent ions are produced very efficiently at 250 nm and 222 nm with pulse energies as low as 1 mJ. The multiphoton ionization signals can easily be made larger than those obtained by electron impact ionization, even though the duty cycle of the laser is a million times less than the electron beam. FT-MS provides mass resolution greater than 200 000, which is far higher than time-of-flight mass spectrometers which have previously been used for MPI studies. High sensitivity is achieved because with FT-MS all ions are detected simultaneously. In addition, alignment and focusing of the laser are not critical because the open geometry of the analyzer cell allows ions to be detected over a volume of several cubic centimeters.

WOD 5 | PULSED-LASER TWO-PHOTON DISSOCIATION

KINETICS IN $C_6H_5Br^+$;

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The two-photon photodissociation of bromobenzene parent ion has been restudied using two new techniques. One uses a chopped, repetitively pulsed light source, giving good photodissociation intensity but a complex kinetic analysis. The other, using a pair of laser pulses separated by a relaxation interval, is more difficult but yields reliable relaxation data. Of particular interest is the infrared radiative rate constant of photoexcited bromobenzene ion, which is found by both techniques to be approximately 3 s^{-1} in reasonable accord with theory.

IONIZATION AND FRAGMENTATION OF BROMOCHLORODIFLUOROMETHANE ($\text{CF}_2\text{C}\ell\text{Br}$):
A PHOTOIONIZATION MASS SPECTROMETRIC STUDY*

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$\text{CF}_2\text{C}\ell\text{Br}$ has been investigated by photoionization mass spectrometry, and ionization and fragmentation processes have been followed over the photon energy range 10–20 eV (provided by conventional hydrogen and helium VUV sources). The computer-controlled spectrometer has been described previously.¹ Photoionization efficiency (PIE) curves - corrected for detector background counts, stray light, and sample pressure variations - have been measured for the $\text{CF}_2\text{C}\ell\text{Br}^+$ parent ion and the fragments: $\text{CF}_2\text{C}\ell^+$, CF_2Br^+ , $\text{CFC}\ell\text{Br}^+$, $\text{C}\ell\text{Br}^+$, CF_2^+ , Br^+ , $\text{CFC}\ell^+$, and CFBr^+ , in order of increasing appearance potential. These are shown in Figure 1.

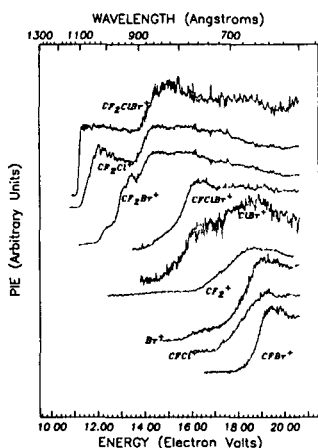


Figure 1. Photoionization efficiency curves for parent and daughter ions from $\text{CF}_2\text{C}\ell\text{Br}$.

The threshold region of the $\text{CF}_2\text{C}\ell\text{Br}^+$ PIE was fit by an Error Function which represents the Gaussian distribution of instrumental and equilibrium rotational contributions to the ion signal. A precise value for the adiabatic ionization energy is obtained from the inflection point; IP = 11.21 ± 0.02 eV. (Previously reported values are 11.83 eV² and 11.51 eV.³)

Because many factors influence the threshold region of fragment PIE curves, there is no suitable fitting function. We therefore obtained estimates for their "vertical" appearance potentials (APs) by adding the rotational contribution to the onset of ionization in the corresponding, smoothed PIE curve. APs determined in this way are listed in the second column of Table I (next page). These values, in turn, were combined with a calculated⁴ heat of formation for the parent neutral and other tabulated thermochemical data⁵ to provide the ΔH_f° values for the daughter ions listed in the last column

of the Table. These numbers are upper limits, since the APs extracted from the experimental data may be too high because of kinetic shift, competition with other fragmentation channels, etc.

* Work supported in part by the National Science Foundation

Table I: Thermochemical Data for Ions Formed upon Photoionization of $\text{CF}_2\text{C}\ell\text{Br}$

ION	Ionization or Appearance Potential [eV]	Products of $\text{CF}_2\text{C}\ell\text{Br} + h\nu$:	$\Delta H_f^0(0^\circ\text{K})^a$ [kcal/mole]
$\text{CF}_2\text{C}\ell\text{Br}^+$	11.21	$\text{CF}_2\text{C}\ell\text{Br}^+ + e^-$	150.34
$\text{CF}_2\text{C}\ell^+$	11.30	$\text{CF}_2\text{C}\ell^+ + \text{Br} + e^-$	126.18
CF_2Br^+	12.00	$\text{CF}_2\text{Br}^+ + \text{C}\ell + e^-$	140.14
$\text{CFC}\ell\text{Br}^+$	14.15	$\text{CFC}\ell\text{Br}^+ + \text{F} + e^-$	200.28
CF_2^+	15.99	$\text{CF}_2^+ + \text{Br} + \text{C}\ell + e^-$	205.41
$\text{CFC}\ell^+$	17.02	$\text{CFC}\ell^+ + \text{F} + \text{Br} + e^-$	237.92
CFBr^+	17.80	$\text{CFBr}^+ + \text{F} + \text{C}\ell + e^-$	255.53
$\text{BrC}\ell^+$	14.63	$\text{BrC}\ell^+ + \text{CF}_2 + e^-$	273.31
Br^+	15.05		

^aUpper limit, calculated from appearance potential and auxiliary thermochemical data: JANAF Thermochemical Tables, NSRDS-NBS 37 (1971).

The count rate for the $\text{C}\ell\text{Br}^+$ ion depends linearly on the sample pressure, which shows that it is the product of unimolecular fragmentation. From the experimental AP and known thermochemical data⁵ we deduce the reaction to be: $\text{CF}_2\text{C}\ell\text{Br} + h\nu \rightarrow \text{CF}_2 + \text{C}\ell\text{Br}^+ + e^-$. The observation of this fragment ion, together with the increased photoionization cross-sections for the parent and earlier daughter ions near the $\text{C}\ell\text{Br}^+$ threshold (Fig. 1), suggest the existence of a rearranged $\text{CF}_2\text{C}\ell\text{Br}^{+*}$ parent ion in an excited electronic state about 3 eV above the ground state of the ion.

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PHOTO-IONIZATION OF 2,3- AND OF 2,6-DICHLOROANILINE

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INTRODUCTION

The photo-ionization of 2,3- and of 2,6-dichloroaniline has been studied both mass-spectrometrically and by He(I) photo-electron spectroscopy to examine the influence of isomerism on ionization potentials and term values for electronically excited states in the molecular ions of dichloroanilines.

EXPERIMENTAL

The mass spectrometric photo-ionization equipment comprises a 1 m near normal incidence VUV monochromator and a quadrupole mass spectrometer (1). To investigate the ionization between 156 and 65 nm, the hydrogen manyline-source and the helium Hopfield continuum were used. The He(I) photo-electron spectrometer is a cylindrical mirror analyser (2).

The 2,3- and 2,6-dichloroaniline samples were commercial products purified by vacuum distillation, whose efficiency was controlled gas chromatographically and by conventional mass spectrometry.

EXPERIMENTAL RESULTS AND DISCUSSION

The ionization efficiency curves, measured both by total ion collection and mass spectrometrically for the parent ions show that ionization sets in at 155.43 ± 0.10 nm or 7.977 ± 0.01 eV for 2,3-dichloroaniline and at 155.37 ± 0.10 nm or 7.980 ± 0.01 eV for 2,6-dichloroaniline.

He(I) photo-electron spectroscopy provides $IP = 7.96 \pm 0.02$ eV and $IP = 7.98 \pm 0.02$ eV respectively for 2,3- and 2,6-dichloroaniline, in complete agreement with the above mentioned values.

The ionization potentials of the two dichloroaniline isomers investigated are thus virtually identical; they further differ but little from that of aniline itself, 7.70 ± 0.02 eV (3).

Term values for electronically excited states in the molecular ions, determined by He(I) PES are 8.5, 9.2, 11.2, 11.8, 12.6, ca. 14.6 eV and 15.8 eV for 2,3-dichloroaniline and 8.4, 9.2, 11.7, 12.8, ca. 15.0 eV and 15.8 eV for 2,6-dichloroaniline. The formation of the corresponding states is reflected in the total ionization efficiency curves and in the ionization efficiency curves for the parent ion. In contrast with the thresholds for ionization, these curves differ markedly for 2,3- and 2,6-dichloroaniline in the wavelength interval investigated.

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PHOTOIONIZATION OF METAL CLUSTERS AND THEIR OXIDES AND CHLORIDES

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Photoionization spectra down to a wavelength of 200 nm are reported for sodium clusters as well as for some sub-oxides and sub-chlorides of sodium and potassium aggregates. Appearance potentials were measured near threshold using a molecular beam technique, a uv light source coupled with a monochromator set at 33 Å resolution, and a quadrupole mass spectrometer detector (see Fig. 1).

Metal clusters were formed by adiabatic expansion of alkali metal vapor with argon or helium buffer gas through a tubular nozzle. Appropriate reactant gases were flowed through an annular nozzle surrounding the inner one containing the metal vapor, whereby desired reaction product clusters were formed. Using reactant gases of different exothermicity enabled a determination of the influence of hot bands on the spectra.

Appearance potentials were measured for sodium metal clusters up to the octomer at higher resolution than had heretofore been reported in the literature on the basis of single photon experiments. A typical ionization curve is shown in Figure 2. The appearance potentials displayed the expected odd-even variations with the values only slowly converging to the work function of the metal (see Table I).

Photoionization spectra of Na_xO and K_xO ($x=2-4$) were also measured. In order of increasing x , the appearance potentials for Na_xO are 5.1, 4.0, and 4.0 eV; those for K_xO are 4.3, 3.6, and 3.6 eV. In comparison to Na_2O , the appearance potential for Na_2Cl (3.9 eV) is much lower. The experimental results of these species compare well with theoretical calculations [1]. Based on the experimental measurements, the appearance potential for Na_2O shows a value close to that for the ionization of sodium or sodium dimer. This finding is consistent with a localized picture of the perturbed $\text{Na}(3s)$ interacting with $\text{O}(2p)$ pointing to a non-bonding level of the extra electron being slightly higher than that of $\text{Na}(3s)$. In the case of the chloride containing species, the binding energy for Na_2^+-Cl was observed to lie midway between that of Na_2-Cl and $\text{Na}_2^+-\text{Cl}^-$. Na_2Cl is electronically equivalent to Na_2O , whereupon the highest occupied level may be spatially localized around Cl and strongly influenced by the electron affinity of Cl. This is seen in the relative position of the ionization potential of Na_2Cl , which falls between the electron affinity of chlorine, 3.615 eV, and the ionization potential of sodium, 5.13 eV.

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Table I
Measurements of Appearance Potentials for Metal Clusters: Na_n

n	A.P. (eV)
1	5.13 ± 0.04
2	4.87 ± 0.04
3	3.93 ± 0.04
4	4.24 ± 0.04
5	3.95 ± 0.04
6	3.97 ± 0.04
7	3.88 ± 0.04
8	3.90 ± 0.04
work function	-2.75

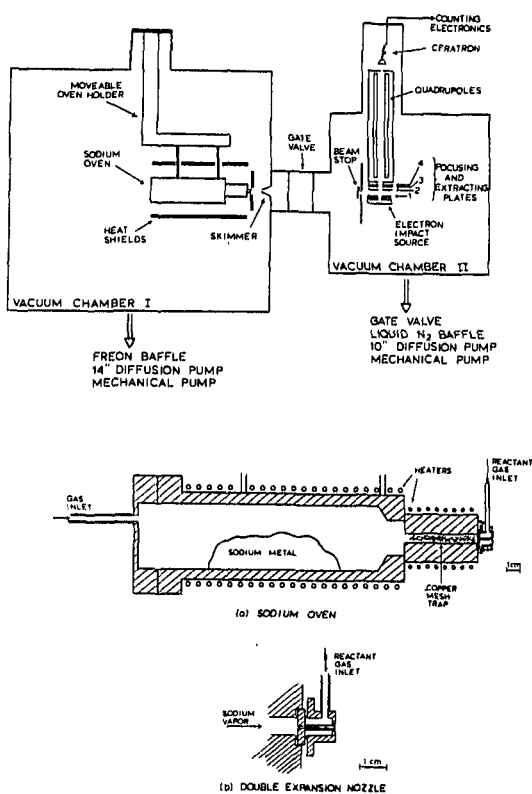


Figure 1

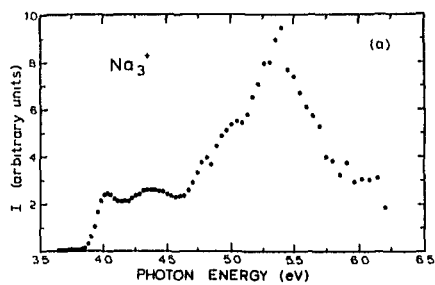


Figure 2

PHOTODISSOCIATION DYNAMICS OF CO_3^- AND ITS HYDRATES

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The technique of laser-induced photodissociation of negative ions and ion clusters combined with analysis of the kinetic energy distributions of the dissociation products provides a sensitive probe of the structure of the species and of the mechanisms of energy deposition and release following laser excitation. We have extended the experiments (1,2,3) on the CO_3^- system to include all the species $\text{CO}_3^-(\text{H}_2\text{O})_n$, $n=0,1,2,3$, a system that has proved to be particularly interesting in that both bare ions and clusters undergo photodissociation in the same photon energy range.

The apparatus used for these experiments is shown schematically in Figure 1, and has been described in detail elsewhere (1). Briefly, CO_3^- ions and clusters are produced in the ion source by sequences of ion molecule reactions. The clusters effuse into the high vacuum portion of the apparatus through an orifice in a platinum disk. The ion optical system then focuses the ions, mass selects them with the Wien filter, and refocuses them into the laser cavity. The identity and intensity of the photofragment ions is measured with the quadrupole mass spectrometer. The energy analyzer, a three screen retarding field type, is located between the interaction region and the quadrupole.

It has recently been established (1) that CO_3^- undergoes laser photodissociation via two mechanisms, this conclusion coming from the observation of a bimodal kinetic energy distribution of O^- photofragments. The electronic potential surfaces involved in both mechanisms are shown in Figure 2. The first mechanism is a sequential two photon absorption in which the first photon takes the ion to the bound electronic excited state (transition 1 in Figure 2), and the second photon takes the ion directly to the repulsive state correlating with $\text{CO}_2 + \text{O}^-$. The second mechanism also begins with photon excitation to the bound excited state, but in this mechanism, the ion undergoes a non-radiative transition to high-lying vibrational levels of the ground electronic state (transition 2 in Figure 2). Redistribution of this vibrational energy among the various modes of the ion (transition 3) leaves the ion vibrationally hot, and dissociation occurs following a collision of the ion with a background molecule.

Photodissociation of all the cluster species $\text{CO}_3^-(\text{H}_2\text{O})_n$, $n=1,2,3$, leads to loss of all water ligands as the dominant dissociation channel.

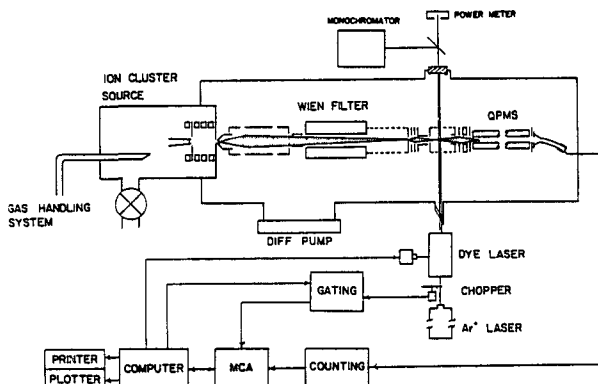
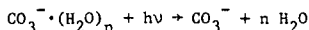


FIGURE 1: Photodissociation Spectrometer. The energy analyzer (not shown) lies between the interaction region and the quadrupole mass spectrometer.

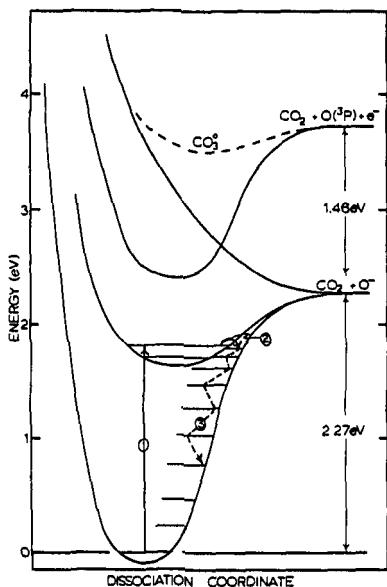


FIGURE 2: Sequence leading to vibrationally hot bare ions and to vibrationally predissociated clusters.

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This process is observed to occur in the same range of photon energies as causes photodissociation in the bare ion, but with a cross section approximately two orders of magnitude larger. There is some indication of vibrational structure in the spectrum of the $\text{CO}_3^-\cdot\text{H}_2\text{O}$ species, but the spectra are generally featureless. In contrast to the unclustered ion, energy analysis experiments show that only one photodissociation mechanism is operable in the clusters, that this mechanism occurs on a time scale short in comparison to the transit time from the laser to the quadrupole, and that the mechanism does not involve collisions with the background gas.

A model of the cluster dissociation mechanism that is consistent with these observations again begins with the sequence of events shown in Figure 2. The central CO_3^- part of the cluster absorbs a photon in exactly the same way the unclustered ion does and proceeds to transfer this excitation energy to the high-lying vibrational levels of the ground electronic state. The primary difference between the unclustered and clustered species lies in the fact that the clusters have many more low frequency vibrational modes available to accept the redistributed vibrational energy. In effect, the clusters are vibrationally predissociated once the initial laser excitation has been transferred to vibrational motion. Transfer of this vibrational energy to the cluster modes is sufficient to dissociate the clusters.

PHOTODISSOCIATION OF IONS WITHIN THE SOURCE
OF A ZAB-2F MASS SPECTROMETER

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Photodissociation of ions has been studied in this Laboratory by introducing laser radiation into double-focusing mass spectrometers. Most of the work to date has been done by irradiating ions in the field-free regions but more recently ions in the source have been photodissociated before they leave the ion chamber [1]. The number density of ions in the ion chamber is considerably higher than in the field-free regions thus resulting in higher interaction rates and higher photofragment-ion yields. In the present work a ZAB-2F mass spectrometer has been modified in a simple way so that radiation from an argon-ion laser can be introduced into a standard VG Analytical EI/CI source. A window has been fitted to one of the sample inlet ports and radiation introduced into the source block via one of the sample inlet holes. Two lenses external to the spectrometer were used to focus the laser beam. As in our previous studies the laser beam was mechanically chopped and photo-induced signals recorded using a lock-in amplifier.

The introduction of laser radiation into the ion chamber results in the absorption of light quanta by ions which have been generated under electron impact. Absorption of photons by molecular ions can cause them to dissociate thus depleting their population within the ion chamber. The height of the molecular-ion peak of the mass spectrum will thus vary at the chopping frequency of the laser light. Since each time the laser beam is on the concentration of molecular ions decreases the lock-in amplifier will record a negative signal at the molecular-ion mass position in the spectrum. On the other hand, increases in the peak heights might be expected for all the fragment-ions formed when the molecular ion dissociates giving rise to positive signals from the lock-in amplifier. However, fragment ions within the source may themselves absorb quanta causing them to dissociate. The total effect on the peak height at any particular fragment-ion mass position may thus either decrease or increase each time the ions are irradiated depending on whether destruction or formation of the fragment ion dominates. As the field of the magnet is scanned the output of the phase-sensitive detector will be a series of positive and negative signals which correspond to photo-induced increases or decreases in the peak heights of the spectrum. The spectrum obtained by recording the output of the lock-in amplifier in such a scan will be referred to as a photo-induced spectrum. We have obtained such spectra for several isomeric compounds to see if they are sensitive to differences in the isomeric structures.

The first compounds investigated were the isomers of xylene. Mass spectra of these are indistinguishable. In previous photodissociation work [2] done here, when the molecular ions were irradiated in the field-free region of a spectrometer, the magnitude of the variation of translational energy released, T , with photon energy, e_p , was found to depend on the structure of the isomeric ion. The photo-induced spectra obtained in the present work show that the ratio of the peaks for M^+ and $[M-1]^+$ is about 1 for *o*-xylene but about 1.6 for *m*- and *p*-xylene. Thus the *ortho*-isomer is clearly distinguishable from the other two. Further useful indications are obtained from the relative photo-absorption values which are determined from the size of the molecular-ion peaks in the photo-induced spectrum relative to the corresponding peaks in the mass spectrum. For the *o*-, *m*- and *p*-xylenes the values are 0.26%, 0.35% and 0.44% respectively. Thus, the *ortho*-isomer is distinguishable from the photo-induced spectra and the *meta*- and *para*-isomers from the relative photoabsorption results.

When the isomeric molecular ions of fluoronitrobenzene were studied previously in the field-free region of the spectrometer, it was found that the dependence of T on e_p was about the same for the ortho- and para-isomers. In the present work the relative photodissociation cross sections have been determined from the relative sizes of the molecular-ion peaks in the photo-induced spectra and their values investigated as a function of wavelength. The results of the ortho- and para-isomers were approximately the same with both sets being markedly different to those for the meta-isomer.

The isomeric bromotoluenes have also been studied. The absorption of radiation by the molecular ions was very large for these compounds and again clearly showed a dependence on molecular-ion structure i.e. 4%, 5.7%, 9.0% for the ortho-, meta- and para-isomer respectively. The photo-induced spectra showed interesting differences in one of the minor peaks - that due to $[M-1]^+$ ions. For the ortho-, meta- and para-isomer these peak heights normalised to the M^{+} peak height of 100 had values of +2.1, -0.6 and -4.5 respectively.

It is clear from the results reported here for a limited number of samples that photodissociating ions in the source of a mass spectrometer is a sensitive technique which could have wide-ranging analytical applications.

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PHOTODISSOCIATION OF CHLOROSTYRENE ISOMERS IN A DOUBLE FOCUSING MASS SPECTROMETER, George Collins and Klara Kiss, Stauffer Chemical Company, Dobbs Ferry, NY 10522 and E. White V, National Bureau of Standards, Washington, DC 20234

Laser photodissociation of metastable ions has been used to study the fragmentation of ions in ICR¹ and double focusing mass spectrometers². In the present work, the laser induced fragmentation of ortho-, meta- and para-chlorostyrene was investigated using a double focusing mass spectrometer. These compounds produce virtually identical EI spectra, and the potential for isomer identification by photodissociation was also investigated.

EXPERIMENTAL

This work was carried out on a Mattauch/Herzog mass spectrometer equipped with a combined EI/FD ion source. Ions were generated in the EI mode at 70 eV ionizing energy and accelerated by 8kV into the first field free region (1st FFR). The spectrometer was operated in the B/E linked scanning mode, allowing detection of daughter ions from 1st FFR fragmentations. The photon beam generated by an Argon ion laser entered the spectrometer through a quartz window covering the FD probe opening. The light then passed through the ion source and into the first FFR. The laser beam was focused by cylindrical lenses so as to be co-linear with the ion beam over the 85 cm length of the 1st FFR. The laser beam was mechanically chopped at 2.4kHz and laser induced fragments were recorded by phase sensitive detection as described by Harris et.al.³ The abundance of photofragmentations measured was normalized to the M/Z 138 molecular ion in all cases.

RESULTS AND DISCUSSION

Dechlorination was the primary photofragmentation mechanism observed for chlorostyrene molecular ions. The photodechlorination fragment ion was at least 30 times more abundant than other ions in the photofragment spectra of the chlorostyrene isomers. It also appeared that the abundance of the M -Cl ion, when normalized to the molecular ion, was different for each isomer. Since more than 95% of the ion current in the photofragment spectra was contained in this M -Cl ion, the relative abundance of the M -Cl ion might be used to approximate relative photoabsorption cross-sections. (This also assumes that photofragmentation is the predominant relaxation mechanism for photo-excited chlorostyrene ions.) The effect of altering laser power (photon flux), ion source pressure (ion current) and laser wavelength (photon energy) on M -Cl intensity was determined for each isomer.

As expected, increasing laser power increased the relative intensity of the M -Cl peak (Figure 1). At 514.5 nm (2.41 eV) the relative intensity increased by a factor of four as laser power increased from 1 to 4 watts. Absolute differences in photofragment intensities between isomers also increased with increased power.

The relationship between ion source pressure (ion current) and M -Cl peak intensity is shown in the following table.

M -Cl INTENSITY AS A FUNCTION OF SOURCE PRESSURE
(4 WATTS; 514.5 nm)

Isomer	1×10^{-7} Torr ¹	2×10^{-7} Torr	4×10^{-7} Torr
<u>Ortho</u> -	77 $\pm$ 1.5 ²	85 $\pm$ 0.5	92 $\pm$ 3
<u>Meta</u> -	52 $\pm$ 3	55 $\pm$ 0.5	60 $\pm$ 1
<u>Para</u> -	47 $\pm$ 3	50 $\pm$ 0.5	56 $\pm$ 0.5

¹Source housing pressure measured by the spectrometer ion gage

²Relative intensity - arbitrary units

It was expected that the intensity of the M -Cl peak relative to the molecular ion would remain constant as ion source pressure increased. However, a small increase in this peak with increased pressure was observed. This could be explained by an increase in the proportion of metastables in the 1st FFR relative to the molecular ion as would be expected for collision induced dissociation, but this has not been established.

The relative intensity of the M -Cl peak determined as a function of wavelength is shown in Figure 2. Each isomer appears to have its own intensity versus wavelength curve but because the measurements were taken at 1 watt laser power, the signal to noise ratio was poor. If photodechlorination remains the predominant relaxation mechanism over a broad range of wavelengths, the plot of M -Cl intensity versus wavelength might be usable as an estimate of relative gas phase photoabsorption cross-sections for chlorostyrene isomers.

In each of the experiments, a difference in the intensity of the M -Cl peak was seen between the three isomers. This difference could be used to identify an individual isomer. These isomers cannot be differentiated by their EI mass spectra.

¹R.C. Dunbar, Gas Phase Ion Chemistry, 2, 181 (1979).

²E.E. Kingston, T.G. Morgan, F.M. Harris and J.H. Beynon, Int. J. Mass Spectrom. and Ion Phys., 43, 261 (1982).

³F.M. Harris, E.S. Mukhtar, I.W. Griffiths and J.H. Beynon, Proc. Roy. Soc., A.374, 461 (1981).

FIGURE 1

Relationship between laser power and photodechlorination ($514.5\text{nm } 2 \times 10^{-7} \text{ torr}$)

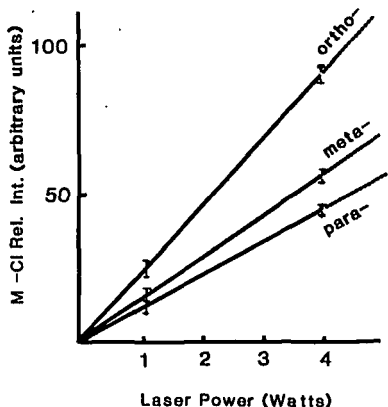
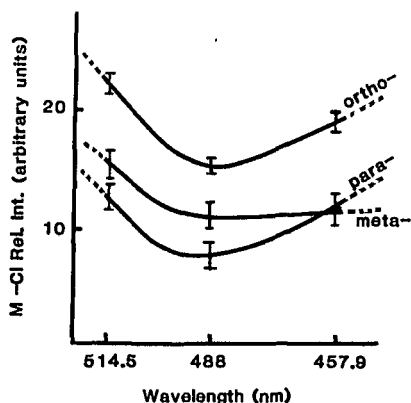


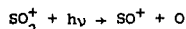
FIGURE 2

Relationship between wavelength and photodechlorination. (1 watt $2 \times 10^{-7} \text{ torr}$)



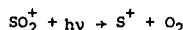
THE PHOTODISSOCIATION SPECTRUM OF SO_2^+ . Timothy F. Thomas*, Fred Dale, and John F. Paulson, Air Force Geophysics Laboratory, Hanscom AFB, MA 01731

The photodissociation spectrum of SO_2^+ has been investigated using a triple quadrupole mass spectrometer employing coaxial illumination of the ion beam over a 15 cm path length by a tunable, frequency-doubled, dye laser (see schematic diagram below). The ions were produced in a simple electron impact ion source at a pressure of 5×10^{-3} Torr. Measured cross sections for the process



were found to range from $\sim 2 \times 10^{-19}$ to $> 5 \times 10^{-19} \text{ cm}^2$ in the 3060-3600 Å region which corresponds to the predicted location of the $\tilde{X}^2\text{A}_1 \rightarrow \tilde{C}^2\text{B}_1$ transition. Vibrational analysis of this transition was hindered by very diffuse peaks and/or a high degree of vibronic congestion.

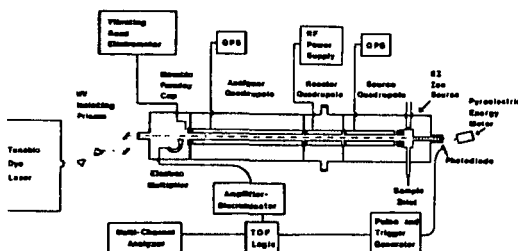
The process



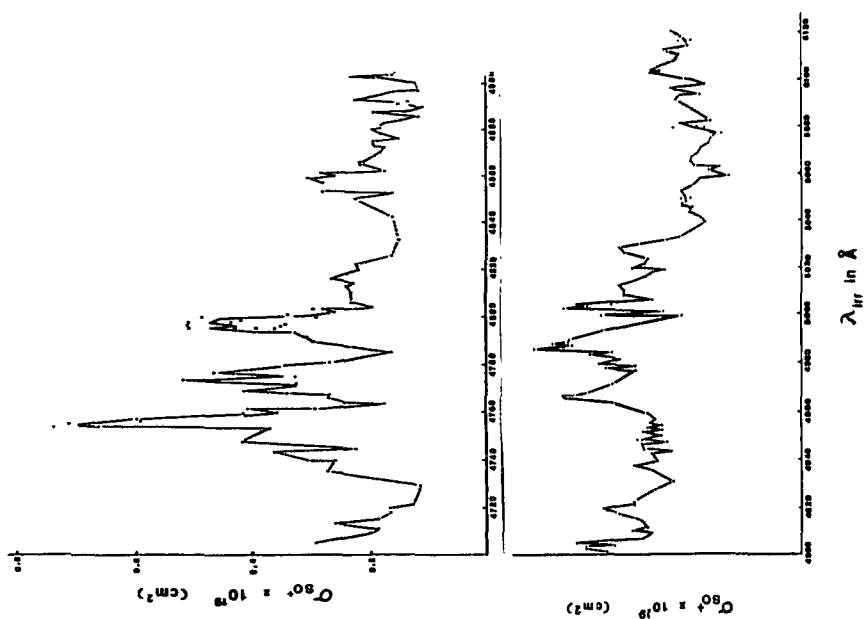
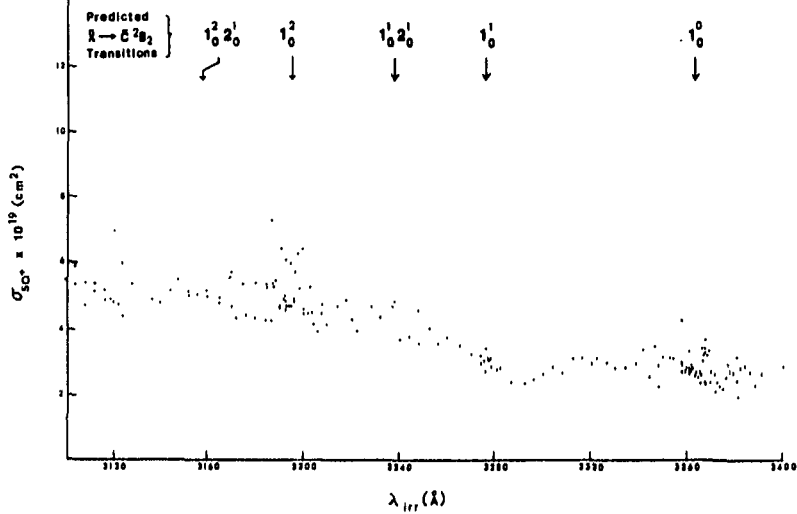
showed an onset near 3080 Å.

While searching for the origin of the $\tilde{X} + \tilde{C}$ transition, it was found that the photodissociation spectrum of SO_2^+ extends to $> 5120 \text{ Å}$. As shown by the figures on the next page, the visible spectrum has considerable vibrational structure, which can be mostly assigned to sequences in the bending mode ($\Delta v = 0, \pm 1$) and a shorter progression in the symmetric stretch. Assuming the visible spectrum is due to the transition $\tilde{A}^2\text{A}_2 \rightarrow \tilde{C}^2\text{B}_1$, population of vibrational levels up to $v_1'' = 4$ and $v_2'' = 6$ in the $\tilde{A}$ state has been identified, amounting to a total vibrational energy content of 0.81 eV in the $\tilde{A}$ state. By varying the ions' residence time in the first quadrupole, the lifetime of the metastable $\tilde{A}^2\text{A}_2$ state has been estimated to be 25 microseconds.

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Photodissociation Spectrum of SO_2^+



WOD 10 | PHOTOFRAGMENT SPECTROSCOPY: INTERCONVERSION OF ELECTRONIC STATES AND THE PARTITIONING OF ENERGY INTO TRANSLATIONAL DEGREES-OF-FREEDOM; R. E. Krailler, T. A. Adams, D. M. Wade and D. H. Russell; Department of Chemistry, Texas A&M University, College Station, Texas 77843.

Photodissociation and photofragment spectroscopy is a powerful tool for probing the structure(s) of gas phase ions and the dynamics of dissociation processes and internal conversion of electronic energy. The translational energy of the photofragment ions is a direct measurement of the partitioning of internal energy into translational degrees-of-freedom. From considerations of the energy gap law, the rate of dissociation (interconversion) of photoactivated ions and the partitioning of internal energy into translational degrees-of-freedom, an estimate of the coupling between electronic states can be made. Those systems exhibiting weak coupling between electronic states, i.e., large energy gap and slow internal conversion rates, would be associated with efficient partitioning of excess energy into translational degrees-of-freedom; whereas, strong coupling favors radiationless decay and much smaller translational energy distributions. The application of these principles to dissociation reactions of highly activated conic systems will be discussed with particular emphasis on 1,3-butadiene ($C_4H_6^+$) and related systems.

WOE 1 | CHEMICAL IONIZATION MASS SPECTROMETRY OF PYRIMIDINE, PURINE AND PTERIDINE DERIVATIVES: Y.Y. Lin, Y. Matsubara, J. Heininger; Institute for Basic Research in Developmental Disabilities, Staten Island, N. Y. 10314.

Some important inherited diseases, such as gout, Lesch-Nyhan syndrome, xanthinuria and orotic aciduria, are known to involve disorders in pyrimidine and purine metabolism; more recently bipterin (2-amino-6-(L-erythro-1,2-dihydroxypropyl)-pteridine-4(3H)-one) has attracted considerable interest because its tetrahydroderivative serves as potent cofactors for several aromatic amino acid hydroxylases and several inherited metabolic defects are attributed to its absence.

CIMS of pyrimidine, purine and pteridine derivatives were examined in order to explore a sensitive and specific analytical technique for the study of metabolic defects. Isobutane CI was found to be most useful: It successfully ionizes the bases as well as their nucleosides without derivatization, and gives intense $M+H^+$ and other characteristic ions. Most pyrimidine, purine and pteridine bases give $M+H$ ions as the base peak, while their nucleosides give three major ions characteristic of the molecules—the protonated molecular ion, the protonated base fragment ion, and the ribosyl fragment ion. For example N, N-dimethyl-guanosine (M.W.311) gave three major ions, m/z 312 ($M+H$), m/z 180 (N, N-dimethylguanine + H), and m/z 133 (ribosyl ion). Direct isobutane CIMS, in combination with HPLC analysis, appears to be useful for the biochemical study of pyrimidine, purine and pteridine metabolism.

The analytical method from GCMS of pteridine TMS derivatives were also studied and compared. Experimental methods, EI and CI spectra will be presented and discussed.

COMPILATION OF URINARY ORGANIC ACID PROFILES AMONG DIFFERENT AGE GROUPS
BASED ON CAPILLARY COLUMN GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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One of the core research efforts in the Analytical Chemistry Center is to establish a data bank on disease profiles of aciduria and compilation of normal urinary concentrations of those organic acids which are relevant for clinical diagnosis.

An improved analytical procedure for quantitative metabolic profiling of urinary organic acids based on capillary column GC/MS techniques was developed. Quantitation and "report" are based on Finnigan Target Compound Analysis (TCA) featuring automatic location and quantitation of compounds in the GC/MS data file. The method is fast and reasonably precise and can be used to obtain organic acid profiles under emergency situations and/or on a routine basis. During the methodology development, it was found that keto acids, even after ethoxymamine derivatization in the aqueous, are not well extracted using ethyl acetate. This problem was solved by including 75 μ l glacial acetic acid in 1 ml urine for sample preparation. Table 1 shows comparisons of preparative methods. Recoveries of pyruvic, 2-keto butyric, and 2-keto isocaproic acids are improved from 20% to 67% in the absence and presence of acetic acid, respectively. Percent precision of measurements are also improved from an average of 72% to an average of 46%. In general, better precisions were obtained for measuring non-keto acids; even so, it appeared that inclusion of acetic acid during sample preparation also improved precision significantly.

Table 1

Quantitation: Comparison of Preparative Methods^a

	A		B		C	
	mean $\pm$	%	mean $\pm$	%	mean $\pm$	%
LACTIC	9.9	5	2.7	7	5.8	6
2-HYDROXY ISOBUTYRIC	10.0	12	4.4	5	8.1	8
METHYL MALONIC	9.6	21	1.4	29	6.9	9
SUCCINIC	9.6	14	2.2	14	4.0	6
	mean $\pm$	%	mean $\pm$	%	mean $\pm$	%
PYRUVIC	9.5	20	1.8	72	6.6	43
2-KETO BUTYRIC	9.5	24	1.9	74	6.6	47
2-KETO ISOCAPROIC	8.9	20	2.3	70	6.8	47

Method A: 4 standards were EO-TMS derivatized directly.

Method B: 5 standards were EO derivatized in the aqueous, extracted with ethyl acetate and TMS derivatized.

Method C: 4 standards were added 75 μ l glacial acetic acid, derivatized and extracted following the same procedures in B.

a Data are mean $\pm$ % precision: Unit is μ g/ml.

Of the 31 organic acids which are included in the study, lactic, 2-hydroxy isobutyric, pyruvic, 2-hydroxy butyric, 2-keto 3-methyl valeric, ethyl malonic, succinic, methyl succinic, maleic, glutaric, 3-methyl glutaric, 2-keto glutaric, p-hydroxyphenyl acetic acids were normally found in urines of five infants, in eight 1-3 year olds, in six 4-6 year olds and in ten 7-13 year olds. The amounts of 2-keto isovaleric, glycerol, glyceric, malic, β -hydroxy β -methyl glutaric, phenylpyruvic, 2-keto adipic acids in the ethyl acetate extracts of normal urines were below detectable levels. However, these were readily detectable in the urine of patients with metabolic disease. 2-Keto butyric, 3-hydroxy butyric, methyl malonic, 2-keto isocaproic, adipic, 3-methyl adipic, o-hydroxy-phenylacetic, and β -phenyllactic acids were found in 1 or 2 samples of the total urine from different age groups.

The viability of the analytical procedures for clinical diagnosis was tested by comparing the urinary organic acid profile of a 5 year old male patient with ketosis relative to normal urine. Urinary concentrations of lactic, 2-hydroxy isobutyric, 2-keto 3-methyl valeric, and succinic acids were in the normal range. However, concentrations of certain organic acids were elevated: 2-hydroxy butyric acid, elevated from a normal range of 0.30-2.6 to 51.8 $\mu\text{g}/\text{mg}$ creatinine; 3-hydroxy butyric, 2-keto isovaleric and adipic acids from below measurable quantities to 1239, 5.4, 53.1 $\mu\text{g}/\text{mg}$ creatinine, respectively.

METABOLIC PROFILE STUDIES OF A PATIENT WITH REYE'S SYNDROME

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In 1963 Reye and associates described a syndrome of acute encephalopathy and fatty degeneration of the viscera in infants and children. It has been emphasized that the diagnosis of Reye's syndrome should be confirmed by direct examination of liver tissues. Hilty et al. and Romshe et al. found that the serum amino acids in patients with Reye's syndrome have unique pattern of hyperaminoacidemia and therefore the quantitation of serum amino acids can be a great help in confirming the diagnosis and is a simple and safe procedure. In addition, the quantitative serum amino acid pattern can be used to differentiate Reye's syndrome from other syndromes clinically mimicking it.

An abnormal organic acid profile of a patient with Reye-like syndrome was first reported by Chalmer et al. in 1981(1). The organic acid profiles of Reye's syndrome may also be unique and, together with amino acid profiles, can be a greater help in the diagnosis of Reye's syndrome. We studied both urinary organic acid and amino acid profiles of a 18 month-old boy with Reye's syndrome, using gas chromatograph-mass spectrometer and automated amino acid analyzer. The levels of lactic acid(35 fold), 2-hydroxybutyric acid(30 fold) and 4-hydroxyphenyllactic acid were greatly elevated. There was only a moderate increase in the concentrations of the ketone bodies. Adipic acid(18 fold), suberic acid and sebacic acid were found in large amounts. Significant amounts of 3-hydroxy-1,10-decanedioic acid, 3-hydroxy-1,12-dodecanedioic acid and 3-hydroxy-1,14-tetradecanedioic acid were found. As the patient was administered aspirin, 2-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid and 2-hydroxybenzoylglycine were also detected. Romshe et al. in 1981 reported that the most striking and consistent elevations in serum amino acid levels occur with glutamine, alanine, 2-aminobutyric acid and lysine. Amino acid analysis of urine from our patient also showed marked increase of alanine, glutamine, glutamic acid and lysine. 2-Aminobutyric acid and tyrosine were slightly elevated. Suprisingly the level of 2-amino-adipic acid, produced by transamination of 2-oxoadipic acid which is a catabolic intermediate of lysine, was greatly elevated; 2.9 $\mu\text{mole/mg}$ creatinine. These data indicates that decarboxylation of pyruvic acid, 2-oxobutyric acid, 2-oxoadipic acid and 4-hydroxyphenylpyruvic acid are blocked. Different from E3 deficiency, the accumulation of branched-chain keto- and hydroxy acids were not observed. In Reye's syndrome, ultra-structure of mitochondria changes and secondary deficiency in the activity of several mitochondrial enzymes has been demonstrated. The activities of pyruvate dehydrogenase and pyruvate carboxylse, which are exclusively

mitochondrial, were low in Reye's syndrome(2). The urinary level of uracil, the most reliable indicator for the chemical diagnosis of ornithine carbamyl transferase deficiency, was not elevated in our patient and his ornithine level was at the lower limit of normal range. These findings are consistent with the fact that Reye's syndrome patients showed marked decrease in the activities of both carbamyl phosphate synthetase and ornithine carbamyl transferase(3). Mitochondrial failure may also results in the inability of fatty acid transport and its metabolism in mitochondria. Peroxisomal β -oxidation system has unique substrate specificity for the chain-length of fatty acids(4). We suggest that secondary impaired mitochondrial β -oxidation of fatty acids may be responsible for the peroxisomal β -oxidation followed by ω -oxidation. In summary, urinary organic acid profile together with amino acid profile can be used to diagnose Reye's syndrome.

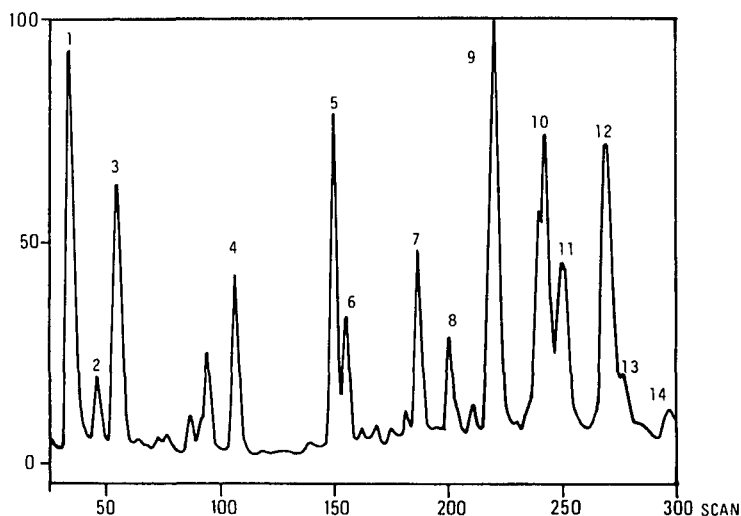


Fig. 1. TIM chromatogram of TMS derivatives of urinary acids from a 18 month-old boy with Reye syndrome. Peak identifications are: 1. lactic, 2. 2-hydroxybutyric, 3. 3-hydroxybutyric, 4. succinic, 5. adipic, 6. 2-hydroxybenzoic, 7. sebacic, 8. 2,5-dihydroxybenzoic, 9. 4-hydroxyphenyl-lactic & sebatic, 10. 3-hydroxy-1,10-decanedioic, 11. n-heptadecanoic (IS) 12. 2-hydroxybenzoylglycine, 13. 3-hydroxy-1,12-dodecanedioic, 14. 3-hydroxy-1,14-tetradecanedioic acid.

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**CAPILLARY GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC PROFILES OF TRIMETHYLSILYL DERIVATIVES OF
SERUM ORGANIC ACIDS FROM REYE'S SYNDROME PATIENTS**

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Reye's syndrome, first described by Reye et al in 1963 (1) is a life-threatening illness that usually afflicts children. It is characterized by a prodrome of viral infection (e.g. chicken-pox or influenza), followed by repetitive vomiting, behavioral changes, rapid onset of fatty infiltration of liver and kidney, and cerebral edema, progressing to coma and death. We have developed procedures for the extraction and GC-MS profiling of biological fluids (2,3). Here we report the profiles of serum organic acids from two Reye's Syndrome patients, one of whom survived but the other died.

Serum samples were obtained from patients 1 and 2 who, upon hospital admission, were diagnosed as Stage I and II of Reye's Syndrome, respectively. An internal standard, phenyl-d₅-mandelic acid was added to a portion (0.8 ml) of each sample to a concentration of 1.5 ug/ml. Three volumes of saturated sodium borate buffer were added to the sample to adjust the pH to 8.5-9.0. Neutral and basic components were removed by extracting 3 times with 5 ml of dichloromethane, the aqueous portion was acidified with 3N HCl to pH 1.0. The sample was again extracted 3 times with 5 ml of dichloromethane. The extracts were rotary evaporated dry and derivatized with N,O-bis-(trimethylsilyl)-trifluoroacetamide. The sample was then analyzed using a combined GC-MS-Computer system (5985 Hewlett Packard), utilizing a fused silica capillary column with bonded liquid phase (Durabond BD-1, 30 m x 0.32 mm, 1 um thickness, J&W Scientific Co.). Mass spectra were obtained by electron impact at 70 eV.

The total ion plots of the trimethylsilylated serum organic acids from patient 1 and patient 2 are shown in Figures 1 and 2, respectively. Besides various short chain keto- and hydroxy- acids and long chain fatty acids normally found in serum, several long chain saturated and unsaturated ω -dicarboxylic acids were detected in these serum samples. These are dodecenedioic, dodecanedioic, tetradecadienedioic, tetradecenedioic, tetradecanedioic, hexadecenedioic, hexadecanedioic, octadecadienedioic, octadecenedioic and octadecanedioic acids. Furthermore, these dicarboxylic acids are at a much higher level in patients 2 who died of the disease than in patient 1 who recovered. These compounds have not been reported previously in Reye's syndrome patients, and the C₁₆ and C₁₈ ω -dicarboxylic acids have not been reported in any dicarboxylic acidemias. The concentrations of these long chain α - ω -dioic acids in the two serum samples are shown in Table 1.

Table 1. Concentration of ω -dicarboxylic acids found in the serum samples (ug/ml).

Acids	Patient 1	Patient 2
dodecanedioic	0.8	12.0
dodecenedioic	-	2.5
tetradecanedioic	0.6	15.5
tetradecenedioic	0.25	6.5
tetradecadienedioic	-	2.3
hexadecanedioic	-	17.5
hexadecenedioic	-	3.0
octadecanedioic	2.0	18.0
octadecenedioic	1.4	27.0
octadecadienedioic	-	2.5

It will be important to determine whether these acids are either specific for Reye's syndrome, or correlates with the mortality of the disease. Starvation appears to enhance the toxicity of hexadecanedioic acids in rats (4). The toxicity of these long chain saturated and unsaturated dicarboxylic acids to Reye's syndrome patients with repetitive vomiting and poor nutritional status remains to be determined.

Figure 1

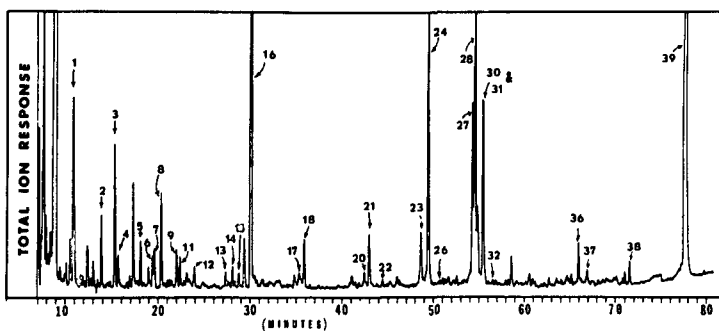
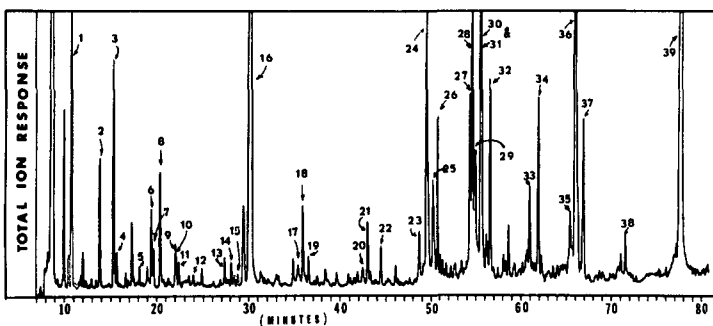


Figure 2



GC-MS profile of trimethylsilylated serum long chain fatty acids from Patients 1 and 2. The ordinate represents total ion response with the most intense component normalized as 100%. The abscissa represents the time axis. (1. lactic; 2. 2-OH-butyric; 3. 3-OH-butyric; 4. 2-OH-isovaleric; 5. benzoic; 6. 2-ketoisocaproic; 7. 2-ketovaleric; 8. phosphoric; 9. 2-keto-3-methyl-valeric; 10. glutaric; 11. 2-ketocaproic; 12. nonanoic; 13. decenoic; 14. decanoic; 15. phenyl- d_5 -mandelic; 16. salicylic; 17. lauroleic; 18. lauric; 19. suberic; 20. myristoleic; 21. myristic; 22. sebacic; 23. palmitoleic; 24. palmitic; 25. dodecenedioic; 26. dodecanedioic; 27. linoleic; 28. oleic; 29. tetradecadienedioic; 30. stearic; 31. tetradecenedioic; 32. tetradecanedioic; 33. hexadecenedioic; 34. hexadecanedioic; 35. octadecadienedioic; 36. octadecenedioic; 37. octadecanedioic; 38. cholestadiene; 39. cholesterol)

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USE OF A GC/MS/DS SYSTEM FOR AUTOMATED METABOLIC PROFILING OF URINARY STEROIDS AND ITS APPLICATION TO STUDIES ON THE EFFECTS OF TOXIC CHEMICALS ON STEROID METABOLISM. J.J. Vrbancac, W.E. Braselton, Jr., B. Holmberg, G. Ristow, M. Martin, H. Humphrey and C.C. Sweeley. Departments of Biochemistry, Pharmacology/Toxicology, and Internal Medicine, Michigan State University, East Lansing, Michigan 48824, and the Michigan Department of Public Health, Lansing, Michigan 48912.

Over the past several years we have been involved in the development of methods for generation of metabolic profiles of compounds isolated from body fluids using a computer program for automated reverse library search of selected mass chromatograms called MSSMET. More recently we have turned our attentions to generation of metabolic profiles of urinary steroids (1,2) and in particular to the effects of certain environmental contaminants on these profiles. In 1973 approximately 1000 pounds of a mixture of polybrominated biphenyls (PBBs) were accidentally mixed with dairy cattle feed in Michigan. Relatively large amounts of contaminated milk and meat were consumed by some farm families or consumers who purchased directly from contaminated farms. Wide spread low level contamination followed involving a large segment of the population of Michigan. The particular commercial mixture of PBBs which contaminated Michigan contained 10 components each of which made up greater than 1% of the mixture, with three of these components accounting for 75% of the mixture. This mixture of components stimulate the induction of hepatic mixed function oxidase (MFO) with characteristic similar to those of both phenobarbital (PB) and 3-methylcholanthrene (3-MC), two agents with distinct stimulating effects. Thus, it would be reasonable to expect that one of the possible effects of this particular mixture of PBBs would be a "PB-like" effect in the exposed human population. Since steroid hormones are metabolized by hepatic cytochrome P-450 mixed function oxidase enzyme systems and since MFO enzyme systems are induced by PBBs, we are in the process of examining the urinary steroid metabolic profiles of PBBs exposed individuals.

Experimental

Samples. Urine samples for both controls and PBBs exposed individuals were obtained from the Michigan Department of Public Health from subjects enrolled in the "Long-term PBB Study" under HEW contract. A high-PBBs subgroup was defined as those individuals with greater than 50 ppb PBBs in plasma and the low subgroup was defined as having less than 2 ppb. Urine samples from non-smoking adult male subjects were obtained from patients of Dr. Ristow of the MSU College of Human Medicine. Isolation of Steroids. Methoxime-trimethylsilyl derivatives of urinary steroids were prepared as previously described (2). Generation of urinary steroid profiles. Metabolic profiles were generated in three different manners. Samples were analyzed by GC with FID using a 60 m DB-1 bonded phase fused silica capillary column (0.242 mm ID, 0.1 μ m film thickness, J and W Scientific) using the following conditions: Temperature programming from 180 to 310 $^{\circ}$ C at 1.25 degrees/min.; 50 minute isothermal period, $\bar{u}$ = 20 cm/sec. Each sample was chromatographed twice, once being co-injected with a mixture of straight chain hydrocarbons for calculation of methylene unit retention indices. Samples were analyzed by GC/MS (EI at 70 ev) using both packed (3%OV-101, 9 m X 2mm) and capillary columns (60 m DB-1 bonded phase fused silica capillary column, 0.32 mm ID, 1.0 μ m film thickness, "on-column" injection). A mixture of straight chain alkanes was co-injected for calculation of methylene unit retention indices.

Results

Certain differences in the urinary steroid metabolic profiles between the three groups were noted. One difference was an apparent increased excretion of 6 β -hydroxycortisol in the PB and PBBs groups. Since 6 β -hydroxycortisol had previously been reported to be a chemical marker in humans for stimulation of hepatic cytochrome P-450 (5), the mass spectra and retention behavior for the MO-TMS derivative of this compound was characterized. Figure 1 shows the mass spectrum for the MO-TMS derivative of 6 β -hydroxycortisol. The molecular ion was observed as were characteristic losses of 15, 31, 90 + 31, 2X90 + 31 and 3X90 + 31. This derivative had a retention index of 3313 on the 60 m DB-1 capillary column and 3315 on the 3% OV-101 packed column. Table 1 shows the data obtained for 6 β -hydroxycortisol by the MSSMET program. These data were obtained from the packed column runs and were normalized to the internal standard and urinary creatinine (6 β -hydroxycortisol was not in high enough concentration to be observed in the capillary column GC/MS analyses).

Since this metabolite of cortisol is a minor component of the mixture the capillary column GC with FID detection proved to be a more quantitative method of analysis. Figure 1

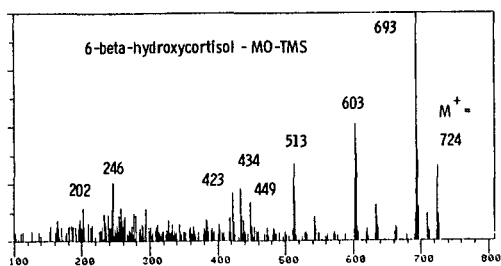


FIGURE 1

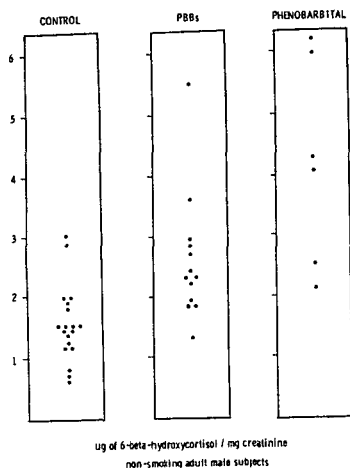


FIGURE 2

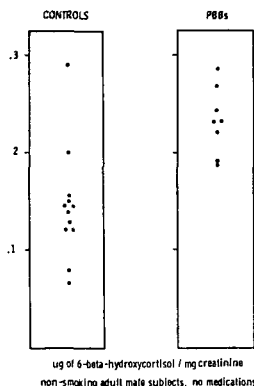


FIGURE 3

TABLE 1
Determination of 6-beta-hydroxycortisol
by automated reverse library search of
selected mass chromatograms.

	Average	S.D.	(n)
Controls, no medications:	1.28	1.8	(8)
PBBs, no medications:	2.52	2.9	(8)
Phenobarbital:	14.1	25.7	(6)

Data normalized to the internal standard,
cholesteryl butyrate and urinary creatinine.

shows the data obtained for all non-smoking males in all three groups. Means $\pm$ SD for control, PBBs and PB were 0.158 ± 0.064 , 0.259 ± 0.105 and $0.765 \pm .958$ ug 6 β -hydroxycortisol/mg creatinine. The comparison that we were most interested in making was between non-smoking, non-medicated controls and non-smoking, non-medicated PBBs exposed individuals. This data is shown in figure 3. Statistical analysis (t-test) revealed these groups to be different at the $p < .005$ level.

In conclusion, these data indicate that PBBs exposure in humans might result in an effect on hepatic cytochrome P450, assuming that urinary levels of 6 β -hydroxycortisol are really an indicator of enzyme stimulation. If PBBs do have the effect of stimulating hepatic microsomal MFO systems in humans, the effect appears to be "mild" at the blood levels examined. We are presently expanding our sample size of non-smoking, non-medicated male subjects to verify these findings. Finally, we would point out that it would be highly erroneous to characterize such an effect as a "toxic-effect" of PBBs, since situations are well documented where stimulation of hepatic P-450 microsomal enzyme systems can be either beneficial, detrimental or of no consequence to an individual.

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GC/MS INVESTIGATION OF POLYOL METABOLISM IN UREMIA

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SUMMARY

The polyols in the urine and serum of uremic patients were analyzed by gas chromatography-mass spectrometry. Several new deoxyalditols and inositol isomers, namely, 4-deoxythreitol, 4-deoxyerythritol, 5-deoxyxylitol, 5-deoxyarabitol, 2-deoxyribitol, 6-deoxymannitol, 6-deoxygalactitol, neoinositol, and chiroinositol, were identified in the normal urine and in the uremic urine. The uremic patients showed the markedly elevated urinary excretion of myoinositol and chiroinositol as well as the elevated serum levels of myoinositol, chiroinositol and scylloinositol compared with the healthy subjects, whereas the uremic patients showed the significantly decreased serum level of 1-deoxyglucose. The results suggest the metabolic disorder of chiroinositol and 1-deoxyglucose in the uremic state.

INTRODUCTION

Polyols in human physiological fluids has been studied in the various diseases. In the uremic state the serum and the urinary levels of myoinositol has been reported to be increased. The accumulation of myoinositol was considered as a cause of the uremic polyneuropathy. By our detailed study of polyol in the uremic urine and serum, new deoxyalditols and inositol isomers were identified and the abnormal metabolism of chiroinositol and 1-deoxyglucose was demonstrated in the uremic state (1).

MATERIALS AND METHODS

Sample: Twenty-four hour urine samples were obtained from 5 healthy adults, 4 uremic patients on hemodialysis and 6 undialyzed uremic patients. Serum samples were obtained from 8 healthy adults, 9 uremic patients on hemodialysis and 3 undialyzed uremic patients.

Sample preparation: Serum was filtered through cone membrane filter. One ml of serum ultrafiltrate or urine sample equivalent to 1 mg of creatinine was applied to Dowex 50W x 8 column after the addition of 50 µg of ribitol as an internal standard. Anion and neutral compounds were eluted with distilled water. The eluate was applied to Amberlite IRA 400 column. Neutral compounds were eluted with distilled water. After lyophilization of the eluate, the neutral compounds were dissolved with hot methanol, then transferred to test tubes and concentrated into small volume with N₂ stream. The concentrate was transferred to sample vial and evaporated. The sample was then trimethylsilylated with 90 µl of BSTFA and 10 µl of TMCS at 60°C for 20 min.

GC/MS: A Hewlett-Packard 5710A gas chromatograph was directly coupled to the source of JMS D-300 mass spectrometer (JEOL, Tokyo, Japan). The gas chromatograph was equipped with a 30 m x 0.25 mm i.d. OV-101 open-tubular glass capillary column. The column temperature was programmed from 120°C to 260°C at 3°C/min.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatograms of polyols in urine of an uremic patient and of a normal subject. Peaks were identified as follows:
1. glycerol, 3. 4-deoxythreitol, 4. 4-deoxyerythritol, 10. threitol, 11. erythritol, 12. 5-deoxyxylitol, 13. 5-deoxyarabitol, 15. 2-deoxyribitol, 19. xylulose, 22. xylitol, 23. arabitol, 24. ribitol (I.S.), 26,28. isomers of 6-deoxyhexitol, 27. 6-deoxymannitol, 29. 6-deoxygalactitol, 30. fructose, 31. 1-deoxyglucose, 36,45. glucose, 37. β-galactose, 39. neoinositol, 42. mannitol, 43. sorbitol, 44. chiroinositol, 46. epi- or cisinositol, 47. scylloinositol, 48. myoinositol. 4-Deoxythreitol, 4-deoxyerythritol, 5-deoxyxylitol, 5-deoxyarabitol, 2-deoxyribitol, 6-deoxymannitol, and 6-deoxygalactitol were newly identified by comparing the mass spectra and the retention times with those of the synthesized authentic compounds. The metabolism and the physiological significance of these deoxyalditols are

not yet known. Neoinositol, chiroinositol and scylloinositol were identified by comparing the mass spectra with those reported in the literature (2).

Table I shows the daily urinary excretion of polyols in uremic patients and healthy adults. Daily urinary excretion of erythritol, xylitol, and arabitol in uremic patients were significantly decreased as compared with healthy subjects. However, the daily urinary excretion of myo-inositol, chiroinositol and scylloinositol was increased in uremic patients as compared with healthy subjects. The increase of myo-inositol and chiroinositol in the uremic urine was remarkable.

In uremic serum the levels of threitol, erythritol, arabitol, mannitol, sorbitol, myo-inositol, chiroinositol and scylloinositol were increased as compared with normal serum. Xylitol, neo-inositol, and epi- or cisinositol were not detected in uremic serum nor in normal serum. The serum concentration of 1-deoxyglucose was, however, significantly decreased as compared with that in the normal subjects. The metabolism of 1-deoxyglucose is unknown so far.

The increase of chiroinositol in the uremic urine and serum suggests that chiroinositol is formed from myo-inositol or that the catabolism of chiro-inositol takes place exclusively in the kidney as myo-inositol. Scylloinositol is considered to be formed from the reduction of myoinose-2 which originates from the dehydrogenation of myo-inositol. The increase of scylloinositol in the uremic urine and serum suggests the increased formation of scylloinositol from myo-inositol.

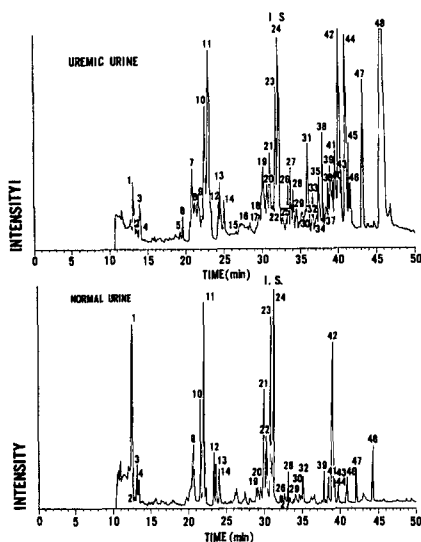


Fig. 1 Gas chromatograms of the polyols in the urine of an uremic patient and of a normal subject.

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Table I Daily urinary excretion of polyols in uremic patients

	normal (n=5)	uremic (n=10)
Threitol (mg/day)	5.7 ± 3.3	4.0 ± 3.3
Erythritol (mg/day)	66 ± 15	20 ± 13***
Xylitol (mg/day)	6.9 ± 4.8	2.0 ± 2.4**
Arabitol (mg/day)	46 ± 9.5	16 ± 12***
Mannitol (mg/day)	81 ± 37	94 ± 85
Sorbitol (mg/day)	8.6 ± 2.1	12 ± 14
Myo-inositol (mg/day)	100 ± 71	430 ± 290**
Neoinositol*	55 ± 44	29 ± 36
Chiroinositol*	130 ± 140	750 ± 270***
Epi- or Cisinositol*	44 ± 53	23 ± 48
Scylloinositol*	200 ± 140	290 ± 260

*Peak area ratio to I.S. per day. **p<0.05. ***p<0.01.

URACIL: AN INDEX FOR GC/MS SCREENING OF ORNITHINE CARBAMOYL TRANSFERASE DEFICIENCY

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Ornithine carbamoyl transferase (OCT) deficiency, known as hyperammonemia type II, is urea cycle disorder. The block in detoxication of ammonia causes hyperammonemia which in turn leads to high levels of glutamine. Since glutamine is not specific for OCT deficiency, and ammonia also accumulates in other urea cycle disorders or some organic aciduria, these two compounds can only be used for the initial screening of this disorder. It was reported that OCT deficiency leads to orotic aciduria, presumably due to intramitochondrial carbamoylphosphate accumulation behind the block and the spill-over to the cytosol where pyrimidine synthesis takes place(1). Again, this compound is not specific indicator for the diagnosis of OCT deficiency. For the purpose of establishing a more accurate chemical diagnosis, we attempted to investigate the urinary organic acid profiles of three patients with OCT deficiency and five patients with other urea cycle disorder, using GC/MS/COM system.

Two abnormal peaks were detected on the TIM chromatogram of trimethylsilyl derivatives (TMS) of organic acids extracted with ether from the urine of all patients with OCT deficiency. They were identified as uracil-di-TMS and orotic acid-tri-TMS respectively. Then we quantified urinary uracil concentrations using n-heptadecanoic acid as an internal standard. The calibration curve for the mass chromatographic quantification of uracil was linear in the range between 0.1 to 6.0 mg/mg creatinine. Uracil concentrations of two patients with severe OCT deficiency were 0.6 and 0.7 mg/mg creatinine respectively, in one supposed to have OCT deficiency was 0.5 mg/mg creatinine and in one patient with confirmed 50% OCT activity using rectum was 0.1 mg/mg creatine. Uracil was not detected in the urine of healthy controls and hundreds of patients with various disorder except for the 8 patients studied here; three of them confirmed to have OCT deficiency, one supposed to have this disorder, while for the others OCT deficiency could be clearly denied on the basis of their abnormalities of amino acid profiles. The amounts of the urinary uracil varied 0.1 to 0.4 mg/mg creatinine in the latter four patients.

OCT deficiency has been clinically diagnosed by the presence of neonatal hyperammonemia without a specific abnormality of the plasma amino acid pattern, plus severe orotic aciduria. Orotic acid was also detected but not quantitatively in our method. Therefore, by the confirmation of normal or decreased citrulline level by amino acid analysis, uracil proved to be a most

sensitive and reliable indicator for OCT deficiency in our organic aciduria screening system. Since hyperammonemia is also found in some organic acidemia and other liver diseases, it is important to distinguish congenital urea cycle disorders from organic acidemias such as propionic acidemia or methylmalonic acidemia. These organic acidemia can be simultaneously denied using this system. Since OCT deficiency is a X-linked disorders of urea cycle and complete OCT deficiency of male infant may be absolutely lethal, and partial OCT deficiency in female infant is also very serious, it is essential that a rapid diagnosis can be made, especially in newborn females at risk for OCT deficiency. For these reason, our screening system using GC/MS/COM is, by combination with amino acid analysis, most reliable for diagnosis of OCT deficiency.

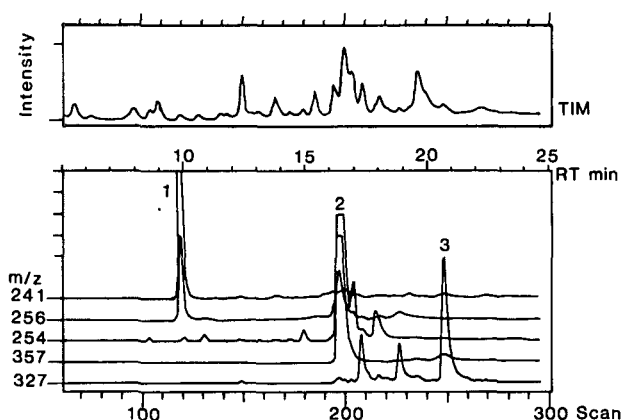


Fig. 1 Mass chromatogram of uracil and orotic acid of a patient with OCT deficiency. Peak identifications are: 1, uracil; 2, orotic acid; 3, n-heptadecanoic acid (internal standard).

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COBALAMIN (VITAMIN B₁₂) DEFICIENCY DETECTION BY URINARY METHYLMALONIC ACID QUANTITATION

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Patients with cobalamin deficiency have elevated levels of urinary methylmalonic acid (MMA) since cobalamin is required for conversion of MMA to succinic acid. If cobalamin deficiency is not detected early it can lead to irreversible neurological damage such as leg paralysis and mental deterioration (dementia). The resulting care for these patients is a great economic and social burden to society.

The past few years at this meeting we have described a rapid, reliable mass spectrometry method for quantitating urinary MMA. The method consists of derivitizing a volume of a spot urine equivalent to 0.05 mgs creatinine along with 25 µgs of ethylmalonic acid, the internal standard.¹ Quantitation is accomplished using a Finnigan 3200 GCMS with a 3% OV-17 packed column programmed from 180°C to 270°C, 15°C/min. The system is interfaced to a Teknivent 29K data system.

We have applied this procedure to over 1600 patients and have detected over 50 proven cobalamin deficient patients.^{2,3} Unfortunately, over one-third of these patients now have permanent neurological disability which could have been prevented if earlier testing had been accomplished. This illustrates how the use of a mass spectrometer at a medical center for MMA testing would prove cost effective to society. In our opinion, GCMS is the only practical and reliable method for quantitating low levels of urinary MMA.³ The "improved" serum cobalamin radioassay is reported to yield a high level of "false low" values and is therefore not feasible for a screening test.^{4,5} The wide scale noninvasive screening of "risk" patients for elevated urinary MMA would be a highly beneficial medical application of the mass spectrometer.

We thank Mr. Maiernd R. Sorensen for technical assistance.

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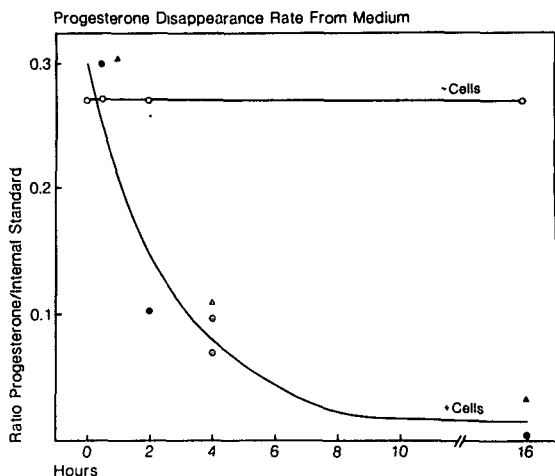
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APPLICATIONS OF STEROID PROFILING IN CLINICAL MEDICINE; *Pike, A.W., Klein, J., Fennessey, P.V. and Horwitz, K.; Univ. of Colorado Sch. of Medicine, Mass Spectrometry Resource, Denver, CO 80262.

The growth and differentiation of steroid dependent target tissues is regulated by well-defined mechanisms. Steroids diffuse through the cell membrane and bind specifically to cytoplasmic receptor proteins. These steroid-receptor complexes then translocate to the nucleus, bind to nuclear acceptor sites and alter transcriptional processes, m-RNA synthesis, and DNA replication.

We have been studying this mechanism of progesterone action in human breast cancer cells, using a permanent cell line called T47D, that synthesizes unusually high levels of progesterone receptors (PR). We have shown that a brief pulse (30-60 min) of progesterone depletes total cellular PR by more than 90%, and that a subsequent replenishment step restores PR to control levels in ~ 16 to 24 hrs. We were surprised to find however, that PR replenishment to high levels occurred even during continuous 24 hour treatment with progesterone; such treatment would be expected to chronically suppress PR. To explain this replenishment in the presence of continuous progesterone, we postulated that progesterone was rapidly metabolized and therefore removed from the culture system.

To show whether this was the case, progesterone levels in medium exposed to cells for varying time periods were analyzed by gas chromatography (GC). Cells were incubated in medium containing 1 μ M progesterone for 5 min to 16 hrs. At 5 minutes, a chromatogram of the medium showed a single peak, presumably progesterone. However, after approximately 2 hours, the concentration of this progesterone peak was halved and by 16 hours, progesterone was undetectable but was replaced by an unknown metabolite.



Media extracts from the 5 min and 16 hr time-points were then subjected to combined GC-MS. The spectrum of the peak seen at 5 min was confirmed to be progesterone by comparison to an authentic standard. The spectrum for the metabolite seen at 16 hrs had a base peak of m/z 100, with additional fragments at masses 296, 386, 476 and 492. These fragments suggest a sequential loss of two trimethylsilanol moieties (mass = 90) indicating the presence of two hydroxyl functions. The base peak at m/z 100 is characteristic of a steroid with a ketone function at C-20 and no other functional groups at C-21, C-17 or C-16. The spectrum suggests a structure having a reduced A-B ring, with hydroxyl functions at positions 3 and 11.

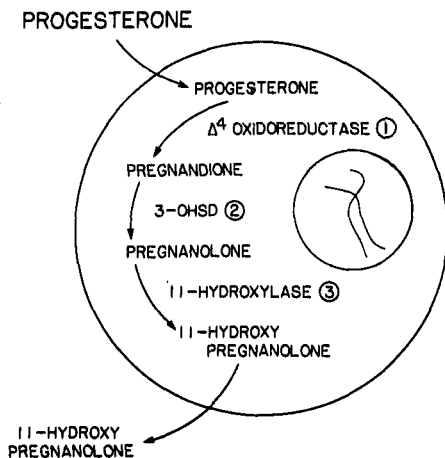
The spectra of the authentic standards, 5 β -pregnan-3 α ,11 α -diol-20-one, 5 α -pregnan-3 α ,11 α -diol-20-one, and 5 α -pregnan-3 β ,11 α -diol-20-one were very similar to that of the unknown metabolite. The standards differed only in their mass spectral relative intensities, and their GC retention times. We have therefore tentatively identified this unknown metabolite as an 11-hydroxypregnanolone whose stereochemistry remains to be determined.

We also conclude from this series of experiments that the metabolism of progesterone is intracellular, since hormone was not metabolized in media incubated for 16 hours at 37°C, in the absence of cells.

We tentatively propose the following metabolic scheme: 1) In order to form 11-hydroxypregnanolone, a series of enzyme reactions are required involving Δ^4 -3-ketosteroid-5-oxidoreductase, 3-hydroxysteroid dehydrogenase, and 11-hydroxylase.

2) We postulate that all the intermediate metabolites are retained intracellularly, and that only the final product is released into the medium.

Hypothetical Intracellular Metabolism of Progesterone in T47D Human Breast Cancer Cells



JUSTIFICATION OF SEQUENCE:

① AND ② KNOWN FROM ANDROGEN AND PROGESTERONE METABOLISM.

① AND ② ARE FAST ENZYMES; ③ IS SLOW.
FINAL REACTION PRODUCT EXPECTED IN MEDIA.

The cytosol and nuclear fractions of cells incubated with progesterone are currently being analyzed by GC and GC-MS to determine the precise sequence and location of its metabolism and to search for the intermediate metabolites. These intermediates may have important biological functions.

The demonstration of 11 hydroxylase activity in extra-adrenal tissue is surprising. However, the results we have presented confirm this unique finding. Previous applications of GC-MS to cell culture have been hindered by the lack of sensitivity and specificity. The advent of fused silica capillary columns coupled to a dry injector allows the analysis of trace amounts of biological sample previously considered difficult to measure by GC-MS. This work indicates that GC-MS can play an important role in the analysis of active trace compounds isolated from biological systems.

NEONATAL BILE ACID BIOSYNTHESIS

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Since it was first established by Bloch *et al* in 1943 (1) that cholesterol is metabolised to bile acids in dogs, considerable effort has been made to elucidate the metabolic intermediates in the formation of the primary bile acids, cholic and chenodeoxycholic acids, in man (see 2). Many of these studies have been carried out in animals fitted with biliary fistulas to avoid the complication introduced by the enterohepatic circulation of secondary bile acid metabolites produced by intestinal microflora.

Recently we investigated the bile acid content of bile present in the gastric contents of neonates as the result of high intestinal obstruction (3). It was hoped that in the absence of bacterial intestinal metabolism the bile acid composition would reflect primary hepatic synthesis from cholesterol and provide information on hepatic biosynthetic intermediates in the pathway. In the present study, improved isolation and separation techniques have been applied to establish the identity and conjugation state of individual bile acids. Gas chromatography-mass spectrometry has been essential to distinguish and characterise the many possible isomeric structures.

A sample of gastric contents from a 4 day old infant (gestational age 40 weeks) with duodenal bands (malrotation) was diluted with 0.1 mol/l sodium hydroxide to pH >10 and applied to a cartridge of reverse phase octadecylsilane bonded silica (4) in order to extract bile acids and their conjugates. Following cation exchange chromatography on SP-Sephadex, unconjugated, glycine/taurine conjugate and sulphate fractions were isolated on DEAP-LH-20 chromatography. The methyl ester-trimethylsilyl ether derivatives of each fraction were separated by capillary column gas chromatography: 25 m glass column (0.4 mm ID) coated with silicone OV-1 and temperature programmed from 225°C at 2°C/min.

Figure 1 shows the capillary column GC-MS trace of the derivatised bile acids obtained from the glycine/taurine fraction. This fraction contained >90% of the total bile acids present. The quantitative composition of the bile acids in this and in previous neonates with intestinal obstruction studied (3) were remarkably similar although the total concentration of bile acids differed from one patient to another.

As expected chenodeoxycholic and cholic acids (peaks 5 and 6 respectively) were the most abundant constituents in all fractions. Their 3 β epimers were also present. 7 α ,12 α -Dihydroxy-3-oxo-5 β -cholan-24-oic (peak 13) and 7 α ,12 α -dihydroxy-3-oxo-chole-4-en-24-oic (not marked) were interesting in that they may indicate a pathway from cholesterol to cholic acid in which oxidation of the side chain precedes some of the biosynthetic steps involving modification of the nucleus. The significance of homocholeic acid (peak 10; mass spectrum - Figure 2; the analogue of cholic acid with a 6 carbon acid side chain) is not known. It may be a product of side chain shortening in the neonate which does not persist as the liver matures.

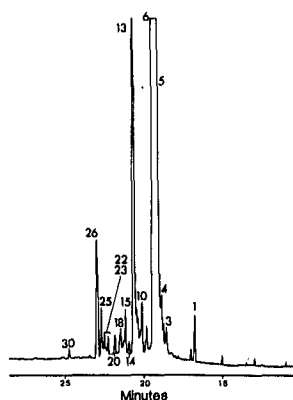
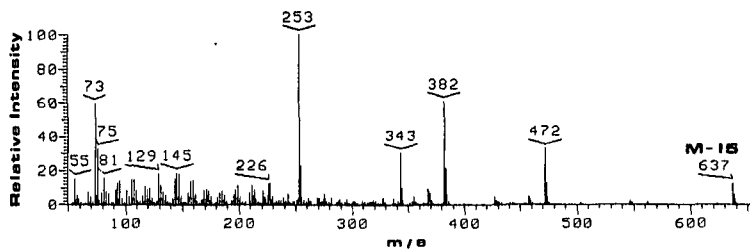


Figure 1: Bile acids identified in glycine/taurine fraction (see text of GC conditions), 1. 3 β -hydroxy-5 β -cholestane (internal standard); 3. 3 β ,7 α -dihydroxy-5 β -cholan-24-oic; 4. 3 β ,7 α ,12 α -trihydroxy-5 β -cholan-24-oic; 5. 3 α ,7 α -dihydroxy-5 β -cholan-24-oic; 6. 3 α ,7 α ,12 α -trihydroxy-5 β -cholan-24-oic; 10. homocholic; 13. 7 α ,12 α -dihydroxy-3-oxo-5 β -cholan-24-oic; 14. 1 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β -cholan-24-oic; 15. Dihydroxy-oxo-cholanoic; 18. 3 α ,12 α -dihydroxy-7-oxo-5 β -cholan-24-oic; 22. Dihydroxy-dioxo-cholanoic; 23. Dihydroxy-dioxo-cholanoic; 25. 2 β ,3 α ,7 α ,12 α -tetrahydroxy-5 β -cholan-24-oic; 26. tetrahydroxy-cholanoic and 30. (25 ξ)-3 α ,7 α ,12 α ,25-tetrahydroxy-5 β -cholestan-26-oic.

Figure 2: mass spectrum of peak 10, homocholic acid (methyl ester-trimethylsilyl ether derivative)



The capacity for nuclear hydroxylation in the neonatal liver is reminiscent of infant steroid metabolism as evidenced by the presence of 1 β (peak 14), 2 β (peak 25) and 6 α (not marked) hydroxylated trihydroxycholanoic acids. Peak 26 has not been fully identified but its four hydroxyl groups are attached to the ring system, possibly at carbons 3, 7 and 12 and one other, most likely on ring A.

A number of 27-carbon bile acids were detected, mainly in the unconjugated fraction and included the C₂₅ and C₂₆ hydroxylation products of 3 α ,7 α ,12 α -trihydroxy-5 β -cholestanic acid. Their importance in the chain shortening process in the biosynthesis of bile acids has still to be determined.

Blood from children with liver disease often contains bile acid components not found in children of ≥ 1 year old with normal liver function. We are presently investigating the possibility that some of the unusual bile acids observed in neonates may represent more primitive pathways in biosynthesis which may be re-activated in some diseases of the hepatobiliary system.

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WOF 1 | METASTABLE DECOMPOSITION OF IONS PRODUCED
IN HEAVY PARTICLE BOMBARDMENT MASS SPECTROMETRY, B.T. Chait
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High (Mev) and low (Kev) energy ion bombardment of organic compounds in the condensed phase are found to give rise to secondary molecular ions in the gas phase containing appreciable internal energy of excitation. This paper presents the results of time-of-flight mass spectrometric studies of the subsequent metastable decomposition of these excited ions from a variety of organic compounds including alanylalanylalanine, guanosine, 5'-adenosine monophosphate, erythromycin, chlorophyll a, pheophytin a and the polypeptide β -Endorphin. The observed reaction pathways, rate constants and internal energy releases for both positive and negative ions will be described. The implications of the findings on the fragmentation mechanisms involved in heavy particle bombardment mass spectrometry will be discussed. The fragmentation observed when the molecule is ionized by protonation and with alkali metal cationization will also be compared.

THE ROLE OF FIELD STRENGTH AND TEMPERATURE
IN FIELD DESORPTION MASS SPECTROMETRY

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In the past the formation of ions in FD MS and its dependence on field strength and temperature has been a subject of controversial discussions. In particular the contribution of ionization processes of low threshold fields in which the emission field strength is self generated gave rise to underestimates of the role of field strength in FD MS. In the following a brief summary of the present knowledge about the effect of field strength and temperature on ion formation is given.¹⁻⁵

1. Field ion formation without thermal activation is possible in field desorption or field evaporation of metal atoms from metal tips⁶ but is not possible in FD MS of organic and inorganic samples.⁷

A purely thermally induced desorption of ions has been observed for some cations and anions of complex salts such as quaternary ammonium salts. However, this latter emission phenomenon is restricted to a rather limited number of compounds. In addition the temperature required for ion emission by purely thermal desorption was found to be significantly higher than for an FD process. Under normal operating conditions the contribution of ion formation processes not principally dependent on high external fields is negligible.

2. Two distinctly different mechanisms of ion formation denoted as "FI mechanism" and "desolvation mechanism" contribute to the ion formation in FD MS. They show a significantly different dependence on field strength and temperature. In case of the "FI mechanism" i.e. ionization of molecules via removal of electrons by tunneling the threshold field strength of molecular ion formation is in the range of $2-6 \cdot 10^9$ V/m. A thermal activation of the sample is mainly needed for supplying molecules to the needle tips via the gas phase or by surface diffusion. For this mechanism the optimum sample or anode temperature is related to the vapour pressure of the sample and/or the surface mobility of molecules. M^+ ions are typical ionization products of an "FI mechanism".
3. In the desolvation mechanism ions are extracted from condensed layers such as viscous solutions and amorphous or crystalline solids. A prerequisite of this mechanism is a sufficient ionic conductivity of the layer and a strong adhesion of the deposit to the substrate avoiding a quick removal of the sample by the electrostatic stress of the field. The field ion emission from deposits of different physical state have been investigated by combining an optical microscope with an FD ion

source. For viscous solutions the following observations have been made: The field stress first gives rise to electrohydrodynamic instabilities which result in a disintegration of the layer via the emission of charged droplets and the formation of field enhancing projections. The role of sample heating is first to decrease the viscosity of the layer, thus promoting the process of disintegration, and second to increase the rate of solvent evaporation from the surface of the projections. The emission of desolvated ions was found to take place from a nearly amorphous or glassy state in, at least, the solvent depleted surface layer of the projections. The temperature of the molecular ion emitting projections was always below the melting point of the organic compound. For an aqueous solution of sucrose the temperature range was 85 - 130 °C. Measured onset temperatures, again for aqueous sample solutions, were 125 °C for raffinose and 145 °C for stachyose respectively. There is significant evidence that thermally labile polar compounds mostly behave in a similar way, i.e. give rise to an ion emission from a nearly solid or glassy state of field enhancing projections grown from viscous solutions. The threshold field strengths required for the growth of projections from viscous deposits is of the order of 10^8 V/m and the self generated maximum field at the ion emitting projections is typically below the onset field strength of FI processes.

4. The emission of ions from crystalline solids has been established for alkali halides and NaOAc by optical microscopy. The onset of ion emission depends on a sufficient ionic conductivity, i.e. on the thickness of the layer. Hence, partial loss of the layer by sublimation was often found to precede the ion emission. Field enhancing projections are suggested to be formed by surface diffusion of molecules and/or disruption of the sample layer. Ion emission from layers of crystalline small peptides (e.g. glycyl glycine) was found to parallel a thermal degradation of the sample. Ion emission from solid deposits is usually weak.
5. The emission of desolvated ions from sample solutions can be significantly enhanced by the addition of so-called matrix compounds such as polyglycols, polyethers, polyvinyl alcohols, polyhydroxy compounds, glycerol, tartaric acid etc. They mainly affect the viscosity of the sample solution, favouring the formation of ion emitting projections via electrohydrodynamic disintegration of the layer. As a result the desolvation field strength and temperature decreases and field ion emission can be observed over a broader temperature range with less intensity fluctuations. The admixture of a matrix compound also prevents crystallization of a sample in vacuum and hence avoids the above mentioned problems encountered in ion emission from a deposit in the crystalline state. For many compounds the ion emission temperature can be reduced to room temperature by addition of a suitable matrix compound.

Further matrix effects worth mentioning are: suppression of FI processes on tip of microneedles of activated emitters; reduction in fragmentation and improved adhesion of the sample to the substrate. It may be added that the formation of negative ions by FD has been mainly accomplished by a matrix effect, i.e. a reduction of the desolvation field strength below the onset field strength of electron detachment from negative ions.

6. The effect of field strength and temperature on fragment ion formation is rather complex and not easy to rationalize. Of course, overheating of a sample gives rise to the ionization of thermal decomposition products. However, even below the temperature range of true thermal decomposition of the sample there may be an effect of temperature on fragment ion formation, well known for field dissociation of ions in FI MS but less so for the desolvation mechanism in FD. In the latter case fragment ions are generated by temperature sensitive proton induced solvolysis reactions in the acidic space charge region of a sample layer. In the negative ion FD mode a basicity results from negative surface charging of the layer.

Although the principal role of field strength and temperature in FD MS is now fairly well understood, considerable work has still to be done in order to optimize the use of FD MS in analytical applications.

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CESIUM ION LIQUID SECONDARY ION MASS SPECTROMETRY AND MULTI-SCAN ACCUMULATION FOR DETERMINATION OF MASS-RESOLVED MOLECULAR IONS OF HIGH-MASS BIOLOGICAL SUBSTANCES

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INTRODUCTION

The last fourteen years have witnessed steady progress in the invention of new mass spectrometric techniques and instrumentation which have made possible the detection and study of chemically and thermally labile biological substances directly without the need for chemical derivatization of polar functionality. These include field desorption, plasma desorption, thermospray, and secondary ion mass spectrometry. While all of these techniques have some analytical attributes in common, their diversity has quickly led to the ability to analyze directly the majority of compound types previously inaccessible by mass spectrometric techniques, including polar, labile bio-organic substances, salts, organometallic complexes, etc. The considerable success of some of these, such as field desorption, plasma desorption and secondary ion mass spectrometry, has recently led to much excitement in developing further capabilities for analysis of higher molecular weight substances. Thus, field desorption has had considerable success in obtaining molecular ions and some fragmentation information in the mass range up to 3,000 daltons for free substances and into the 4,000 mass range for derivatized substances (1). It presently appears limited by lack of continuous feed of the substance to be analyzed to the emitter for field desorption. The development of thermospray ionization shows promise in providing field desorption-like spectra of molecules in the mass range to at least 2,000 daltons from a continuous HPLC effluent (2). ^{252}Cf plasma desorption has provided pioneering data and a continuing thrust for obtaining molecular weight information on classically most intractable substances of ever increasing molecular size (3).

However, in the last two years, the introduction of liquid matrix secondary ion mass spectrometry provided a routinely practical experimental approach to the study of a very wide variety of polar, chemically and thermally labile biological materials of increasing molecular size. Once again the first demonstration of the ability to determine the molecular weight of intact bovine insulin was obtained as the isotopically unresolved molecular ion cluster by plasma desorption techniques utilizing an accelerator-produced I-127 beam (4). This was followed almost immediately by detection of the unresolved molecular ion isotopic cluster using liquid SIMS technique for the same molecule (5) and, more recently, the molecular ion cluster for human proinsulin (9390 daltons) (6). While these methods are clear in their demonstration of the feasibility of determining molecular size in this high mass range, the techniques used by these authors resulted in poor mass resolution and accuracy of molecular weight determination. We now wish to report determination of the molecular ion region of methyl glucose polymer (at mass resolution $\sim 10,000$) and that for bovine insulin (at mass resolution $\sim 6,000$) using cesium ion liquid SIMS and high precision, accelerating voltage multiscan averaging techniques.

EXPERIMENTAL AND RESULTS

Previously, this laboratory has reported on the design and performance of a cesium ion gun used to produce the primary bombarding beam for liquid matrix sputtering of biological substances (7). A refined gun design (8) was used, mounted as previously described (7) inside the ion source housing in a Kratos MS-50S mass spectrometer equipped with a 23 kGauss magnet and a post-acceleration detector system. For single scans, the normal magnet scan function was employed. In addition, an LSI Nova data system was used to collect repetitive, high precision accelerating voltage scans over limited mass ranges to serve as a multichannel averaging device. The comparison of peak shape and mass resolution for a single scan vs. several accumulated high precision voltage scans is illustrated by observation of the molecular ion region of methyl glucose polymer (MGP) shown in Figs 1a and 1b. In Fig. 1a the cationized molecular ions

of MGP are observed at $M + Na^+$, 3538; $M + K^+$, 3554; and $M + Na_2^+$ at 3560 at a scan rate of 1,000 sec/decade; in Fig. 1b, the results for accumulation of 30 scans over the same mass region are depicted. Fig. 1c is an expanded and enlarged view of the $M + Na^+$ cluster at mass 3538. A comparison of secondary molecular ion yield was made using bovine insulin applying identical instrument operating conditions for both the commercially available xenon neutral and our cesium ion guns in order to establish that comparable sensitivities were being obtained for these measurements. The results are shown in Fig. 2a for the unresolved molecular ion isotopic cluster of bovine insulin using a scan rate of 3000 sec/decade. With these conditions, the cesium gun maintained a somewhat higher sensitivity, as reported previously for lower mass substances (7). Results for a single scan over the mass-unresolved molecular ion isotopic cluster for bovine insulin are illustrated in Fig. 2b which contains an estimated $1-3 \times 10^3$ ions/sec at a scan rate of 1000 sec/decade. Analogous data for a 32-scan accumulation at mass resolution $\sim 6,000$ is shown in Fig. 2c.

In addition, signals were observed for bovine proinsulin in the accumulation mode, centered at 8699 daltons. The mass was determined using the cesium iodide cluster peak at 8706 daltons and its corresponding value offset by 2000 ppm.

From these data it can be seen that precision multichannel averaging techniques are effective in producing stable isotope cluster-resolved molecular ions distributions at mass resolutions in excess of 10,000 for high molecular weight naturally occurring biological substances. It also appears that the accurate of mass of all the individual nominal masses in each stable isotopic cluster can be determined with accuracies in the low ppm range.

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MGP

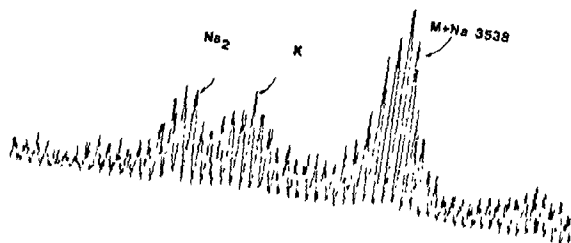


Figure 1a

Wavelength (nm) 4000 4200 4400 4600 4800 5000

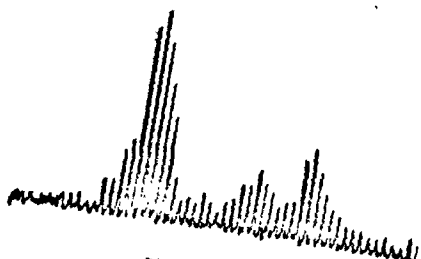


Figure 1b

Wavelength (nm) 4000 4200 4400 4600 4800 5000

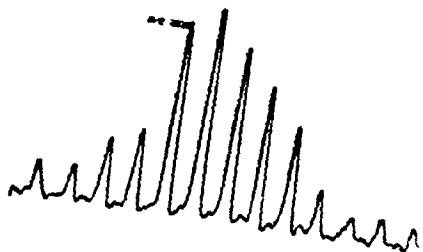


Figure 1c

bovine insulin

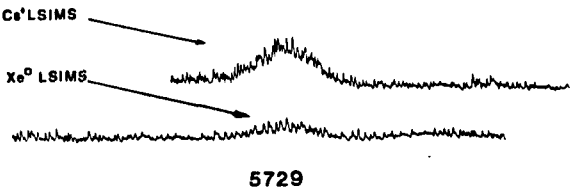


Figure 2a

b. Insulin

1000 sec/dec



Figure 2b

INSULIN 10K 200 1000 1000-1000 1000-1000
5729 25

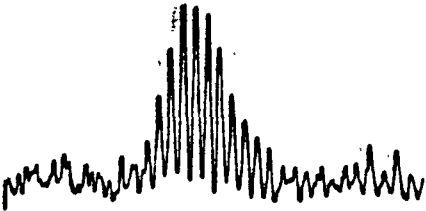


Figure 2c

While the fragmentation evident in spectra obtained by several powerful desorption ionization techniques (SIMS, PD, LD, FAB,...) provides valuable structural information, it can prevent detection of a molecular or quasi-molecular parent ion for some samples, and generally complicates or even precludes direct mixture analysis without additional separation instrumentation or analysis steps. It is unclear what fraction of the internal energy promoting fragmentation results directly from the process of removing an ion from its matrix. Although the detailed mechanism(s) of desorption ionization remain under study, most clearly offer means for increasing analyte internal energy incidental to the actual desorption process. For example, collisions with bombarding particles may result in thermal energy transfer, as may the heating of a field desorption emitter.

This question has gained additional importance with the popularization of desorption from liquid matrices in experiments such as FAB or liquid SIMS. The liquid matrix is believed to facilitate these experiments by providing a diffusional means of replenishing sample at the surface bombardment sites. The effects of the matrix on the energetics of the desorption process have not received as much attention, at least in part because of the convolution with other energetics, as described above. However, a better understanding of this role and the factors affecting it would facilitate the identification of a "good" matrix solvent for a given experiment from the growing list of solvents that have been tried, and should also aid in the selection of new solvents for trial.

Because it does not involve sample bombardment or other heating, electrospray ionization (ESI)¹ offers a means of probing liquid desorption with relatively little interference from other energy deposition processes. Diquaternary ammonium salts are attractive samples for tests of energy deposition, because the coulombic repulsion between their charge centers makes them susceptible to fragmentation by deposition of even small amounts of internal energy. The detection of parent ions in the ESI mass spectra of glycerol solutions of a series of these salts² suggests that the desorption process is extremely "soft". Fragments observed in corresponding FAB spectra² may be attributable to internal energy increases resulting from bombardment. Despite the softness of the desorption process, several factors have been identified which can affect its efficiency and the overall sensitivity of ESI³. For example, sample solubility in the matrix employed improves emission stability and spectral precision, although acceptable spectra have been obtained from suspensions in ESI (e.g., ref. 4) as in FAB.

Beyond simple solubility, solvent-solute interactions appear to play a large role in determining ESI sensitivity. In a homologous series of simple methyl ammonium salts, the detected M.S. signal was observed to increase with increasing methylation, suggesting that the weaker interactions with solvent (glycerol) experienced by the more substituted ions promoted selective emission.

Two additional factors become evident with increasing analyte charge or charge density. To illustrate these, an average charge ($\bar{Z}$) and average degree of solvation ($\bar{N}$) can be defined, as follows:

$$\bar{Z}_m = \frac{\sum I_{m,j} Z_j}{\sum I_{m,j}} \quad \bar{N}_x = \frac{\sum I_{x,n} N}{\sum I_{x,n}}$$

where $I_{m,j}$ is the detected intensity of the j th ion of charge Z_j , containing the species m (including ion pairs and solvated ions), and $I_{x,n}$ is the intensity of an ion x solvated by n solvent molecules. In a glycerol solution containing BaI_2 , $Z_{\text{Ba}} = 1.44$; most of the barium detected was present as the ion pair BaI^+ , rather than the atomic cation. Furthermore, ions containing barium were heavily solvated: $N_{\text{Ba}^{2+}} = 6.7$ and $N_{\text{BaI}^+} = 3.8$. This suggests that the preponderance of BaI^+ may have been due to its lower charge and resulting weaker interactions with solvent. Of course, if ion pairing (or higher order association) neutralizes analyte charge, sensitivity can actually be reduced.

An alternative explanation for the preponderance of BaI^+ is that ion pairs are the predominant preformed ions in solution, in view of the moderate dielectric constant of glycerol (42.5 vs 78.3 for water). However, when the potential of the electrostatic analyzer (ESA) was lowered to accept the products of fragmentation in the first field-free region, the spectrum was dominated by solvated Ba^{2+} ($Z_{\text{Ba}} = 2.0$). This provides

an example of another factor affecting sensitivity: ions which are heavily solvated (generally those with high charge density) may undergo in-flight desolvation⁵ and so be undetected in spectra of ions with full acceleration energy.

One final factor which should not be overlooked is the chemical reactivity of the matrix. Early attempts to observe ferric ethylenediaminetetraacetate were foiled by the esterification of EDTA with glycerol, catalyzed by the Lewis acid Fe^{3+} . Successful sampling was achieved only when the metal acidity was reduced by preforming and isolating the complex (as a solid sodium salt) prior to dissolving in glycerol⁶.

While reactivity is obviously a chemical consideration, other factors affecting sensitivity (ion pairing and interactions with solvent, including in-flight desolvation) are also basically physicochemical in nature, and may be expected to vary with the chemistry of the analyte and matrix. For example, in the spectrum of a glycerol solution containing an equimolar mixture of $(\text{CH}_3)_4\text{NBr}$ and the diquaternary salt $(\text{CH}_3)_3\text{N}(\text{CH}_2)_2\text{N}(\text{CH}_3)_3\text{I}_2$ (diquat, MI_2), the tetramethylammonium ion was much more abundant than the diquat or its singly charged ion pair³ $[\text{MI}]^+$. This is reflected in the selectivity ratio, $S_{\text{M}2+}/(\text{CH}_3)_4\text{N}^+$:

$$S_{\text{A/B}} = \frac{\sum I_{\text{A}} / \sum I_{\text{B}}}{\text{mol A/mol B}}$$

where I_{A} and I_{B} are the intensities of all detected ions incorporating A and B, respectively. For the mixture above, $S_{\text{M}2+}/(\text{CH}_3)_4\text{N}^+ = 0.05$, suggesting lower sensitivity to the diquat. This is probably because of more extensive solvent-diquat interactions, as evidenced by the substantial increase in S (to 2.3) when the ESA potential was lowered. However, the role of ion pairing is difficult to assess in such an experiment. It will reduce sensitivity for the single quat by neutralizing it, and may neutralize or merely reduce the charge of the diquat; charge reduction without neutralization could actually increase sensitivity.

This issue clearly requires further study. A useful experiment would be to vary ion pairing of a single analyte by changing the dielectric of the matrix. Such an experiment would give more direct insight into the role of the matrix in desorption ionization. Of course, variation of matrix dielectric requires flexibility in using a variety of matrices, which heretofore has not been possible in EHMS. However, recent refinements reducing the size of the emitter orifice (by using capillary bonding tools) have greatly improved our flexibility. In fact, Figure 1 shows a portion of a spectrum obtained from an aqueous solution of NaI . The extensive solvation probably reflects the solvent properties of water. A detailed study is underway.

Figure 2 shows a surprising effect of the matrix on the mass spectrum: the width of conventional metastable peaks (arising from desolvation in the second field-free region) appears to depend on the nature of the matrix. The figure shows metastables for water desolvation corresponding approximately in apparent mass and in weight fraction loss to a metastable observed in a spectrum obtained with glycerol. The substantial narrowing with water is evidently due to the lower internal energy release accompanying removal of that solvent. This effect may even contribute to the initial kinetic energy spread of sampled ions. Figure 3 shows stable ions of similar mass sampled from glycerol and from dimethylsulfoxide (DMSO). The apparent resolution in the latter case is significantly higher, although no changes in spectrometer parameters (accelerating potential, slit width, etc.) were made.

Detailed studies of these matrix effects are under way. They will include systematic variation of such solvent properties as dielectric constant and viscosity. In this way, we hope to remove some empiricism from the selection of the optimum matrix for a given desorption ionization experiment.

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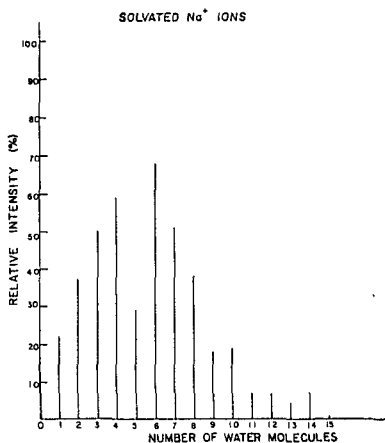


Figure 1: Portions of the EH mass spectrum of aqueous solutions containing Na^+ (Average of 12 spectra).

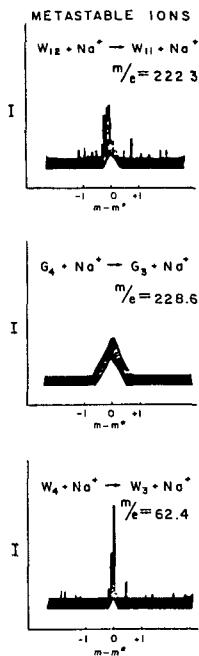


Figure 2: Oscillographic recordings showing conventional metastable ions (arising from desolvation in the second field-free region) for glycerol and water solutions containing Na^+ .

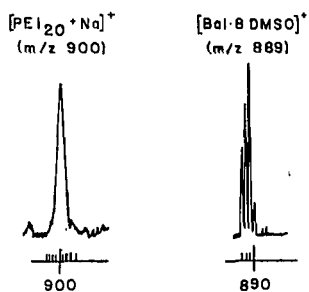


Figure 3: Oscillographic recordings showing portions of EH mass spectra of poly(ethyleneimine) 600 in glycerol (peak shown is for the 20-mer) and of BaI_2 in dimethylsulfoxide (DMSO).

RAPID ANALYSIS USING DIRECT SUPERCRITICAL FLUID INJECTION-MASS SPECTROMETRY

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Direct probe introduction methods for mass spectrometry have the advantages of convenience and rapid analysis times. However, conventional heated probes are limited by the volatility and thermal stability of the sample. A major trend in mass spectrometry continues to center about the development of methods for analysis of nonvolatile or labile compounds. As a result there has been intense interest in the range of "soft" ionization techniques which result in ion formation at or near a surface. These techniques include field desorption, laser desorption (LD), secondary ion mass spectrometry (SIMS), fast atom bombardment (FAB), californium-252 plasma desorption, "in-beam" chemical ionization, particulate impact, electrohydrodynamic ionization, and a variety of rapid heating techniques. These techniques, however, all suffer from difficulties which include: substantial matrix effects; often complex mass spectra with abundant fragmentation (and, in some cases, ions produced at nearly every m/z value); response which varies greatly with compound polarity; quantitation which is difficult at best; spectra which are difficult to predict and interpret; and sensitivity to the precise chemical and physical nature of the surface or sample matrix. While these problems limit the potential for quantitative, and in many cases even qualitative, analyses they have greatly extended the range of mass spectrometry to include thermally labile, non-volatile and highly polar compounds not compatible with conventional introduction techniques and ionization methods. However, it must be remembered that the failure of conventional ionization methods is due to the inability to easily transfer these "difficult" molecules intact to the gas phase.

The direct fluid injection (DFI) method developed at Battelle-Northwest circumvents difficulties associated with ionization from a surface and allows the direct transfer of material to the gas phase before ionization⁽¹⁻²⁾ without the aid of rapid heating. The method involves injection of a solution of the material into a high pressure solvent flow which is then rapidly heated to supercritical conditions. The supercritical fluid is chosen so that that material of interest is sufficiently soluble. The solvating power of the fluid may be controlled by selection of both the temperature and pressure. The supercritical fluid transports the material to a chemical ionization (CI) source where an efficient injection into the CI plasma assures almost uniform sensitivity regardless of compound volatility. Fluids such as carbon dioxide with low critical temperatures (31°C) can be used for the analysis of thermally labile compounds.

Instrumentation developed for supercritical fluid chromatography-mass spectrometry (SFC-MS) is used in this method with a submicroliter injector and 0.1 mm I.D. platinum-iridium tubing between the injector and the point of injection into the ion source. The direct fluid injection (DFI) method uses a water cooled injector valve to insert the sample into the fluid stream at ambient (below supercritical) temperatures after which it is heated rapidly to supercritical conditions. The fluid stream, propelled by a pressure regulated high pressure syringe pump, moves through a 25 cm transfer line at supercritical conditions to a chemical ionization (CI) source, at a flow rate determined by the DFI probe pressure restrictor⁽⁴⁾. Fluid flow rates are chosen to provide an optimum CI source pressure. Sample solvent and volume are chosen so that supercritical conditions are maintained for the transfer line temperature. Solvent injections which result in subcritical transfer degrade the DFI process, reduce sensitivity, and often result in plugging of the DFI orifice.

The DFI chemical ionization mass spectrometric technique offers a potentially powerful alternative to direct heated probe and "soft" ionization techniques involving direct ion formation at a liquid or solid interface. Experience with SFC-MS has indicated that DFI allows one to obtain mass spectra of thermally labile and nonvolatile compounds under relatively gentle ionization conditions. The process involves a rapid injection of the dense gas stream directly into the CI plasma. Hence, problems associated with heterogeneous processes and limited volatility are avoided. This approach also offers advantages for quantitation and allows the detection of essentially all fluid phase components. In addition, one can vary the amount of fragmentation by simply changing the CI reagent gas. The only

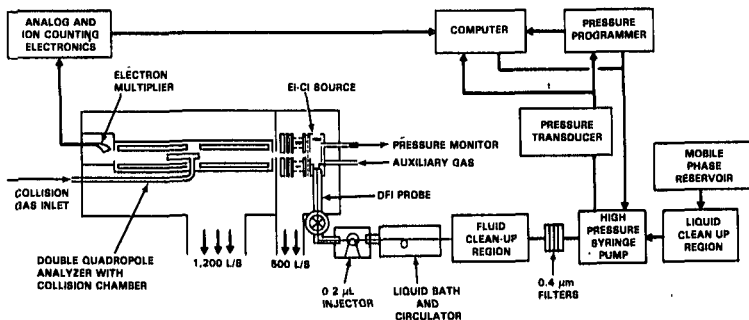
restriction on application of this method appears to be the requirement of solubility in the supercritical fluid system.

The DFI-MS method has been successfully applied to a wide range of compounds and complex mixtures using a variety of fluid systems. For example, mycotoxins of the trichothecene group have been analyzed using supercritical carbon dioxide and a variety of CI reagent gases. For more polar systems supercritical ammonia has proved useful and mass spectra for a variety of compounds including selected nucleotides and amino acids have been obtained. In addition complex materials, such as the polar fraction of various fuels and humic and fulvic acids, have been examined and mass spectra obtained using the DFI-MS method. The extension of these methods to additional fluid systems and evaluation of optimum conditions is continuing in our laboratory⁽⁴⁾. An important aspect of this is the interfacing to a magnetic instrument with an extended mass range to fully explore any limitations associated with the DFI process.

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DIRECT FLUID INJECTION - MASS SPECTROMETER



SURFACE IONIZATION OF VOLATILE ORGANIC COMPOUNDS ON HOT FILAMENT

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Surface ionization is defined as the ionization process described by the Saha-Langmuir equation. It has been used for chemical species with low ionization potential because of its high sensitivity and simplicity. However, its analytical use has been restricted to metals so far.

Recently, Cotter et al. (1981) reported the production of R_4N^+ ions from quarternary ammonium salts by a process that is named thermal desorption ionization. A mechanism producing the principal ions in this study is almost certainly the surface ionization following the decomposition through the rupture of chemical bonds or otherwise pyrolysis on the hot metal surface. This work stimulates the investigation of surface ionization mass spectrometry of organic materials.

Here I report on the study of surface ionization for 10 kinds of volatile organic materials with low ionization potentials. An emphasis is given to the comparison of molar sensitivity of these compounds as well as to the filament temperature dependence of the ionization efficiency. The ionization mechanism is the surface ionization of radicals produced by the interaction of the gaseous substance with the incandescent Re filament.

Experimental The experiments were performed with a Finnigan 3300 gas chromatograph-quadrupole mass spectrometer equipped with a home built combination ion source to allow an easily interchangeable operation both in surface (SI) mode and in electron impact (EI) ionization mode. As can be seen in Figure 1 the design of surface ionization source assembly, direct insertion probe, is such that the Re ribbon filament (6mm x 0.75mm x 0.025mm) can be placed in the center of the EI ion source chamber when the probe is inserted. With this configuration, therefore, gas chromatographic introduction can easily be coupled.

The organic gas was admitted to the mass spectrometer from a reservoir via a variable leak valve (series 203, Granville-Phillips) and sample gas pressure over a range of 1 to 5×10^{-7} torr was measured by the ionization gauge (VG-51, Wakaida) calibrated with capacitance manometer (315BHS-1, MKS).

Molar Sensitivity The predominant ions in the SI spectrum are shown in Table I for the organics under study. All the compounds except isopropanol and o-chloroaniline produce an intense $(M-1)^+$ peak. $(M-1)^+$ intensity is highest for most of these compounds with few, if any, other peaks at the low-intensity level. However, indene forms a molecular ion of approximately 40 % of $(M-1)^+$ and as many as 14 large peaks appear in the α -pinene spectrum.

Molar sensitivity is expressed in the resulting ion current of the base peak in the SI spectrum observed in the presence of 2×10^{-5} torr O_2 gas per torr of sample (A/torr). The ion current measurement was made by the electron multiplier with a gain at 5×10^3 under the condition of the optimum filament current (i.e. temperature) which gives the maximum ion signal. The result shows a very wide variation from 5.5 of α -methyl-naphthalene to 1.8×10^{-5} A/torr of indene. It is to be expected because, according to the Saha-Langmuir equation, the molar sensitivity (ionization efficiency) depends strongly on the difference between the ionization potential of the vaporizing radicals formed on the hot filament.

The SI/EI, the ratio of the base peak intensity obtained in the SI mode to that in the EI mode, is listed in the final column. The α -methyl-naphthalene yields 13.7 times larger peak in the SI mode. This result makes SI mass spectrometry potentially useful as an analytical method for specific organic compounds.

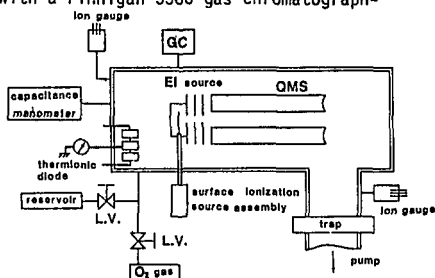


Figure 1. Experimental Set-up

Table I. Molar Sensitivity of Some Volatile Organics by Surface Ionization

compound	intense peak (m/e)	molar sensitivity (A/torr)	SI/EI
isopropanol	43	4.3×10^{-5}	2.5×10^{-4}
trimethylamine	(M-1) ⁺ , 30	1.6	8
xylene	(M-1) ⁺ , 91	6.1×10^{-2}	3×10^{-1}
ethylbenzene	(M-1) ⁺ , 91	2.0×10^{-2}	1.1×10^{-2}
m-ethyltoluene	(M-1) ⁺ , 105, 91	1.5×10^{-2}	3.1×10^{-2}
p-methoxytoluene	(M-1) ⁺	1.7	5
α -methyl-naphthalene	(M-1) ⁺ , 155 (?)	5.5	13.7
indene	(M-1) ⁺ , M ⁺	1.8×10^{-5}	3.2×10^{-5}
o-chloroaniline	M ⁺	7.4×10^{-4}	2.3×10^{-3}
α -pinene	133, (M-1) ⁺ , 131, 129, 119, 105, 107, 93, 91, 79, 58, 55, 43, 29	-	-

Sensitivity vs Re Filament Current

Variation of filament current (temperature) results in change in the ionization efficiency as expected. A plot of the ion signal of the base peak against emitter current is shown in Figure II for p-methoxytoluene (marked as 1) xylene (2) isopropanol (3) and trimethylamine (4). The maximum which occurs at the filament current of 0.9 to 2.5 Ampere varies from sample to sample. The observation that ion currents decrease with filament current after reaching the maximum is mainly attributable to the decrease in work function of Re filament with O₂ desorption from Re surface which takes place due to the temperature rise.

GC-SIMS Preliminary experiments were conducted for the gas chromatograph-surface ionization mass spectrometry using the 0.032 % diethylamine solution as a sample. Table II shows the SI and EI spectrum and the detection limit at S/N = 2, demonstrating that SI provides much simpler spectrum with more than 50 times better detection capability.

The linear dynamic range is limited on the side of large concentrations by the possibility of poisoning of the Re emitter filament. The dependence of the response on the concentration of diethylamine at the emitter temperature less than about 1200K is linear up to at least 1 μ g/ml with O₂ gases at 2×10^{-5} torr.

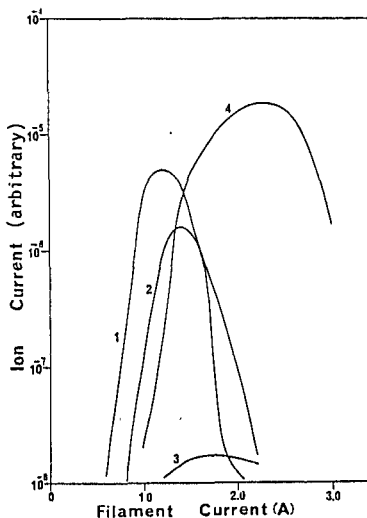


Figure II. Filament temperature dependence of ionization efficiency

Table II. SI and EI spectrum of diethylamine

	mass spectrum, m/e (intensity)	detection limit (S/N=2)
GC/SIMS	72(100), 58(7)	0.05 ng at m/e 72
GC/EIMS	58(100), 30(100), 44(22), 73(20), 72(15)	2.6 ng at m/e 73

Water Desorption From Thin Biomolecule Samples
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The desorption of water on five different model biological surfaces has been investigated by a thermal desorption technique. The basic experiment consists of coating a platinum ribbon with a thin layer of the biological macromolecule under consideration, cooling to liquid nitrogen temperatures in vacuum, thermally desorbing the adsorbed water molecules by ohmic heating of the sample ribbon, and detecting the desorbed water molecules with a quadrupole mass spectrometer detection system. The resulting plot of the desorbed water flux as a function of the sample temperature exhibits a series of peaks corresponding to different binding states of the adsorbate molecules on the sample. The observed peaks divide naturally into three groups on the basis of the sample temperature: the low temperature peaks between -140°C and -50°C, the middle temperature peaks between -50°C and 210°C, and the high temperature peaks between 210°C and 560°C.

The samples investigated were a protein (bovine serum albumin), a polar and a nonpolar polypeptide (poly-L-lysine hydrobromide and poly-L-alanine), a carbohydrate (glycogen), and a completely nonpolar lipid (tristearin).

The low temperature peak was present in all samples as well as controls and no sample specificity was observed. At high coverages this peak can be interpreted as the sublimation of ice. Adsorption into this peak took place with a sticking coefficient very close to unity for sample temperatures during adsorption ca 125°K, for coverages between ca 0.1 and ca 300 monolayers of water.

The middle peak groups gave very strongly sample specific peak patterns, with major peak energies from 16.5 to 22.2 kcal/mole, and desorption in the range of energies from 14.5 to 27.5 kcal/mole. The peaks each contained a range of activation energies for desorption, and the peak patterns were consistent with the assumption that the number and types of polar groups present in the sample determine the amount of water adsorption in this peak group and the complexity of the peak patterns, thus giving the observed sample specificity. This peak group appears to correspond to the water adsorption traditionally measured by water adsorption-desorption isotherms taken near room temperature and often described by the Brunauer-Emmett-Teller (BET) theory of multilayer adsorption, with the important differences that kinetic rather than equilibrium parameters were measured and that different adsorption states could be differentiated. Lower limits on the sample specific water adsorption in this peak group of ca 15% of the BET monolayer values were established, and the observed coverages were shown to correlate well with the trends obtained from the BET monolayer values. The size of these peaks was proportional to sample amount, and the peak locations were reproducible even when the amount of sample applied was varied.

For the BSA, this peak group was found to be capable of repeated reconstitution after desorption by exposure to ca 500 Pa of water vapor at room temperature if the sample temperature had not exceeded ca 190°C during desorption. In contrast, this peak group was not readily repopulated by exposure to a low intensity water beam for adsorption temperatures between -100°C and +10°C, indicating a sticking coefficient of less than ca 7×10^{-6} for adsorption under these conditions.

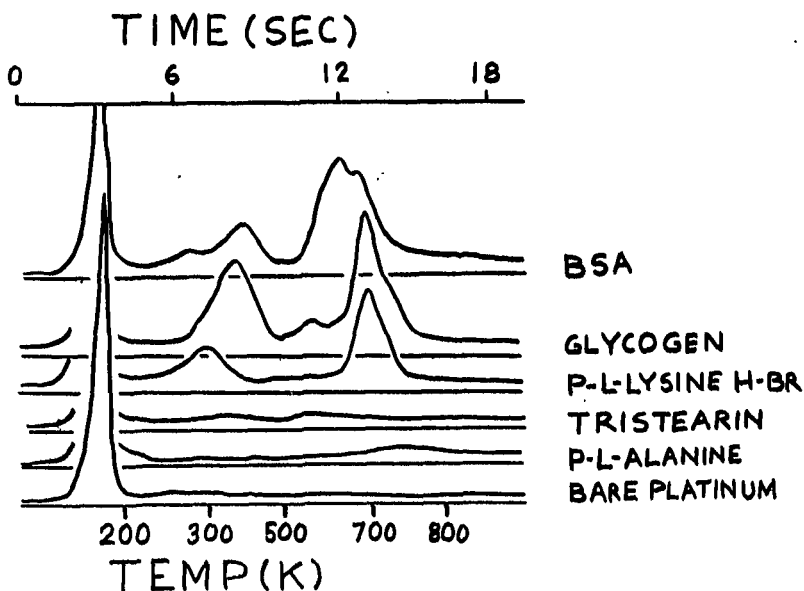
The high temperature peak group gave sample specific peak patterns representing pyrolysis of the sample.

A few representative desorption curves of water from the substances indicated were traced from original recordings and are shown below. These are, in effect, plots on a linear scale of the amplified output of the ionizer-mass spectrometer combination tuned to mass 18 (and thus the instantaneous rate of water desorption) versus temperature on a distorted scale.

A few features to be noted: starting at low temperature (ca 77K) all surfaces adsorb and desorb physically adsorbed water at ca 118K. This peak decays more rapidly than it rises as the physisorbed water is depleted. These peaks are qualitatively similar but can be expected to yield information concerning the interaction of water molecules with their image dipole in the surface and hence the average surface polarizability.

The next set of peaks, lying between 200K and 500K, are characteristic of the substance (absent for lipids and metal surfaces) and will be most useful for obtaining surface composition information with Scanning Desorption Molecule Microscopy. One must stain at 200K (to avoid physisorption) and not exceed 500K to avoid irreversible damage to the sample, pyrolysis, when additional tightly bound water is driven off as shown in 8.10, 8.12 and 8.14.

Further studies of this kind are being done with better data recording and data processing techniques, with more samples, with attention to sample treatment and handling, and with various stain molecules.



WPA 4 | PERFORMANCE CHARACTERISTICS AND ANALYTICAL
APPLICATIONS OF ADVANCED ION TRAP M.S. P.E. Kelley and
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New developments to the ion trap have resulted in significant improvements in sensitivity, mass range and resolution. These improvements allow the utilization of the ion trap mass spectrometer as a low cost universal gas chromatograph (GC) detector. The Ion Trap Detector (ITD) is designed to interface to commercial capillary column GC's.

The desirable characteristics of a universal GC detector are generally recognized as the following: 1) high sensitivity for all compounds amenable to GC separation (1 ng detection limit); 2) adjustable specificity for different compound classes and individual compounds; 3) sufficient stability to produce precise quantitative results; and 4) approximately the cost of the gas chromatograph. Chromatographers currently employ a variety of GC detectors (FID, NPD, ECD, TCD, etc.) to obtain the benefits of these four attributes because except for ITD no other single detector embodies all four.

This detector, which is micro-computer controlled, is reliable, simple to operate and gives identification and quantitation for known and unknown analytes.

The applicability of this detector to a range of analytical problems will be presented.

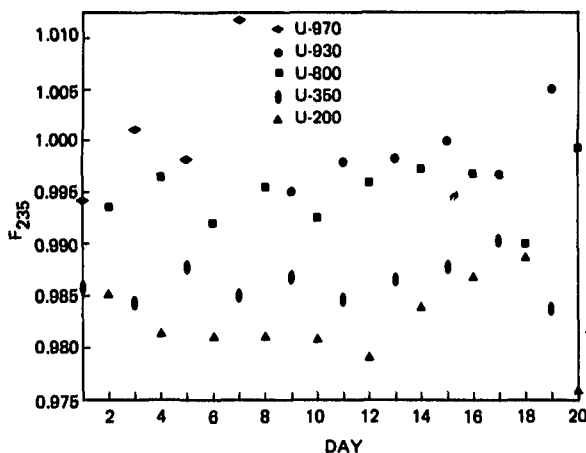
AN EVALUATION OF CONTINUOUS DYNODE ELECTRON MULTIPLIERS
FOR ISOTOPE RATIO MASS SPECTROMETRY

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A Galileo 4700G channel electron multiplier (CEM) was evaluated as a replacement for a 17 dynode-stage electron multiplier in an isotope-ratio mass spectrometer. The experiments were performed on an AVCO single-stage mass spectrometer semi-automated with a DEC PDP-8 computer. Sixteen NBS uranium standards were analyzed in a three-day period; this constituted one "run". Four runs were taken. The U-235 mass discrimination factor, F_{235} , was calculated from the isotopic data by dividing the experimentally determined U-235/U-238 weight-per-cent ratio by the NBS value of the U-235/U-238 weight-per-cent ratio. In each of the four runs, the mass discrimination factor is linearly dependent on the weight-per-cent of U-235. Comparison of the values of F_{235} among the four runs shows that as the multiplier ages, the values of F_{235} decrease for the NBS standards under 50 weight-per-cent U-235, while the values increase at the higher weight-per-cent levels. Another series of experiments provided a large, self-consistent data base for statistical evaluation. The NBS U-930 and U-350 standards were analyzed as a pair every other day. The NBS U-800 and U-200 standards were analyzed on the alternate days. The mass discrimination factor, F_{235} , was calculated for each analysis and plotted versus the day of analysis.

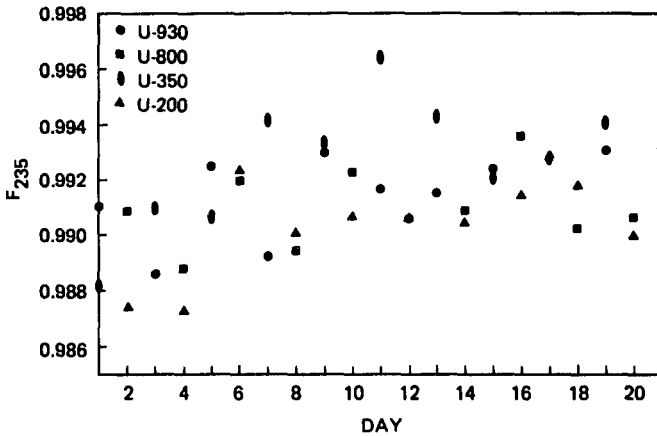
TEMPORAL VARIATION OF ^{235}U CORRECTION FACTOR FOR CEM



The statistical analysis of these data shows a large variation in F_{235} ($\bar{x} = 0.9910$, $2s = 0.0161$) and a correlation of F_{235} with per-cent U-235.

The experiments described in the preceding paragraph were performed with a new dynode multiplier. The 95% confidence limit ($2s$) has decreased by a factor of 10. The mass discrimination factors are observed to increase from days 1-9 and remain constant from days 9-21. The difference in the mean F_{235} for days 1-9 and

FOR DYNODE MULTIPLIER



the mean F_{235} for days 9-21 is greater than the 95% confidence limit of the difference of the means. Hence, these data may properly be evaluated as two distinct groups. In either group, the calculated linear-correlation coefficient is much smaller than the linear-correlation coefficient at the 0.5% confidence level for the same number of observations. There is no dependence of F_{235} on the weight-per-cent of U-235 using the 17 dynode-stage multiplier. The F_{235} remains constant for days 9-21.

This evaluation has shown the CEM to be unsuitable for ion detection in this specific application. These experiments also demonstrate the necessity of complete characterization of instrumental modifications under rigidly controlled conditions in the production laboratory.

ACKNOWLEDGMENT

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CHARACTERISTICS OF NEGATIVE IONS FORMED BY A HOLLOW CATHODE DISCHARGE SOURCE
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A hollow cathode discharge (HCD) ion source has been utilized to generate molecular negative ions of interest in high temperature environments. This ion source was mated with a tandem mass spectrometer¹ in order to characterize the ions formed. A schematic diagram of the HCD source developed is shown in Fig. 1. The discharge apparatus consists of a quartz tube, of 10 mm. internal diameter (I.D.), and two stainless lids placed at both ends of the tube. A metal tube, 1 cm. in length and 8 mm. I.D. is used as the hollow cathode. This cathode, located inside the discharge tube, is movable, so that the distance from the anode can be varied. A glow discharge is generated between this hollow cathode and a stainless-steel sampling slit, which is used as the anode. The ions which pass through this slit are then accelerated to 180 eV, and are focused on the object slit of the tandem mass spectrometer by an electrostatic lens.

Four different configurations of the HCD source, as shown in Fig. 2, were employed to study the discharge ion source characteristics. Fig. 3 shows graphically the relative intensities of the positive and negative ions which were obtained with each discharge source configuration for an oxygen discharge. These ion beam intensities were measured with the first stage magnetic mass filter of the tandem mass spectrometer. For these experiments, the discharge gas pressure was typically 0.5-5 Torr, and the discharge current was in the range from 4 to 40 mA. Under these conditions, the ion source usually exhibits an optimum discharge current for a given pressure which corresponds to the maximum ion beam intensity.

As seen from Fig. 3, the major positive ion formed in the oxygen discharge is O_2^+ , for all source configurations. For negative ions, however, the major ion in the discharge is O_3^- for source configurations 1, 2 and 3, but O_2^- is the major ion for configuration 4. It was also observed that O^- becomes the major negative ion for source configuration 1 at higher discharge pressures. These differences observed for the various source configurations are presumably due to the fact that ions are sampled from somewhat different regions of the discharge with these different arrangements. For example, with configuration 1, ions are sampled primarily from the positive column region of the discharge, because the sampling slit is located far away from the cathode. The experimental results in terms of ion composition in this case agree qualitatively with the results of recent calculations based on a numerical simulation of the positive column plasma². On the other hand, with source configuration 4, the ions sampled originate principally from the negative glow region of the discharge, in which the electron energy is much higher than in other regions of the discharge. In this region, the electron attachment reaction, $O_3 + e \rightarrow O_2^- + O$ is apparently enhanced because of the higher electron energy.

The HCD ion source appears to offer considerable versatility in terms of its ability to generate various types of ions, which can be achieved by varying the discharge parameters and the configuration of the source.

ACKNOWLEDGMENT

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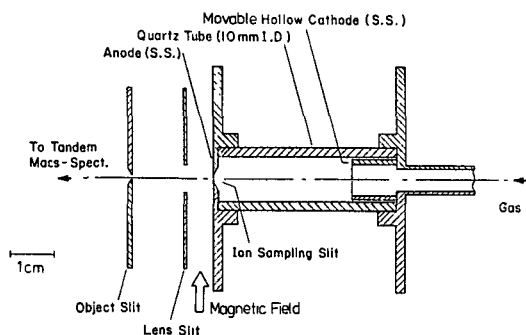


Figure 1. Schematic diagram of hollow cathode discharge ion source.

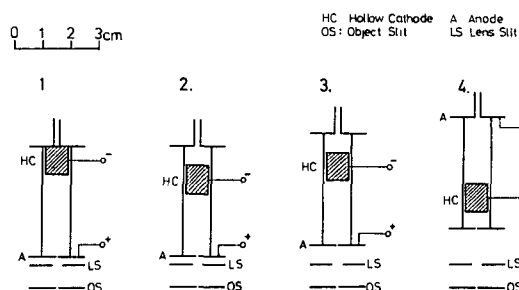


Figure 2. Four different configurations of discharge source.

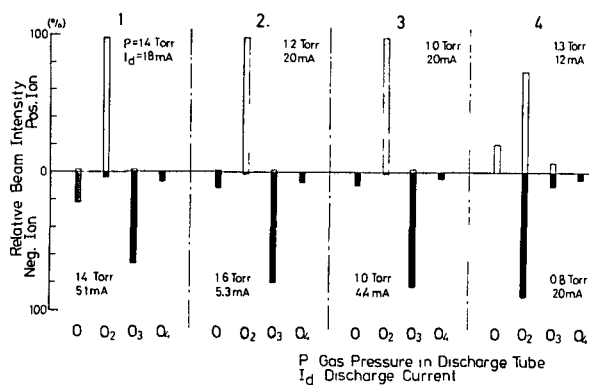


Figure 3. Comparison of relative ion beam intensities obtained with four different source configurations for an oxygen discharge.

MOLECULAR SYNTHESIS BY MASS SPECTROMETRY

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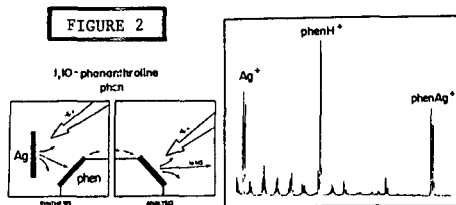
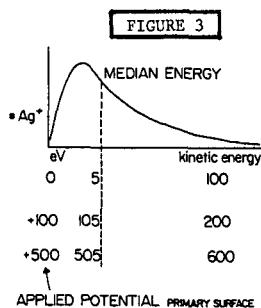
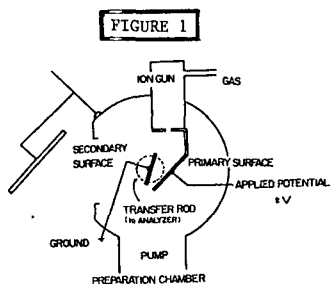
An ion gun has been installed in the preparation chamber of a commercial SIMS spectrometer. The ion gun is modified by addition of an insulated extension which comprises the replaceable primary surface. A 3 keV beam of argon ions bombards this primary surface, which consists either of a metal foil, or a graphite foil onto which an organic compound has been burnished. A primary ion flux of about 1×10^{-6} A is focussed into a spot size on the primary surface of 2 mm diameter; the angle of incidence is 45 degrees. The primary surface can be biased to several thousand volts. The secondary surface is the original sample stage of the instrument, held at the end of a magnetic transfer rod, which allows transfer of the sample under vacuum into the main chamber of the SIMS instrument for analysis via bombardment with a second beam of primary ions. This secondary surface is held in the chamber 2 cm away from the primary surface, and at a relative angle of 30 degrees (Figure 1). Transfer of material from the primary to the secondary surface can be remarkably efficient. With a primary beam of 10^{-6} A on a silver foil, a measured ion current of 10^{-7} A can be measured by a picoammeter attached to the secondary surface. Under such conditions, the transfer rate of silver ions is approximately 3 ng per minute. Complete coverage of the secondary surface with the transferred species can be obtained during preparation times of less than one hour. Analysis of the secondary surface takes place under static SIMS conditions. For primary surfaces of organic materials, the bombarding current must in some cases be reduced to avoid damage to neat samples, or the organic material is prepared in solution (as in FAB) or in a matrix such as ammonium chloride which increases both the absolute and relative intensities of the ions of interest. Reduction in primary ion current lengthens the time required to modify the secondary surface, but the demonstrated subnanogram sensitivity of SIMS permits analysis of surfaces prepared in reasonable times.

Both inorganic and organic species have been transferred from the primary to the secondary surface. Metal ions such as Fe^+ and Ag^+ are transferred with high efficiency. Figure 2 shows that silver ion transferred to a secondary surface prepared with 1,10-phenanthroline reacts to form the Agphenanthroline (Agphen) complex observed in the SIMS spectrum with good S/N ratio. Total preparation time for this sample was one hour. Time resolved data which follow the relative abundances of the Ag^+ and the Agphen $^+$ ions with time of analysis show changes which can be interpreted in terms of formation of layered structures on the secondary surface, consisting of a layer of Ag^+ under which is found Agphen $^+$, and finally unreacted phenanthroline. Different SIMS spectra are recorded from secondary surfaces exposed to sputtered Cu^+ dimethylphenanthroline, Cl_2 (CuDMP^+ , Cl_2). When the secondary surface is silver foil, the CuDMP^+ ion is observed, but no Cl_2 reaction with the silver is evident. Under the same conditions, a secondary surface of silver nitrate reacts to form AgDMP^+ .

The ability to apply a potential to the primary surface makes it possible to vary the kinetic energy of the ion species transferred to the secondary surface. This is accomplished by shifting the normal kinetic energy distribution of the sputtered species by an amount equal to the applied potential, as illustrated in Figure 3 for an approximate distribution of energies of sputtered silver ions, modified by imposition of positive potentials to the primary surface of 100 or 500 V. The energy distribution of sputtered organic ions is narrower than that of atomic species, and thus the shifted distribution can be expected to be narrow even without energy analysis of the transferred beam. Changes in the relative abundances of Ag^+ , Hphen^+ , and Agphen^+ with applied voltage have been noted; the data suggests that silver ions accelerated by an applied potential of 300 V react more readily to form Agphen $^+$ than do those transferred at a lower kinetic energy.

Proximity of the primary and secondary surfaces increases the collection efficiency for sputtered ions. However, more sophisticated experiments will involve a second generation soft landing instrument which permits mass and energy analysis of the transferred ions, and removal of line of sight between the surfaces to prevent the transfer of neutrals. A hybrid sector/quadrupole mass spectrometer has already been used in experiments of this type using Fe^+ transferred to a 1,10-phenanthroline surface. Construction of the second generation instrument is underway. The primary advantage of instruments which transfer material from surface to surface is the ability to use the unique ions (cluster ions, cationized organics, and atomics) created directly from the condensed phase in a controlled synthetic process. This ability is complemented by the use of the sensitive surface technique of SIMS for the subsequent analysis. Mass analysis of the transferred species results in a stoichiometric tailoring of the secondary surface, as well as a precisely controlled isotopic enrichment or depletion. Implications for both mechanistic studies of the sputtering process itself, and practical applications in heterogeneous catalysis are evident.

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A CONVERSION DYNODE SYSTEM FOR THE DETECTION OF POSITIVE AND NEGATIVE IONS

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Detection of negative ions with a conventional secondary electron multiplier (SEM) at increasing gain is a problem due to retardation of the negative ions. This is especially a problem when using fast atom bombardment (FAB) ionization. A conversion dynode eliminates many of these problems by providing optimal conditions for the detection of the negative ions. A conversion dynode modification has been developed for the MAT 311A and 731 mass spectrometers in this laboratory. The systems are identical except for the model of the SEM. The results for the 311A will be reported.

Beuhler¹ and Friedman have suggested the use of aluminum as a surface for conversion dynodes. A piece of aluminum foil approximately 0.25 mm thick was cut to fit by friction over the first dynode of the SEM. The first resistor in the SEM chain was removed and the connections to the SEM were modified. A new high voltage connector was built for the first dynode incorporating both the first resistor for the SEM chain and a cable to the second dynode for positive ion detection. In the negative ion detection mode the first dynode is biased positive with an external power supply and the second dynode is connected directly to the 311A SEM supply.

The performance of the conversion dynode was measured using positive and negative ions in the FAB spectrum of glycerol. The modified system provided a factor of nine increase in gain for the m/z 91⁻ FAB peak of glycerol compared to the connection used for positive ions. There was no measurable decrease in the positive ion detection mode performance and the switch between the two connections takes about 10 seconds to perform.

This work supported by NIH grant GM 27029.

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OPTIMIZATION OF EXPERIMENTAL CONDITIONS FOR THE DETECTION OF LABELED CARBON BY CAPILLARY GC/MS

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Previously, we reported on a chemical reaction interface for capillary gc-ms utilizing a low pressure microwave induced plasma to convert carbon containing compounds to simple di-or triatomic species for detection of labeled carbon (1,2). We saw reaction with oxygen to form CO and CO₂ or with H₂ to form predominantly CH₄. Two systems have been compared to determine their possible advantages for the detection of labeled carbon. One is a unit resolution quadrupole mass filter detecting the reaction products with oxygen as the reagent gas. The other is a high resolution double focusing magnetic sector instrument measuring the products with hydrogen as reagent gas.

Experimental

Low resolution system: A Varian 3700 gc was interfaced to a Finnigan 1015/Extranuclear SpectrEl mass filter with a chemical ionization pumping system. The quadrupole analyzer was modified to effect a differentially pumped electron impact ion source and quadrupole assembly. The Bowman microwave cavity was of a split construction to permit prior assembly of the quartz interface line with rigid glass-to-metal seals and a metal compression bellows with helium leak-tested nickel gasket seals. The microwave cavity is assembled around the quartz interface line and tuned via adjustable threaded end-caps for maximum power transmission and auto-ignition. The cavity was powered by an Ophos MPG-4 power supply producing 10-20 watts at 2.45 Ghz. The microwave cavity interface assembly is supported on a vacuum flange, and encased in a heater jacket oven. Wide bore, thin-film fused silica capillary columns were used and extend from the injector area directly into the region of discharge. Oxygen reagent gas is metered through a tee-assembly and flows around the 17-20 ion section of column in the interface. The total system is leak tested to preclude air background interference, and high purity helium and oxygen transmitted through stainless steel lines are used for all experiments. Either a solventless dropping needle type or direct injector are used, depending upon the volatility of the material to be analyzed. During direct injection, the ion source filament is turned off while the solvent elutes, although volumes of 0.2 ul do not extinguish the discharge.

High resolution system: A Tracor Microtek MT-220 gc was interfaced to a DuPont 21-492 high resolution double focusing magnetic sector mass spectrometer equipped with a chemical ionization pumping system and an electron impact ion source. A Bowman microwave cavity is mounted on the gc oven wall and a heated stainless steel transfer line used to transmit combustion products into the ion source.

Results

Low resolution oxygen discharge: The system is useful for the detection and analysis of carbon labeled compounds in complex mixtures as previously demonstrated (1,2). Excellent chromatographic resolution is maintained through the interface compatible with the performance of capillary columns. Total carbon is detectable at the low nanogram level and labeled carbon at approximately 1000 dpm. Sensitivity enhancement expected for going from carbon to oxides of carbon is less than anticipated due to instrument carbon background preventing the use of high multiplier gain and maximum recording sensitivity. Use of oxygen as a reagent gas (0.2 ml/min) has not had a detrimental effect upon filament lifetime. Use of ¹⁸O₂ as a reagent gas demonstrated that most carbon background is evolved from instrument materials.

High resolution - hydrogen discharge: Separation of background interferences by moderate resolving power (1000-2000) facilitates high sensitivity detection of labeled carbon. For example, at m/z 18, ¹⁴CH₄ (18.0345) is readily resolved from H₂O

(18.0106). The hydrogen discharge offers both advantages and disadvantages. Hydrogen is a convenient carrier gas for capillary work, removing the need for a second gas in the system, and utilizing a benign gas for instrument operation and lifetime. However, discharge chemistry with hydrogen produces several products (CH_4 , C_2H_2 , and C_2H_4). Traces of nitrogen in the system produce HCN from CH_4 ; high pressure in the ion source leads to H_3^+ which reacts to produce CH_2N^+ . Detection limits for labeled carbon are similar to those for the low resolution oxygen discharge - sub nanogram for ^{13}C and 1000 dpm for ^{14}C .

Conclusion

Either system offers a useful method of carbon nuclide detection for gc-ms studies of labeled compounds in complex mixtures with excellent chromatographic integrity. High resolution mass analysis is required for hydrogen discharge work to eliminate nominal mass interferences from oxygen or nitrogen species. For a specific laboratory, the instrumentation choice should be based upon availability rather than any clear advantage of discharge chemistry.

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2. S.P. Markey and F.P. Abramson, Anal. Chem. 54, 2375, 1982.

THE ROUTINE DETERMINATION OF MASS TO 0.1 PPM. J.C.Bill, B.N. Green, P. Kershaw and N. Lynaugh., VG Analytical Limited, Tudor Road, Altrincham, Cheshire, England.

Introduction

The precise measurement of mass as a means for determining the elemental composition of organic compounds has been an important use of mass spectrometry for many years. Many developments, particularly the application of data systems, have tended to produce more comprehensive and accurate data. Nevertheless, routine measurements on commercial instruments do not often show better than 1 ppm accuracy, a value insufficiently exact to give an unambiguous composition in many cases.

Ion statistics and instrument stability are well known factors which limit the accuracy. By making maximum use of the ions available, together with the employment of more stable instrumentation, an automated technique based on the well known peak matching method has been developed, which has the ability to achieve accuracies down to 0.1 ppm when internal reference masses are used.

Method

The technique is essentially an automated development of peak matching in which the mass to be measured is bracketed between two reference masses and the electric sector voltage/ion energy controlled by an 18 bit digital to analogue convertor (DAC). The voltage is jumped to one side of each peak in turn and then scanned through it. Centroids are used to estimate the electric sector voltage at which each peak occurs and hence calculate the mass to be measured. In this way, the subjectivity usually associated with visual peak matching is avoided and the accuracy may be improved significantly by averaging. In order to obtain the required linearity, the full 18 bits of the DAC change the electric sector voltage by only 1% to 5% in 0.04 - 0.1 ppm steps.

Results

To assess the technique, various masses were measured at 25,000 resolution on a VG MM ZAB at a rate such that one mass measurement (3 peaks) was made every second. Thus, ion statistical errors remained relatively small and the 25-100 measurements needed to reduce random errors to 0.1 ppm could be made in a reasonably short experiment time.

Table 1 shows typical data from 20 sets of 10 measurements each of the $C_3^{13}C^{35}C_1^{37}$ ion using the $C_3^{13}C_1^{37}$, C_1^{37} and $C_4^{35}C_1^{37}$ ions as references. The total data acquisition time was about 3 minutes. The results show that accuracies of about half a ppm are achievable on 10 measurements and approach 0.1 ppm on 200 measurements.

Table 2 summarises the results of 4 sets, each of 100 measurements of the masses of 2 ions. Both sets of data show overall errors of about 0.1 ppm.

TABLE 1. Measurement of $C_3^{13}C^{35}Cl_4^{37}Cl$ (225.844668) using $C_4^{35}Cl_4^{37}Cl$ (224.841313) and $C_4^{35}Cl_3^{37}Cl_2$ (226.838363) as references.

Mean Error (ppm) (10 measurements/result)	
-0.19	
0.85	
0.10	
0.28	
-0.59	Mean (1st set 10 results) = 0.06 ± 0.15
0.19	
0.80	
-0.41	
-0.13	
-0.33	
-0.08	
0.54	
0.03	
0.09	
-0.04	Mean (2nd set 10 results) = 0.12 ± 0.09
0.11	
-0.37	
0.12	
0.02	
0.68	
Overall mean =	0.09 ± 0.09

TABLE 2. Results of 400 measurements on 2 masses

Reference Masses	Measured Mass	Error (ppm)
C_4Cl_6 (257.813116)	$C_3^{13}C^{35}Cl_6$ (258.816471)	0.15 ± 0.10
$C_4Cl_5^{37}Cl$ (259.810166)		0.16 ± 0.16
		-0.19 ± 0.11
		0.08 ± 0.11
	Overall mean	+0.05 ± 0.16
$C_4^{35}Cl_4^{37}Cl$ (224.841313)	$C_3^{13}C^{35}Cl_4^{37}Cl$ (225.844668)	0.06 ± 0.15
		0.12 ± 0.09
$C_4^{35}Cl_3^{37}Cl_2$ (226.838363)		0.01 ± 0.09
		0.03 ± 0.14
	Overall mean	+0.06 ± 0.05

VALUES OF α , β , γ FUNCTIONS FOR THE
DESIGN OF QUADRUPOLE FILTERS OR TRAPS

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Trajectories in quadrupole mass filters or traps are governed by Hill type differential equation which has the form

$$\frac{d^2x}{dt^2} + F(t)x = 0$$

where $F(t)$ is a periodic function of the time. The simplest case is the well known Mathieu equation where:

$$F(t) = a + 2q \cos \omega t$$

It can be shown [1-3] that the solution of Hill type equation has the form

$$x = \beta^{\frac{1}{2}}(t) \cos(\psi(t) + \delta)$$

where

$$\psi(t) = \frac{d\tau}{\beta(\tau)}$$

β function can be evaluated by calculating two independent trajectories over one cycle. The knowledge β function and the associated α and γ ones enable the calculation of many properties of the trajectories such as maximum overall extension, maximum and minimum velocities. More than that, the position and velocity of any charge particle can be evaluated at any time very easily and precisely when these functions are known. We show on the next page five graphs of typical parameters associated with these functions: $\beta_{\max}$, $\beta_{\min}$, α , $\gamma_{\max}$. Since β function can be expressed without too much error by the expression

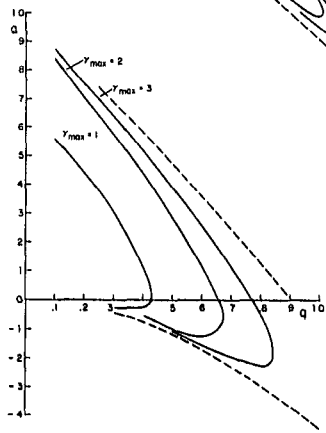
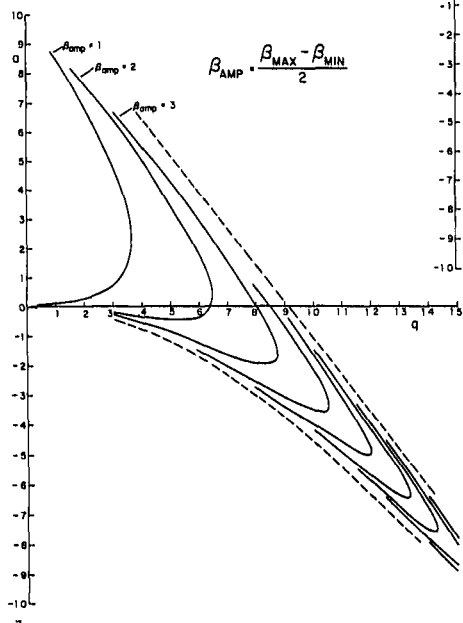
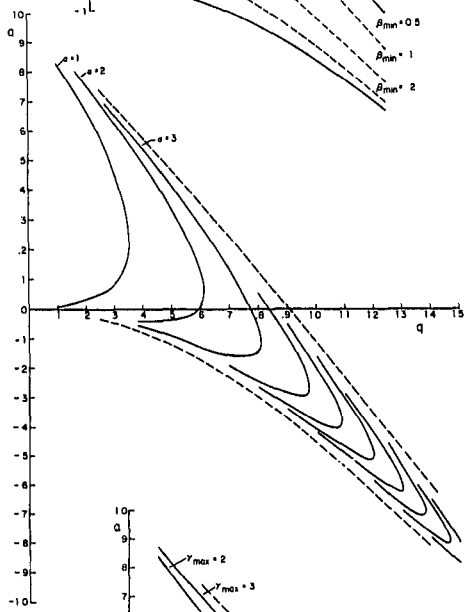
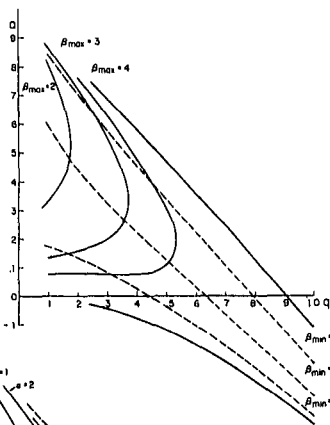
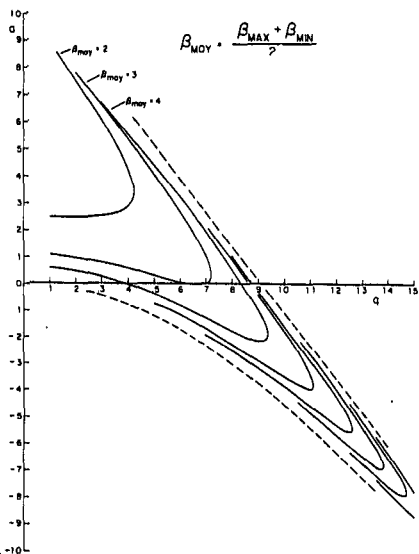
$$\beta(\theta) = \beta_{\text{moy}} + \beta_{\text{amp}} \cos \theta$$

we have also included graphs of the two parameters β_{moy} and β_{amp} which are defined by the following equations:

$$\beta_{\text{moy}} = (\beta_{\max} + \beta_{\min})/2$$

$$\beta_{\text{amp}} = (\beta_{\max} - \beta_{\min})/2$$

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- [3] BARIL, M., Dynamic Mass Spectrometry, Vol. 6, p. 33-43 (1981), (Heyder & Son).



WPA 12 | A SIMPLE HEATING CURRENT PROGRAMMER FOR A
MASS SPECTROMETER SAMPLE PROBE USING A GAS CHROMATOGRAPH
TEMPERATURE PROGRAMMER MODULE. F.A. Bencsath, and Y.T. Ng,
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An inexpensive device was designed for programming the heating current of a mass spectrometer probe during an MS run. Temperature programming is highly desirable in many measurements, especially for multicomponent mixtures and for achieving uniformity desirable in comparing served samples.

We worked with our DuPont Model 21-492 magnetic sector instrument. We inserted a silicon controlled rectifier circuit [1] in the line providing power for the probe heater. Appropriate insulation precautions were taken. The circuit consist of only few components, among which is a potentiometer to regulate the output current of the unit. This potentiometer is driven by a motor which is controlled by the power supply of the temperature controller in the Varian Model 1400 gas chromatograph which is part of the GC/MS combination. Thus the programming speed and the temperature gradient is regulated at the GC module. A manual over-ride of the circuit is provided.

The total cost of the components is on the order of \$100. The unit has operated satisfactorily for six months to date.

Ref.: 1. S. Ciarcia, D.C. Motor Controls, BYTE May 1981 pp 76-80.

AN ION COUNTING SYSTEM

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An ion-counting system has been built and installed on a Kratos MS-50 double-focussing mass spectrometer. The purpose for developing such a system is the improvement of the S/N ratio for weak signals where other sources of noise besides quantum noise become important. The improvement in the S/N ratio would facilitate ultra-trace picogram analysis and isotope ratio analysis at high resolution ($>30,000$), the latter having the advantages of separating ions of the same nominal mass and resolving ambiguities that arise from $M-H^+$ and $M-D^+$ ions and the assumption that rates of H and D loss are similar. Since most of the components of the ion-counting system are transferable, future applications include low-level high-mass studies and MS/MS metastable and collisionally-activated dissociation applications on the Kratos MS-50TA.

Improvements to the S/N ratio of weak signals as afforded by ion-counting are: (1) elimination of the noise due to gain fluctuations in any multiplying detector, (2) discrimination against dark current and other similar secondary events, (3) discrimination against large scale single events, eg. cosmic rays, and in the case of a sampling data system, (4) elimination of the noise due to the random interval between ion pulses and sampling, and (5) increase of sampling duty cycle since ion counting is an integrating procedure.

Important design goals for the system were: (1) modifications and additions to be transparent to the normal operation of the Kratos MS-50, (2) continued use of the Daly detector which has long-term gain stability which has been important in ultra-trace analysis, and (3) operation with peripherals to the MS-50: (a) a custom-built computer-interfaced MID, and (b) the Nova 4X-based data system.

The implementation of these goals into an ion-counting system is illustrated in the Figure. The Kratos Collector Preamplifier was modified by (1) adding LC filters to the power, controls, and analog signal-output lines to eliminate high-frequency feedback paths and by (2) switching by reed relays the output from the photomultiplier anode of the Daly detector between the standard preamplifier and the high-speed pulse preamplifier. Additions to the detector electronics include (1) an Ortec 9301 high-speed pulse preamplifier mounted on the detector, (2) a 3-pole Pi-section low-pass filter with cutoff frequency of .5 MHz, (3) an Ortec 9302 high-speed pulse amplifier-discriminator, and (4) a custom-built unit to perform as (1) pulse-pair separator to separate pulses by >100 nsec, (2) pulse translator to drive TTL logic, (3) analog signal synthesizer to generate a signal for scope display, and (4) controller to manage all functions necessary to activate ion counting. A custom-built computer-interfaced MID (1) originates all control signals for peak switching and ion counting, (2) controls selection of mass ratio, sweep time, and in the analog mode, signal gain and filter frequency, and (3) routes pulse train to a 10 MHz data counter in the interface. Data processing is accomplished on a Data General Nova 4X which is part of the Kratos DS-55 system.

The ion-counting system is programmed to operate in two basic modes. In the ultra-trace analysis mode, the MID, whether operated either stand-alone or computer-controlled, assigns equal sweep times to all channels and data is collected under these conditions. In the ratio analysis mode, the MID is computer-driven and first, a "scout" data collection is performed with equal sweep times per channel and the intensity or # of counts of each peak is determined, then the sweep times are programmed such that sweep time is proportional to $(\text{intensity})^{-1/2}$ or $(\# \text{ of counts})^{-1/2}$ for each channel, then collection is performed.

In using the Kratos Daly detector, a serious problem was discovered.

WPA 14 ELECTRICAL LEAKAGE IN ION-SOURCE INSULATORS:
A SUBTLE PROBLEM AND A SIMPLE SOLUTION. Wendy G. Linn and
Thomas J. Eskew, Nuclide Corp., State College, PA 16801

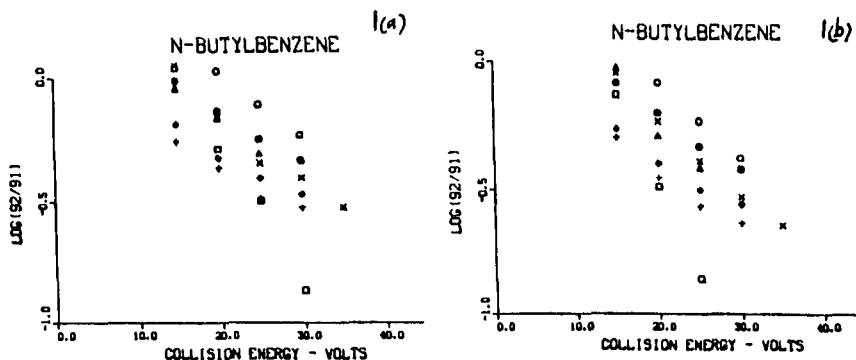
The electrical leakage across ion-source insulators is not proportional to the applied voltage: in voltage-scanned mass spectrometers (e.g. CEC 21-103, 21-104 etc.) we have found that this leakage causes several problems. Because the potentials for the repellers, the ionization chamber, and the focus electrodes are usually obtained through a resistive voltage divider (a high-impedance source) the leakage non-proportionality will cause the source electrode potentials to be non-proportional to the ion acceleration potential and to each other as the IAP is scanned. Some consequences are as follows: 1: a simple two-point mass vs HV calibration will cause errors in mass assignment. For example, in a CEC 21-104 with moderately dirty insulators, we measured $<1\mu\text{A}$ at 500V, $8\mu\text{A}$ at 1000V, $28\mu\text{A}$ at 2000V, and $100\mu\text{A}$ at 2,800V. With mass 69 set at 3000V and mass 269 at 769.52V, the error at mass 169 exceeded 2 amu. 2: Due to residue buildup, the relative sensitivity of high vs low masses will gradually deviate over time from that measured when the instrument was calibrated using standard mixtures. 3: A bias will be introduced into peak-intensity measurements when using a data system which assumes a constant time-width of peaks (i.e., precisely exponential scan).⁽¹⁾ We have designed a circuit, using a recently available high-voltage op-amp, which drives the critical source electrodes from a low-impedance source so that the leakage currents do not measurably alter the ion-source's behavior. The details of the circuit are described. We are now obtaining data to determine the length of time that instruments can be operated between insulator replacements with the old vs new circuits.

(1) T.J. Eskew, "Minimum-noise Data Acquisition with Variable Scan Rate: A Digital-Filtering Method." Paper presented at the 29th Conference on Mass Spectrometry, June 1981.

A Round-Robin Test of Reproducibility of Operating Conditions for Generating MS/MS Libraries Using Triple Quadrupoles

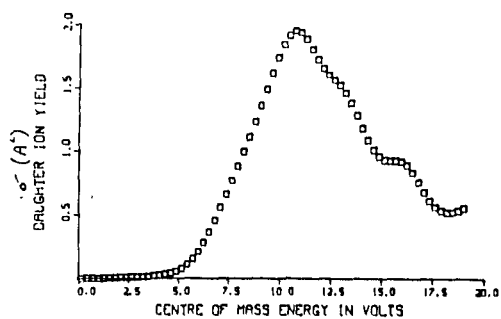
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Following the 1982 ASMS Workshop on standardised operating conditions for generating MS/MS libraries with triple quadrupoles, a round robin test of the reproducibility of conditions between different laboratories and different types of quadrupoles was undertaken. Of the 20 laboratories requested to take part, seven responded and two types of instrumentation are included (the crossed collision jet configuration used by the Sciex Taga 6000 and the closed collision cell configuration used in the Finnigan TSQ). The two parent ions investigated were n-butylbenzene ($C_{10}H_{14}^+$) and protonated dimethylphthalate ($C_{10}H_{11}O_4^+$). These ions have some useful characteristics. For example with $C_{10}H_{14}^+$, the $105^+/91^+$ and $78^+/91^+$ daughter ion ratios are not very dependent on collision conditions whereas the $92^+/91^+$ ratio depends on collision energy but not very much on collision gas density and the $65^+/91^+$ ratio is a very strong function of collision gas density. Participants were required to set the collision energy at 25 eV, to add sufficient collision gas (argon and Nitrogen) to reduce the parent intensity to (a) 1/3 or (b) 1/10 of its original value and then, in each case, to record the spectra as a function of collision energy. Fig. 1 shows the data for the $92^+/91^+$ ratio from n-butylbenzene at the two collision gas densities. The open points represent one type of instrument (crossed jet), the filled points the other (closed cell). Fig. 2 shows data for the $78^+/91^+$ ratio with excellent agreement except for one laboratory. The $65^+/91^+$ ratio, however, showed wide divergences from one laboratory to another as illustrated in Fig. 3. The conclusions were as follows (a) when collision conditions were not critical, good agreement was obtained even with different types of instruments (b) the collision energy was fairly well defined but a better method of verifying its value might give more precise agreement (c) the collision gas density was not adequately defined. This was very evident in comparison of the two types of instruments but even also between instruments of the closed collision cell configuration. This was presumably due to different degrees of parent ion loss from the system by gas scattering. A revised procedure for standardising operating conditions is being proposed. This uses a fixed target density for all parent ions obtained by adjusting the $65^+/91^+$ ratio of daughters from amylbenzene to 2%. The collision energy is arrived at by using the $92^+/91^+$ ratio from the same parent. MS/MS spectra of amylbenzene taken in several different laboratories (equipped with SCIEX TAGA 6000 systems) using this standardisation procedure are shown in fig. 4. A number of different compounds have been tested and the procedure seems quite promising.



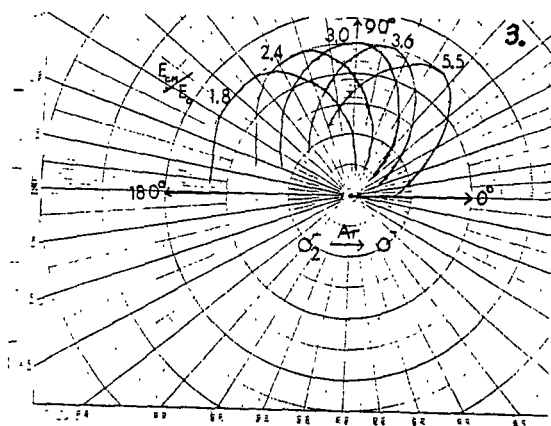
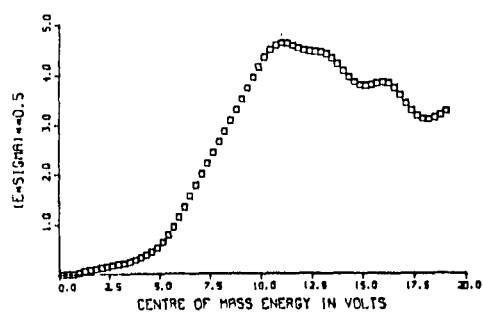
O₂MINUS DISSOCIATION

1.



O₂MINUS DISSOCIATION

2.



DATA ACQUISITION SYSTEM FOR SLOW SCANNING MASS SPECTROMETERS

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Slow scanning mass spectrometers (MS), such as the CEC-103, are still widely used for quantitative organic analyses in the petroleum industry. Until recently, an IBM Model 1800 mainframe computer was used to collect data and compute a mass spectrum from our CEC-103 MS. The computed spectrum would subsequently be transferred to a second mainframe computer for calculation. Commercial MS data systems are designed for high sampling rates, in excess of 40,000 points per second, and thus are underutilized if connected to a slow scanning system. Powerful, low cost personal computers make possible the implementation of low cost, full function data acquisition systems compatible with these slow scanning MS systems.

A data acquisition system for slow scanning MS using personal computers can be designed in at least two ways. A single personal computer can perform all of the control and data processing functions or a dual personal computer system can use one personal computer that is dedicated to instrument control and the other personal computer is dedicated to data processing functions. The former has the advantage of lowest initial cost while the latter has the advantage of greatest throughput. In our laboratory we chose to follow the latter path.

The design we implemented uses a Rockwell AIM-65 as an intelligent interface to control the CEC-103 and collect its data and a Hewlett-Packard (HP) Model 87XM as the host computer. In order to minimize changeover problems between the new design and our previous system, we were constrained to (1) scan the accelerating voltage and (2) use peak intensity maxima for our calculations. Thus, we programmed the interface to act as a "peak picker." The interface, when commanded to, will initiate a scan and store the peak data according to user-selectable parameters. Following completion of a scan, the peak data (intensity and accelerating voltage) and the magnetic field is transmitted to the host computer and a new scan may be started.

The host computer was designed for ease of use. The HP 87XM is well-suited to this need with a set of 14 programmable, special function keys as well as the ability to mask out the function of any key. In all segments of the software, each of the special function keys are labeled and a brief description of their function is displayed. The software was designed in modular, menu-driven form for greatest flexibility. The main program module permits user access to all of the other modules. In addition, this module has a "HELP" function which provides the user with extensive information concerning the operation of the data acquisition system. The main program also includes a set of utility routines to handle disk access functions such as

storing data files, loading previously stored data files, disk initialization, etc. One program module controls the interface. Its functions include setting the parameters that define when a peak is found, initiating a scan of the accelerating voltage, and transmitting the peak data from the interface to the host computer. A separate program module manipulates collected data; e.g., display the mass intensity pairs, plot the spectrum, modify the mass scale if needed, or compute standard tuning parameters. Another module, in conjunction with the modem, turns the host computer into an intelligent terminal for the Company Timeshare computer system. This permits data files, programs, and other information to be transmitted between the data acquisition system and a mainframe computer. Yet another program module allows the user to process the collected data via standard calculations. For convenience, the calculations selected were the "high volume" analyses accounting for about 90% of the work load for this MS. Finally, a full set of diagnostic procedures has been built into a separate module. Transfer between all of these modules is accomplished by use of the appropriate special function keys.

The programming of the host computer is entirely in BASIC to permit easy implementation of new functions and correction of problems. The programming of the host computer and the interface computer was complete within about three man-weeks exclusive of the calculation programs.

The interface personal computer was programmed in machine language to obtain sampling rates of up to 1000 points per second. In addition to operation as a "peak picker," the interface will also acquire profile data. The latter is used primarily for diagnostic operation.

In summary, a data acquisition system for a slow scanning MS has been successfully implemented. The hardware cost for the two personal computer designs used was about \$10,000, with about three man-weeks of effort to develop and implement the necessary software. This system has been in use since December 1982 and has doubled the sample throughput of our CEC-103 MS compared to the previous data acquisition system.

AUTOMATED PROCESSING OF LARGE QUANTITIES OF GC/MS DATA

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This report discusses the Mass Spectral Information System (MSIS)^{1,2}, an aid to chemists in the interpretation and management of GC/MS data. The system is uniquely suited for a GC/MS facility where large quantities of GC/MS data or complex mixtures are analyzed on a routine basis.

In the MSIS, the procedure for analyzing a GC/MS sample consists of several steps. The initial steps are designed to require a minimum of operator intervention and include: the conversion of raw GC/MS data from diverse commercial data systems to a MSIS format; the extraction of pure component mass spectra free from background noise, solvent effects, and interferences from neighboring elutants; and the comparison of those spectra with the spectra from NIST/EPA Mass Spectral Database using the PBM method. In addition these mass spectra are further compared with a special library containing the mass spectra of previously unidentified compounds. This allows one to note the repeated detection of unidentified components. The results of the mass spectral library search are then compared against a library of relative retention time (RRT) values, which is organized according to the internal standard and the GC column. If a library compound matches the unknown sufficiently well in terms of both spectrum and RRT, then the MSIS assigns an identification to the unknown. The criteria used for an automatic identification are that the ratio of the similarity index to the maximum similarity index from the PBM search must be greater than 0.44 and that the observed RRT of that component be within 0.06 of the RRT from the historical RRT data. In practice, we have found that 20% of the components in a diverse series of 800 GC/MS runs have been automatically identified.

Following these initial steps, the need for human interaction becomes important and the MSIS is designed to take this into account in the SELECT step. SELECT permits interactive manipulation of GC/MS data and provides many helpful features to aid in the review of the automatically identified components and in the identification of other chemical components not selected by the PBM and RRT criteria. Facilities are provided for manual extraction of the mass spectra by background subtraction. Library spectra may also be subtracted from the raw or pure spectra. In addition, spectra from different GC/MS runs may be compared. A large number of display options are available including the display of reconstructed gas chromatograms (RGC), selected ion chromatograms, library mass spectra, and mass spectra of the previously unidentified compounds. Routines are also available to predict the molecular weight of a compound according to the method of Dromey et al,³ and to determine whether or not chlorine is present in a compound according to the method of Meisel et al⁴. The RRT library may be searched to list all compounds occurring in a specific RRT window (for a particular GC column and internal standard), or RRT values on different columns for a particular compound. Displays containing the component spectra and the corresponding library search results are provided automatically. At any point a summary of the current status of a run (the detected components, the components identified, and the components for which no action has yet been taken) may be displayed. These and other analysis tools allow the analyst great flexibility in the interpretation of the GC/MS runs.

An important feature in the MSIS is the capability for several chemists to perform simultaneous, but independent, analyses on different GC/MS runs stored in the system. It is also possible for several chemists to access the system simultaneously to examine the same run. In this way, personnel can be allocated in the most efficient manner to get the required analyses completed. The system has an additional advantage in that it automatically checks for consistency and for completeness in the analysis results if they are performed by multiple users. The MSIS also contains report generation functions with reports delivered either to an offline printer or an interactive terminal. Reports can be made of all the components detected in a specific GC/MS run or at a specific location, including

the chemical formula, name, and CAS Registry Number of all compounds. If relative response factors are known, relative concentrations of all compounds can also be reported. Alternatively, *statistics* can be compiled on the frequency of occurrence of each compound in a series of GC/MS runs and reported either by the number of occurrences or by the CAS numbers of the compounds.

Finally, as analyses are completed, the data can be archived on magnetic tapes. The MSIS stores the location of the data so that regardless of whether the data is stored online or is in archival storage, it can be accessed simply by entering the run name.

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THE NIH/EPA MASS SPECTRAL DATABASE SEARCH SYSTEM: IMPROVED DATA COLLECTION PROCEDURES

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The NIH/EPA Mass Spectral Search System (MSSS) is one of the components of the NIH/EPA Chemical Information System (CIS). The CIS is a collection of various types of chemical data which may be searched and retrieved using the commands of the system. In addition to mass spectral data, both EI and CI, the CIS contains C-13 NMR, IR, and crystallographic data. There are also a number of databases in the CIS which contain toxicity values, spill response information, Federal Register citations, and chemical structure and nomenclature. The CIS, including MSSS, is available on a commercial computer which may be accessed via both the TYMNET and TELENET networks.

MSSS consists of a set of programs to search databases of EI spectra, CI spectra, and proton affinity/gas-phase basicity values. The EI database contains spectra of nearly 40,000 compounds taken from an archival file of over 70,000 spectra. The spectra for the public file are chosen on the basis of their quality index(1). Spectra are received from laboratories around the world and are used to update the database approximately once a year. The EPA, through its Cincinnati office and with the assistance of Radian Corporation, is attempting to add to the database spectra for 2,500 compounds from the Toxic Substances Control Act (TSCA) Inventory List and from the list of chemicals for priority testing for which mass spectral data are not available. The current database contains 1,600 spectra from that project which have been measured under very carefully controlled conditions. Each day, prior to measuring the spectra of any reference compounds, the instrument must be calibrated using the compound bis(pentafluorophenyl)phenylphosphine (CAS RN 5074-71-5). The spectrum of the calibration compound must meet the published criteria(2). Sample purity must also be carefully checked and where possible samples are introduced by GC. A recent study has shown that the quality indices of these spectra are considerably higher than the average quality index of the rest of the database(3). The quality index ranges from 0-1000, and is the product of nine individual quality factors. The average value for the entire library is 472. In addition, about 1,674 spectra received a quality index value of zero. In the data collected by Radian Corporation, the average quality index was 731, with only seven receiving quality index of zero. It is interesting to note that the reasons for a quality index of zero are due largely to the quality factors involving illogical losses and the lowest mass reported and these seem to have been addressed by the quality assurance criteria used here. The quality factor with the lowest average value is based on the number of peaks in the spectrum. The average for this factor has been raised slightly in the Radian spectra, from .75 in the database at large to .86, but is still significantly lower than the other quality factors. This suggests that the fairly simple relation theorized between the number of peaks expected in a spectrum and the number of atoms in a molecule is not completely adequate, and that a more complex treatment is actually needed.

In addition to being accessible through MSSS, the EI database is also available on magnetic tape from the National Bureau of Standards(4), and in printed form from the U.S. Government Printing Office(5).

The CI database contains approximately 1,500 spectra, most of which were obtained using methane as a reagent gas. An effort is currently underway to develop a quality index algorithm for CI spectra. The CI algorithm is based on the EI algorithm(1), but a number of changes are being made to account for the differences between the methods. For CI spectra, electron voltages greater than 70 eV are allowed, specific ions greater than the molecular ion are possible and are dependent on the reagent gas, and illogical losses are based on $(M+1)^+$ as well as on M^+ . In addition, the number of peaks in a CI spectrum should, in general, be less than for an EI spectrum.

The NBS file of proton affinity and gas-phase basicity values contains approximately 650 measurements for 420 compounds. Citations to the literature describing the measurements are also included.

The MSSS programs allow one to search the database in a number of ways to retrieve information. Compounds may be retrieved on the basis of molecular weight, molecular formula, and partial formula. Because of the capabilities of other components in the CIS, compounds may also be retrieved on the basis of chemical names, trade names, CAS Registry Number and structure. The mass spectrum may be used as the basis for an interactive peak search, in which compounds containing specific peaks with specific ranges are retrieved. The KB and PBM searches retrieve spectra similar to an unknown spectrum based on algorithms comparing abbreviated forms of the spectra.

Although the database of EI spectra contains many compounds, it is by no means complete. Therefore, a number of pattern recognition programs have been developed to provide certain information about a compound even when the library searches fail to provide any good matches. The MOLION program, written by Dromey and co-workers at Stanford University, determines likely molecular weights for a compound based on the spectrum. The MSTREE option, developed by Technology Service Corporation, uses a decision tree approach to determine the presence or absence of specific structural groups and assigns a probability to each determination(7). An examination of the entire database yields spectral features which are useful for distinguishing compounds containing a given structural group from those which do not. When an unknown spectrum is tested for these spectral features in an established sequence (following the paths of the decision tree), the compound is classified as either containing or not containing the structural group. While the generation of the decision tree is a time-consuming process, the actual analysis of an unknown spectrum is very fast. The program currently checks for chlorine, bromine, nitrogen and phenyl.

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A Vectored Architecture MS/MS Database Management System

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The quantity of data obtainable from routine MS/MS analysis, with its extended dimensionality, makes strong demands upon an automated storage and retrieval system. Even without the additional dimensions, exhaustive searches of extensive data bases are time-consuming, are inefficient in interactive modes and are often practical only with the storage of abbreviated spectra. A Multiple Vector Inverse Data Base Management (MVIDBM) system has been developed in order to provide a real-time data storage and retrieval system for MS/MS, as well as more conventional forms of mass spectrometry, including GC/MS.

The MVIDBM system is centered upon two types of files, archival and inverted. The archival data base file (Figures 1 and 2) is organized on a compound by compound basis, where each chemical species comprises a data set, and is identified by a unique registry number within the data base. The data set header contains descriptive information including the compound name, empirical formula, molecular weight, chromatographic parameters and structure. Relevant spectral data are divided into two classes, primary and secondary; the distinction is that some aspect of each secondary data set entry is found within the primary data set entry. For example: in most cases, the EI or CI mass spectrum is classified as primary data, while CAD (daughter) spectra are classified as secondary data. The required relationship is established from the fact that the parent ion of each daughter spectrum must be present in the primary spectrum for that compound. Several independent sets of secondary data may be maintained, perhaps indicating the pressure dependence (reaction order) or energy dependence of specific fragmentation pathways. A complete set of instrumental parameters is recorded with each spectrum, providing an archival record of the experimental conditions under which the data were obtained. It is essential to note that archival spectra are not abridged; this was necessary in order to (a) provide an unmodified experimental record, and (b) represent all possible parent peaks for CAD spectra.

The inverted file (Figure 3) contains linked streams of reverse indices (vectors) derived from the archival data base, each of which pertains to some relevant characteristic (e.g., primary or secondary spectrum peaks, empirical formula, molecular weight, retention time, etc.). Each vector stream is the subset of "hits" that would have resulted from the corresponding exhaustive search of the archival data base for that particular characteristic. By structuring these inverted records as the positive elements of a bit map spanning the entire data base, composite queries may be resolved as the set intersection (Boolean AND) or other logical combination of their component individual queries. Complex queries involving several inversion characteristics may therefore be resolved easily and rapidly.

The internal structure of MVIDBM was designed to be amenable to the needs of the mass spectrometry research laboratory (as opposed to, but not exclusive of, clinical applications). Routine "house-keeping" functions such as the addition of new compounds, the deletion of old ones, or the update of selected spectra are easily performed on the archival data base file. The corresponding inverted files are updated incrementally to reflect the changes in the archival data base; a complete re-inversion is not required.

Although the archival data base reflects the experimental history of a given chemical laboratory, individuals may have interests of considerably narrower scope. In these instances, any number of masks may be applied to the data base during inversion, thus generating application-specific inverted files. For example, indexing can be restricted to compounds containing specific hetero-atoms or falling within a range of molecular weights or retention times.

Using an "automatic" matching procedure, typical unknown spectra may be matched against the library (presently containing 20,000 compounds) in five to ten seconds. A "manual" mode is also available, through which the user may steer the program along individual retrieval and matching criteria. Although the "manual", or interactive, mode may take considerably longer than the "automatic" method, it provides the user with the capability of coupling specific chemical information into the matching process. For example, peaks which may be attributed to a known contaminant may be ignored, while those known to be characteristic of a given structural moiety may have their significance accentuated. Once the registry numbers of candidate matching spectra have been determined, the archival data are retrieved from the library at a rate of 100-200 msec. per spectrum for further analysis and/or graphical output.

The data base management software and retrieval primitives are written entirely in MACRO-11 assembly language, in order to maximize run time speed, minimize the task image size and take full advantage of the RSX-11M operating system on DEC PDP-11/ or LSI-11/ series computers. Higher level matching algorithms are written in FORTRAN-IV. Additionally, the retrieval primitives are structured as FORTRAN-compatible subroutines, allowing them to be easily incorporated into user-written programs with alternate "front-ends" and/or matching algorithms.

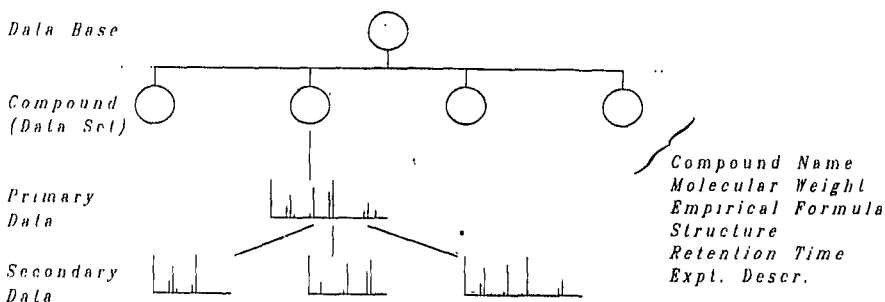


Figure 1

Master Header	
Data Base	Firmware
Global Data	
Directory	
Data Set Header - Reg. 1	
Compound Name	Molecular Weight
Empirical Formula	Structure Pointer
GC Column ID	Retention Time
1 ^o data pointer	2 ^o pointer 2 ^o pointer
2 ^o pointer 2 ^o pointer	2 ^o pointer etc
2 ^o pointer 2 ^o pointer	2 ^o pointer etc
Concatenated Data for Data Set 1	
Data Set Header - Reg. n	
1 ^o and 2 ^o data pointers	
Concatenated Data for Data Set n	
Concatenated Data for Data Set 1 (cont'd)	

Figure 2

Master Header
Directory
Linkage
Vector Stream "i"
Linkage
Vector Stream "k"
Linkage
Vector Stream "j"
Linkage
Stream "j" (cont'd)
Linkage
Vector Stream "l"
Linkage
Stream "i" (cont'd)

Figure 3.

IMPROVEMENTS TO COMPUTER ALGORITHMS FOR IDENTIFICATION OF UNKNOWN MASS SPECTRA; D. B. Stauffer, M. A. Sharaf, R. G. Dromey, C.-J. Guo, and F. W. McLafferty, Chemistry Dept., Cornell Univ., Ithaca, NY 14853

Improvements to the performance of the Probability Based Matching (PBM) and Self-Training Interpretive and Retrieval System (STIRS) algorithms for the identification and structural elucidation of unknown compounds based on their mass spectra have resulted from the increase in the size of the data base used, an extension of a technique already used in STIRS, and the implementation of a dynamic search strategy for PBM.

The 1982 expanded Cornell mass spectral data base contains 79,000 spectra of 67,000 different compounds. This includes 5,500 spectra from the NBS data base. In order to keep the time PBM requires to search the data base from doubling along with the number of spectra, the weighting used to order the data base for searching has been modified to reflect more closely the actual distribution of peaks in the collection. In addition, a criterion based on the highest mass peaks in the unknown and reference spectra has been used to speed the search dramatically for low molecular weight compounds. These modifications have resulted in a reduction of the amount of time required for a search. The recall/reliability plots used to measure the retrieval capabilities of PBM show a decrease in performance on the new data base. This results from the elimination of several thousand spectra that were, as measured by the PBM metric, identical. Correcting for this deletion shows that increasing the data base size has had little or no effect on PBM performance. This should not affect the performance of the algorithm on an actual unknown submitted by the user.

The STIRS algorithm can identify the presence of any of 600 preselected substructures in the unknown spectrum. This prediction ability is being expanded from 1 match factor to 18 match factors. Since each match factor concerns a different part of the spectrum, and different parts of the spectrum reveal different information about the structure, this expansion should result in more reliable substructure predictions. Since STIRS' ability to identify substructures depends on the presence of spectra of compounds with that substructure in the data base, the increase in the number of spectra has also resulted in improved substructure prediction capability.

A substantial modification to the search strategy for PBM is being made to increase its ability to distinguish between similar spectra of different compounds. After a normal PBM search of the database, the best matching spectra, usually numbering 10-100, are examined by the computer to find those peaks in this small subset that are statistically most significant for distinguishing between the spectra. The new mass weightings resulting from this peak selection are used to match the subset of reference spectra against the unknown again. Since the new mass weightings are derived from only those spectra that are similar to the unknown, peaks that are insignificant in the whole database, but important in the chosen subset, are selected for use in the matching. As a result, PBM becomes much better at discriminating against spectra that are largely the same as the unknown, but with important small differences.

WPA 21 | THE CREATION OF MASS SPECTRAL LIBRARIES
FOR QUADRUPOLE-BASED TANDEM MASS SPECTROMETERS

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The analysis of complex mixtures by tandem mass spectrometry has to date been mainly in the area of "target" compound analysis as opposed to the determination of unknown components in the mixture. This is not due to any inherent weaknesses in the MS/MS technique, but rather due to the fact that no extensive mass spectral data base exists for MS/MS instruments. Our laboratory is presently constructing a series of collision induced dissociation (CID) mass spectral libraries under a variety of instrumental conditions as a preliminary step in the development of a central data base for quadrupole-based (low collision energy) MS/MS instruments. Although collecting library spectra under different instrumental conditions (parent ion energy, collision target gas thickness) is time consuming, it does offer several advantages including the investigation of collision processes for several compound classes. The preferred instrumental conditions for the collection of library spectra based on the results of this study will be discussed.

A rudimentary artificial intelligence algorithm has been developed to search the data base for common neutral losses or the formation of common daughter ions upon fragmentation. This will aid the MS/MS user in obtaining functional group information on unknown compounds which are not present in a library. This algorithm will become more sophisticated as the fragmentation processes become better understood and as the data base grows.

Some aspects in library search methods for CID mass spectra and deconvolution techniques for overlapping spectra will be discussed.

WPA 25 DUAL PROCESSOR DATA SYSTEM FOR OPTIMIZED
INTERACTIVE GC/MS. L.A. Westover, K. Hollingshead, J.F.
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The instrument control and data collection functions of a quadrupole gas chromatograph/mass spectrometer have been separated from the data reduction, output, storage and transfer routines, with the use of a DEC PDP-11/03 dedicated processor interfaced directly to the instrument. The collected data from each scan are transferred via direct memory access to a DEC PDP-11/23 which serves as the host for the dual processor system. This arrangement provides maximum efficiency for operator interaction simultaneously with data collection. Using a single terminal for command and output simulate a background/foreground mode that lacks the time constraints of software versions. The resident executive program, RSX-11M manages the application programs of scan collection, calibration, SIM, and the various quantitation, library, graphics, and transfer routines. Additionally, the instrument is capable of alternate electron impact and chemical ionization scan modes. The resulting analysis can be observed in real-time or can be processed and output in either separated or interleaved formats.

DIALOGUES BETWEEN AN 'INCOS' DATA SYSTEM AND OTHER COMPUTERS: HOW AND WHY *

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Earlier extensions of our 'INCOS' system (1-4) provided for data transfers between the INCOS system and a 'VARIAN' 620/L-100 minicomputer used for such tasks as acquisition of photoplate-recorded data. Further, compatible extensions provide for communication with 'APPLE II Plus' or 'CROMEMCO' computer systems and for direct control of a 'CALCOMP' Model 81 digital plotter.

All these data exchanges with the INCOS system are usually by an interconnecting line to an otherwise unused serial I/O port (intended for modem interfacing) on the second of two MS-10 boards in our system. Hardware additions further to extensions described earlier (1,2) now allow a switch on the front panel to select any of seven data transfer rates from 300 to 19200 baud. A similar switch allows the operator to connect the port to any of three cables (6 to 60 meters length) for transferring data between the INCOS system and the other computers or the plotter.

INCOS-resident software developed earlier (2,4) to support communications with the VARIAN minicomputer system was a set of subroutines used through a main program, 'LINE', written in FORTRAN IV to be compatible with INCOS 'MSDS' conventions. This software also supports communications with the APPLE AND CROMEMCO systems, and has been extended to support direct control of the plotter by INCOS.

New software developed for the CROMEMCO (FORTRAN; Z80 CPU) and the APPLE ('Applesoft' BASIC; 6502 CPU) systems handles communications with the INCOS system. Both conform to the byte-by-byte send, then receive or 'dialogue' strategy which our 'LINE' program can use to avoid timing problems which would otherwise make it difficult to transfer data at high rates between such different systems.

A main use of our INCOS system's extended abilities to communicate with other systems has been to allow special MSDS graphics hardcopies of higher quality to be made on a CALCOMP Model 81 plotter. This capability avoids the drafting otherwise needed when slides, transparency overlays, or graphics for publication are needed. Our 'LINE' program was extended to provide for the particular INCOS 'monologue', with occasional commanded CALCOMP responses, needed for the plotter to be controlled directly by the INCOS system. The 'speech-writer' for this monologue is a program 'CPLT', written in FORTRAN IV to be compatible with MSDS conventions.

'CPLT' reads the temporary files of graphics information (files 'BOPLOTEMP.99' or 'B1PLOTEMP.99') created by MSDS applications programs to be read by the MSDS 'PLOTH' overlay called to operate the graphics hardcopy devices usually interfaced with the INCOS system. An obedient speech-writer, 'CPLT' allows the user to modify or edit the graphics data in several ways before handling nearly all details of producing a file of CALCOMP commands which 'LINE'

will use in its monologue with the plotter to produce the final graphics product. 'CPLT' can handle the graphics output from any MSDS program.

Some of the graphics modifications which can be done by 'CPLT' are: independent expansions, reductions or offsets in either X or Y directions; rotated (0 to 360 degrees) or isometric views; composite plots combining several graphical outputs either as one file or as exactly registered overlays; changes of CALCOMP plotting pens (different widths, colours); text message modifications (specific or global deletions, exactly positioned additions, manually positioned messages, slanted font, character height and width changes).

An alternative output file can be created by 'CPLT'. This conforms to the conventions of data files (.XX files) readable by the MSDS 'PICT' program released as part of the Triple Stage Quadrupole system software. Since the 'PICT' program can create and edit graphics in an 'on-screen' way, this second type of 'CPLT' output file allows very flexible, user-friendly editing of MSDS graphics and the addition to them of other graphics created on-screen or from files. The result can be output on the usual hardcopy device, saved as a file, or again processed by 'CPLT' so that 'LINE' can command a product from the CALCOMP plotter.

Data transfers between the INCOS system and the other computers mainly allow interchange of acquired analytical data and for collation and final processing of 'translated' forms of these in the INCOS system. Transfer of data reports to APPLE-resident word-processing software is useful. This latter software also aids development of FORTRAN source for use in the INCOS system. The smaller systems also benefit from access to the INCOS data storage peripherals (now 52 megabytes for our system; expandable to 212 megabytes).

Work is in progress to enable transfers to the INCOS system of any data acquired by a VARIAN Vista 401 system used for GC and HPLC experiments. Other work will assess the extent to which AA (CROMEMCO system) and ICP-AES (PDP11/03) data can be collated or processed within the INCOS environment.

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DIRECT COMPUTER-COMPUTER DATA TRANSFER

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This paper describes an intercomputer communication system designed to transfer data between two (or more) Data General "NOVA" computer systems, although it will in principle function between any two computers (not necessarily of the same type) with "reasonable" operating systems. This work was undertaken to permit our data system (Kratos DS-50-S) to be expanded to accommodate additional mass spectrometry equipment at a reasonable cost. The major objectives have been to provide a rapid and inexpensive method for transferring data and programs that could be activated by either operator without disturbing the other. In addition, the transfer operation should fully overlap other processing on both computers and allow the operator the option of specifying data by the data system's "RUNNAME". Also, either operator should be able to delete data from the other computer's disk.

There are alternative methods to transferring data between computers, but they are all impractical for a number of reasons. Removable disk cartridges cannot be swapped from one computer to the other on systems which have different types of drives. Even if all drives are identical, this method is extremely inefficient and time consuming. A communication line is available from Data General, but, of the six objectives listed above, it fails all but the first and fourth (it is, in fact, more "rapid" than the system described here, and while transfers only partially overlap other processing, the speed of the transfer is fast enough that this is not important). In addition, no System Generation capabilities are available for our version of the data system, so we would have to purchase an otherwise unnecessary "upgrade" merely to obtain this capability. Dual porting of a single large disk drive (connecting it simultaneously to two computers) is often more convenient; but, it is not possible for all types of drives (particularly older models), it costs much more when it is possible, and (probably most important) it prevents independent operation of each system. (If one computer crashes, the other is likely to be taken down; also, if the disk malfunctions, both systems are down, etc.)

The hardware connection between the two computers has been made by using standard "RS-232-C" interfaces modified to increase speed to 33,000 baud and to implement hardware handshaking on a character by character basis. This handshaking was absolutely essential as the operating system in use was unable to properly execute transfers using standard RS-232-C boards, even at the relatively slow rate of 4800 baud without regularly dropping bytes. The line has been tested successfully at 45,000 baud and can probably operate even faster than that, depending on the distance between computers (which is only five feet (170 cm.) in our installation). With a speed of 33,000 baud we are able to achieve a total throughput rate of 2340 bytes/sec. (including all disk access time), which will increase to roughly 2900 bytes/sec. at 45,000 baud, and will probably be able to be increased further.

Software control is accomplished with a continuously resident foreground "monitor program" which performs all actual data transfers, and a background "scheduling program" which processes all operator requests, handles all transfer errors, and interacts with the mass spectrometry data system to allow data to be specified by "RUNNAME" and type if desired, rather than by operating system "File Name". This method thus permits either computer operator to initiate a data transfer, even if the other operator is busy. Once the request is processed, control is returned to the operating system so both operators may continue with other tasks while the transfer actually takes place. The number of requests that can be "queued up" by the program is limited only by the amount of memory assigned to the transfer program (currently 1.4 K); if more memory is available this could be increased substantially. Our system is a 32 K single-user unmapped system which has only 20.3 K available for user programs. With the transfer line monitor resident only 18.9 K is now left, which must accommodate the entire data system. This is not a problem for general operation, although there are a few specialized application programs that require the full 20.3 K. When this occurs, the monitor can be deleted easily, the

needed programs run, and then the monitor reloaded. Clearly operation would be more efficient on a system with more memory, but the cost of expanding the memory size beyond 32 K is currently prohibitive.

Using the above data transfer system, it now becomes reasonable to compare a single computer, dual-user, foreground-background system (which the majority of DS-50/DS-55 users have purchased) to two separate single-user computers connected by a data transfer line (similar to the University of Pennsylvania system). There are numerous advantages to the dual unmapped system. The first is much greater overall operational speed. While the larger computer typically used in the "mapped" system (ECLIPSE S-120 or NOVA 4-X) is faster than the smaller computers used in the "dual unmapped" system (NOVA 4-C), it is nowhere near twice as fast. The second is totally independent operation. Each user has full access to his/her own computer and cannot be disturbed by the other user. There are no restrictions on program operation such as would occur in a mapped system. Likewise, a crash on one system will not bring down both users. The third advantage is far less "down time". Either system can be repaired without disturbing the other, whereas in the mapped system, if the computer or disk goes down, both users are down. The final advantage is reduced cost. The larger computer costs more than twice as much as the smaller one. While two smaller disk drives (for the two small computers) cost more than a single larger one (for the large computer), the overall package cost of the dual unmapped system is roughly 10% less, depending on the exact hardware selected. This cost can be reduced substantially further by making use of available second-hand components. Since this system has a built-in redundancy, second-hand components are far less likely to increase down-time. There are only two significant disadvantages. First, memory is a little tight, as indicated above, and any memory expansion would raise costs substantially. Second, it does take longer for one user to access the other user's data, but this can be minimized by careful planning, and is compensated for by a faster operational speed once the data has been transferred.

Currently, the system operates between two single-user unmapped systems. Our future expansion plans call for a third computer to be added in several months. This computer will be tied into the network using the above system in a manner which will allow each computer to communicate with each of the others. Each computer will need two transfer line interfaces. The transfer monitor program will be modified to monitor both lines as well as the background program, so any of the three terminals can initiate any type of transfer operation involving any combination of computers.

Operation on a mapped system presents no problem except that since the program requires use of the foreground, no other foreground operation is possible simultaneously with the transfer. Ultimately the transfer line program will be incorporated directly into the operating system so the above restriction is removed. This would allow all four users to operate the systems simultaneously with a data transfer operation. The program would need to be added in a manner which would permit it to be deleted if its memory is required for other programs. Other useful improvements would allocate all buffers required by the program dynamically instead of using fixed buffers as is now the case. The principle advantage would be that these buffers could be enlarged to whatever size was required. This would speed up operation considerably, impose no restraints on the size of a background program, and allow an unlimited number of transfer requests to be "queued up". A careful assignment of priorities would need to be made for this to be effective. Also, multi-transfer operations should occur simultaneously. Since the interface is full-duplex in its entirety, there is no hardware reason why transfers couldn't be made in both directions simultaneously. With more than two computers on the network, many more possibilities are theoretically feasible.

In conclusion a direct computer-computer data transfer line is the quickest and easiest method of moving data from one computer to another. A careful design of the hardware and software bypasses operating system limitations and enables transfers to be fully overlapped with other processing. The operation of the line is not restricted to two computers and could also be modified to operate between computers of different manufacturers. Finally, the overall cost of this system compares favorably with a mapped single computer dual-user system.

PROCESSING OF COMPARATIVE DLI-LC/MS, DCI AND PID DATA AND OF CAPILLARY GC/MS DATA USING CUSTOMIZED PROCEDURES BASED ON THE RPN DATA PROCESSING STACK.

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Hewlett-Packard Company, 1601 California Avenue, Palo Alto, CA 94304

The architecture and implementation of a new approach to mass spectral data processing, the RPN (Reverse Polish Notation) data processing environment, have been described (papers WPA 27 and WPA 29 at this meeting). This data processing environment is based upon a register stack similar to that on an HP hand-held calculator and consists of individual programs each of which performs a single function. Because each of the functions can be executed from the command processor of the host computer (the File Manager), it is possible to combine the single functions into automated procedures which when executed will yield results tailored to the particular technique used to acquire the data being processed. This paper describes customized data processing procedures and the results obtained from use of these procedures for the following applications:

1. display of mass spectral and chromatographic data on a single worksheet for rapid visual confirmation of analysis;
2. compound identification using cross-correlation and quantitation;
3. spectrum averaging with comparative display of mass spectra.

For each of these procedures, the user determined parameters, the procedure file itself (with annotation) and the results obtained are presented. For the second (identify and quantitate), the intermediate contents of the register stack are also presented. The compound identification and quantification procedure file and the results it produces are shown below. Each comment line, beginning with **, in the procedure file itself is a description of the purpose of the steps between that comment and the next comment in the file.

PROCEDURE FILE: DETECT AND QUANTITATE A COMPOUND

The user types in the data file to be processed, the expected retention time window and up to three m/z-abundance pairs for use in identification and quantitation of the desired compound. The run string is:

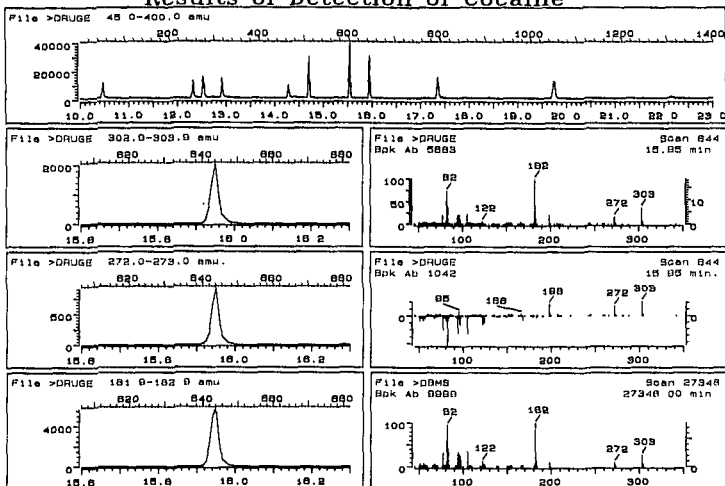
:TR, IQID, data file name, start retention time, stop retention time, m/z #1, abundance #1, m/z #2, abundance #2, m/z #3, abundance #3

In the example illustrated, cocaine was to be detected and quantitated using m/z's 182.2, 272.3 and 303.2. The expected retention time window was 15.6 to 16.3 minutes. The data file was >DRUGE. Thus, the run string used was:

:TR, IQID, >DRUGE, 15.6, 16.3, 182.2, 100, 272.3, 16.2, 303.2, 35.4

```
SV,4,,H
**-----
** Procedure File: IQID                                     <B30414.1313>
**
** Set up data file
:RU,FI,10
:IF,SP,EO,0
:TR
**
** Set up custom window set and graphics
** device (9872 plotter)
:RU,STW,,1.5,100,73.96
:RU,STW,,2.5,52.48,6.72
:RU,STW,,3.5,52.24,3.47,6
:RU,STW,,4.5,52.0,23.3
:RU,STW,,5.5,100.48,6.72
:RU,STW,,6.5,100.24,3.47,6
:RU,STW,,7.5,100.0,23.3
:RU,STW,,10.0,100.0,100
:RU,DEV,9872.58
**
** Draw total ionization chromatogram
:RU,TIM
:RU,EC
:RU,GC,F,23B,173567B,1
:RU,DR,1,,ALL
**
** Find compound in window using cross
** correlation of m/z-abundance pairs
:RU,XS,20,3G,4G,5G,6G,7G,8G,9G,....2
:IF,SP,EO,0
:TR
:RU,INT,,,1
**
** Draw background subtracted mass
** spectrum
:RU,PF,1
:RU,DR,2,2,2G,3G
:RU,L,P
:RU,NEW,2
:RU,PS,OR
:RU,SUB
:RU,MSF,113B,175673B,1
:RU,DR,5,,45,350
**
** Search library (PBM) for best match
:RU,PBM,....5
**
** Set up spooling system and store
** library search results on disc
:RU,RESULT
:CR,RESULT,,4,10
:SL,10,61
:SL,10,RESULT,BO
:RU,SCA
:RU,PRES,....10,,2
**
** Plot best library match and difference
** from compound spectrum
:RU,PS,2R,>DBMS,10G
:RU,DR,7
:RU,NRM,9999,Y
:RU,SUB
:RU,DR,6
**
** Draw extracted ion current profiles
** used for compound identification
:RU,TIM
:RU,MC,4,4G,6G,8G,2G,3G
:RU,DR,2,2
:RU,DR,3,Y
:RU,DR,4
**
** Integrate extracted ion current pro-
** files and store results on disc
:RU,INT,,,1,2,10
:RU,INT,,,1,Y,10
:RU,INT,,,1,X,10
**
** Close disc file
:CS,10
**
** Draw library search and integration
** results
:RU,AR,RESULT,10,2,,2,4
:RU,NEW
:RU,DR
**
** Reset windows, globals and severity
** code
:RU,STW,0
:SE
:SV,0,,H
:TR
```

Results of Detection of Cocaine



Library Search Results

1. 8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benz 303 C17H21NO4
2. Benzene, (2-methylpropoxy)- (BC) 150 C10H14O
3. Bicyclo[2.2.1]hept-2-ene, 2,3-dimethyl- (BC) 122 C9H14
4. Maltotriose, phenyl-4-pyridyl- (BC) 153 C12H16NO
5. 3-Cyclohexene-1-acetaldehyde, alpha,4-dimethyl- (B 152 C10H16O

Sample file: >DRUGS Spectrum #: 644
Search speed: 2 Tiling option: S No. of ion ranges searched: 55

	REL	REC	CON	K-VAL	DK-VAL	FLG	TILT	%	CRN
1.	66*	27348	22918	88	78	2	0	59	DB
2.	15*	7054	7840	30	31	2	0	18	DB
3.	14*	3134	7806	25	30	1	0	20	DB
4.	14*	12817	22840	26	31	2	0	12	DB
5.	14*	7425	7848	27	33	2	0	11	DB

Quantitation Results

>DRUGS BARBITURATES + ALKALINE SPL. 25M FS SE54 .31MMID OPEN SPLIT

302.9-303.9

Peak #	R.T. min	first scan	max scan	last scan	peak width	raw area	corr. area	corr. %
1	15.950	639	644	651	12	5817	5817	100.00

272.0-273.0

Peak #	R.T. min	first scan	max scan	last scan	peak width	raw area	corr. area	corr. %
1	15.950	639	644	649	10	2545	2545	100.00

181.9-182.0

Peak #	R.T. min	first scan	max scan	last scan	peak width	raw area	corr. area	corr. %
1	15.950	638	644	649	11	17289	17041	100.00

ARCHITECTURE OF A NEW DATA PROCESSING ENVIRONMENT: REVERSE POLISH NOTATION

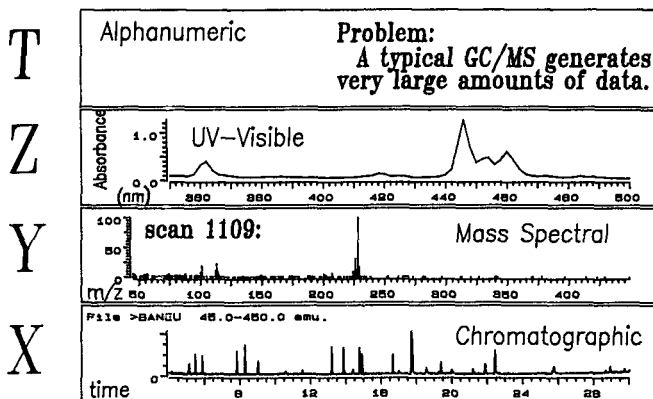
EDWARD F. REUS and DAVID W. PETERSON, Hewlett-Packard Company,
1601 California Avenue, Palo Alto, California 94304.

A typical GC/MS experiment generates very large amounts of data. This data must be efficiently reduced and integrated with data from other data domains (analytical techniques) to generate answers appropriate to very diverse analytical problems. So the data analysis system must be independent of application, flexible, simple in structure (from the user's perspective), and have the ability to be "programmed" for routine analysis.

A new data reduction software environment is proposed for solving these problems. The environment consists of a four level "last-in-first-out" (LIFO) register stack, a set of functions for operating on the data, storage "registers", a flexible subsystem for graphical output, and the capability to be programmed. The operation of this system is analogous to that of a programmable calculator. In fact, the conceptual model for this system is a stack oriented calculator like the Hewlett-Packard HP-41C (ref. 1).

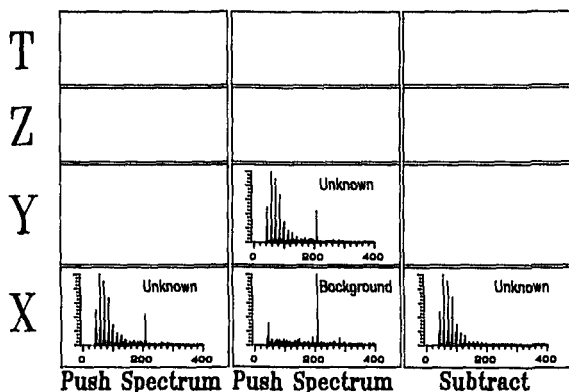
Like that of an HP calculator, there is a four level LIFO register stack for data manipulation. The registers are named X, Y, Z, and T. Unlike the hand-held calculator, each of the registers may hold complex data objects such as entire chromatograms or mass spectra (fig. 1). The register stack operates in a last-in-first-out manner, data is pushed onto the stack through the X register, the previous contents of the X, Y, and Z registers are pushed up one level to the Y, Z, and T registers respectively (the contents, if any, of the T register are lost off the top of the stack).

Figure 1:



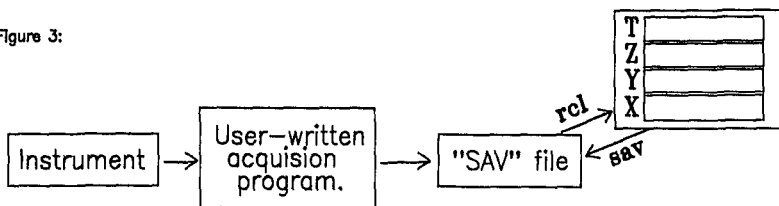
Functions are the basic tools of this environment. They move data into and out of the register stack, and operate on the data within the register stack. They are scaled up to the kinds of operations appropriate for the complex data objects held by the register stack and can operate on entire data objects. The two-register functions give the stack its reverse polish notation (RPN) structure as the X and Y registers are implicitly specified and merged (fig. 2). The functions can be divided into two groups, generalized functions and data-specific functions. Generalized functions will operate on almost any type of data, SUBtract is a good example. Data specific functions are specialized to particular types of data. PBM library search of mass spectra (ref. 2) and STIRS, Self Training Interpretive and Retrieval System (ref. 3), are examples of data-specific functions.

Figure 2:



Storage registers allow storage of the stack registers (intermediate and final results) to disc files. They are also a vehicle for entering data from other data domains (analytical techniques) into the RPN analysis system. That is, any data written in the format of a storage, "SAV" register may be read into the register stack and manipulated (fig. 3).

Figure 3:



The graphics subsystem provides a flexible, device-independent display mechanism for data in the register stack. This subsystem is implemented via a set of functions for the display of data in the register stack to any part of the current display device. The format of plots (for numeric data) as well as text attributes (for alphanumeric data) may also be modified display information in an appropriate manner.

Like other calculators, this one is also programmable. The functions can be strung together to form compound functions. These new "user-defined" functions may be simple often-used tasks or complex interactive (or auto-analysis) procedures.

This environment gives the user several important benefits. The RPN environment defines a generic structure for the processing of data from many data domains. The calculator-like structure makes the system easy to use and very flexible. Combines with a generalized graphics subsystem, this structure provides a powerful tool for solving diverse analytical problems.

REFERENCES

1. "Owner's Handbook and Programming Guide, HP-41C, HP-41CV", Hewlett-Packard Company (1980).
2. F.W. McLafferty, R.H. Hertel, and R.D. Villwock, Org. Mass Spectrom., 9:690 (1974).
3. F.W. McLafferty, Org. Mass Spectrom., 15:114, (1980).

Reverse Polish Notation (RPN) has been well characterized as an efficient environment for performing arithmetic operations in electronic calculators for more than a decade. However, its application for the manipulation of GC/MS data has recently been developed and is now available on a commercial GC/MS system.

The last in-first out register stack (X,Y,Z,T) forms the structure by which ion chromatograms, mass spectra, and alphanumeric data are handled; this structure allows large amounts of spectral information to be handled with facility. In addition many arithmetic and logical functions have been written which have not heretofore been used on data of this type.

These RPN commands either control the register stack, operate on data in any one register, or perform arithmetic or logical operations on two registers together. In addition, a full device-independent graphics subsystem with alphanumeric overlay permits the presentation of these data in a variety of formats.

The operation of the RPN stack and a description of its associated commands will be presented and examples of data so obtained will be shown.

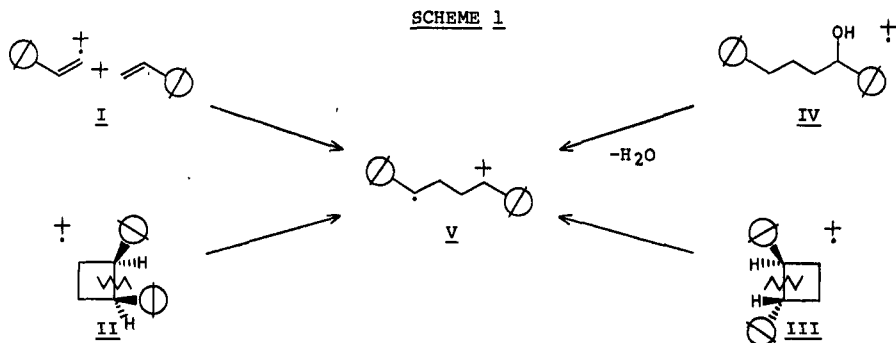
The Structure of the Styrene + Styrene Ion-Molecule Adduct, G.S. Greenewald and E.K. Chess, Department of Chemistry, University of Nebraska, Lincoln, NE 68588.

The structure of the styrene ion-molecule adduct has been investigated because it is a good candidate for an archetypal [1 + 2] cycloaddition. The mechanism of [1 + 2] cycloaddition reactions has been the subject of previous solution-phase research.¹ This work focussed on the reactions of aromatic substituted ethylene radical cations with their corresponding neutrals, which produced 1,2-diaromatic substituted cyclobutane products. These reactions were interpreted in terms of two mechanisms: a concerted [1 + 2] cycloaddition,^{1a} and a stepwise mechanism which proceeds via an acyclic ion dipole intermediate.^{1b} In related ICR research on the gas-phase styrene ion-molecule reaction, styrene ion-molecule adducts were interpreted to decompose via a 1-phenyltetralin intermediate.² This possibility might involve a [3 + 2] or a [4 + 1] cycloaddition mechanism.

In the present research the structure of the styrene ion-molecule adduct has been investigated by first stabilizing the adduct using nonreactive collisions in a high pressure chemical ionization source, followed by isolating the adduct using the first stage of mass analysis (MS-I) of a tandem mass spectrometer,³ and finally analyzing the collision-activated decompositions (CAD) of the adduct using MS-II. The structure of the adduct can be assigned by comparing its CAD spectrum with the CAD spectra of nine isomeric reference compounds, which were chosen to model chemically reasonable structures that are possible from the styrene ion-molecule reaction.

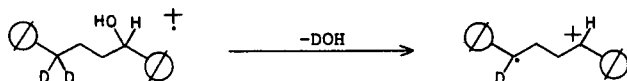
The CAD spectrum of the styrene ion-molecule adduct (I) is nearly identical with the CAD spectra of the C₁₆H₁₆ radical cations generated from compounds II-IV (see Scheme 1). The CAD spectrum of the stabilized adduct is clearly not 1-phenyltetralin, in contrast to the earlier ICR study in which decomposing styrene ion-molecule adducts were examined.² Other C₁₆H₁₆ isomers whose CAD spectra did not match the spectrum of I were 2-phenyltetralin, 1-o-tolyl-3-phenylpropene, 3-o-tolyl-1-phenylpropene, [2.2]-paracyclophane, 1,3-diphenyl-1-butene, 1,4-diphenyl-1-butene and 2,3-diphenyl-2-butene.

The structure of the adduct is revealed by a consideration of the possible structures of II-IV. Cis- and trans-1,2-diphenylcyclobutane (II, III) have significantly different structures and it is unlikely that they would give identical CAD spectra unless they were isomerizing to the same structure prior to CAD analysis. A possible isomerization is an intramolecular epimerization, but this is unlikely because it would require an improbable 1,2-antarafacial hydrogen transfer. The most reasonable isomerization process is a ring opening occurring via cleavage of the 1,2-bond, yielding the 1,4-ion dipole.



The 1,4-ion dipole was also generated by a regiospecific 1,4-water elimination from 1,4-diphenylbutan-1-ol⁺. The water loss mechanism was proven by a study of 1,4-diphenylbutan-1-ol-4,4-d₂ (see Scheme 2), in which the D/H distribution that was observed in the water loss was interpreted in terms of a mechanism that was >99% 1,4-regiospecific. The possibility of I-IV having a cyclobutane structure was unequivocally ruled out by a consideration of the H_f calculated for III and IV. The H_f for III (247 kcal/mole) was found to be at least 8 kcal/mole higher in energy than the H_f of IV (< 239 kcal/mole). Therefore the structure of IV, and hence the structures of I, II and III also, cannot be 1,2-diphenylcyclobutane. This strong evidence that radical cation reactions which produce cyclobutanes proceed via stepwise mechanisms.

SCHEME 2



The distinguishing mass spectral characteristic of the stabilized 1,4-ion dipole is a dissociation to styrene⁺ and neutral styrene. In contrast, earlier ICR studies of decomposing styrene ion-molecule adducts were characterized by the elimination of benzene.² The rate constant for the styrene ion-molecule reaction was measured in order to correlate these observations, and was found $k = 6.0 \times 10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ sec}^{-1}$. The Langevin collision constant⁴ was calculated at $k = 1.23 \times 10^{-9} \text{ cm}^3 \text{ molecule}^{-1} \text{ sec}^{-1}$; therefore the reaction is only 5% efficient. It is emphasized that this efficiency reflects only those reactions which produce a product other than styrene⁺, because this process is not observable in the ICR. Collisional stabilization can intercept this process, but reversion to styrene⁺ (m/z 104) reappears as the most intense peak in the CAD spectrum of the adduct. A small fraction of the adducts rearrange to a structure whose distinguishing characteristic is the loss of benzene. 1-Phenyltetralin is a good candidate for the minor component because it is characterized by intense loss of benzene, in accord with the previous study of Wilkins and Gross.²

Acknowledgement

The authors thank Professor Michael L. Gross for his guidance and inspiration throughout the course of this work.

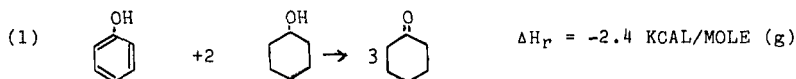
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4. Gioumiosis, G and Stevenson, D.P., *J. Chem. Phys.*, 1958, **29**, 294.

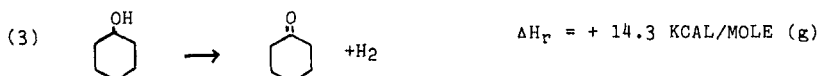
MASS SPECTRAL STUDY OF
CYCLOHEXANONE FORMATION BY TRANSFER HYDROGENATION
E. R. McCarthy, A. Rosan and R. Tang
Allied Corporation, Morristown, NJ 07960

Introduction

We have attempted to prepare cyclohexanone via the reaction of phenol and cyclohexanol according to the following reaction



The direct combination would be an alternative to the following reactions



To determine if cyclohexanone formation is indeed a consequence of H transfer from cyclohexanol (donor) to phenol (acceptor) and not simply arising via cyclohexanol dehydrogenation ($\Delta G > 0$), we performed reaction (1) utilizing perdeuterophenol (4% conversion 16 hr 155°C/N₂).

Experimental

Reaction mixtures were analyzed using a Finnigan 3300 MS (EI mode) employing the following GC conditions

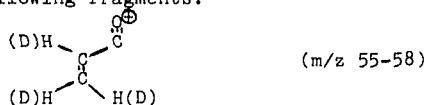
column: 6' x 1/8" 10% Carbowax 20M
 column temperature: $\frac{80^\circ}{2m} \xrightarrow{\frac{8^\circ}{m}} 180^\circ$

carrier gas: helium at $\frac{30cc}{m}$

Observations/Results

GC/MS analysis of reaction aliquots that were examined periodically indicated the following:

- (a) D incorporation into product cyclohexanone was revealed in the molecular ions and the m/z 55-58 region where the latter would correspond to the following fragments.



In Table I and Table II are presented the D distribution for the M⁺ and the m/z 55-60 region respectively of cyclohexanone for both the before and after (3% C) reaction cases

Table I
CYCLOHEXANONE, MOLECULAR ION
Relative Abundance

m/z	#D	Relative Abundance	
		Before Reaction	After Reaction
98	0	100	15
99	1	6.4	63
100	2		100
101	3		65
102	4		20
103	5		10
104	6		12
105	7		9
106	8		2

Table II
CYCLOHEXANONE, m/z 55-60
Relative Abundance

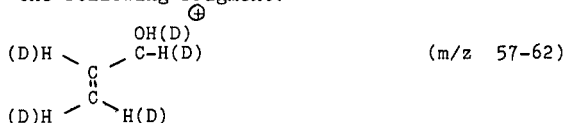
m/z	#D	Before Reaction	After Reaction
55	0	100	84
56	1	9	100
57	2	1	38
58	3		41

(b) A relative decrease of the amount of D in phenol label is illustrated in Table II where the phenol molecular ion region is examined

Table II
Phenol, Molecular Ions
Relative Abundance

m/z	#D	Before Reaction	After Reaction
94	0		
95	1		
96	2		22
97	3		61
98	4	18	100
99	5	100	76
100	6	28	

(c) D incorporation into the cyclohexanol is evident particularly in the m/z 57-59 region of its₂ spectrum. This distribution is revealed in the following fragment.



This enhancement is shown in Table IV

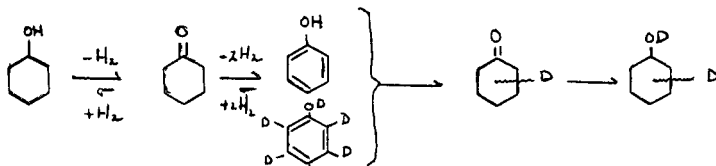
Table IV
CYCLOHEXANOL, m/z 57-59
Relative Abundance

m/z	#D	Before Reaction	After Reaction
57	0	100	100
58	1	3	43
59	2	<0.2	8

Discussion

Product cyclohexanone contains deuterium at C₂ and C₃. The incorporation is in excess of that expected from enolization at C₂. The extent of labeling was addressed by evaluation of the C₃H₃-ND₃O fragment nominal m/z 55. The relative abundances of m/z 55 and m/z 56 (D₀, D₁) vs m/z 57 and m/z 58 (D₂, D₃) indicate that the stoichiometry in reaction (1) is not achieved. At stoichiometry, a m/z 55-58 distribution of 66:10:20:3 is calculated. The experimental distribution is 35:41.5:16:17.

The data is accommodated by the following reaction scheme.



References

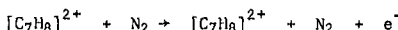
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2. C. G. McDonald, J. S. Shannon & G. Sugowodz, Tet Let 807(63)

DECOMPOSITION AND STRUCTURE OF $[C_7H_8]^{2+}$ IONS FORMED
FROM DIFFERENT PRECURSOR MOLECULES.

M. Rabrenović, F. M. Harris and J. H. Beynon.

Royal Society Research Unit, University College of Swansea,
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The study of $[C_7H_8]^{2+}$ ions, formed from five C_7H_8 isomers and four higher molecular weight monosubstituted benzenes, has been undertaken in an attempt to determine the ion structure. Unimolecular and collisionally-induced decomposition of $C_7H_8^{2+}$ ions formed in the ion source as well as in the charge-stripping reaction



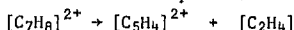
have been studied to provide the structural information. Ionisation energies, obtained from the minimum energy,¹ (Q_{min}), needed to ionize singly-charged $C_7H_8^+$ ions from the compounds studied have also been obtained. The results are given in Table 1

TABLE 1

Precursor Molecule	$Q_{min} =$ $IE[C_7H_8^+ \rightarrow C_7H_8^{2+}]^{\#}$ (eV)	Relative intensities of $[C_7H_x]^{2+}$ ions in CID MIKE spectrum of $C_7H_8^{2+}$ ions.				
		$[C_7H_8]^{2+}$	$[C_7H_7]^{2+}$	$[C_7H_6]^{2+}$	$[C_7H_5]^{2+}$	$[C_7H_4]^{2+}$
toluene	15.7	100	50	83	10	4
1,6 - heptadiyne	15.3	100	7	14	11	1
bicyclo[2,2,1]hepta-(1,5)diene	15.2	62	33	100	24	6
quadricyclene	14.8	68	30	100	14	5
1,3,5 - cycloheptatriene	14.3	100	36	72	8	2
n-butylbenzene	14.3	100	25	45	5	2
phenethylalcohol	14.4	100	30	53	6	3
n-pentylbenzene	14.6	100	26	46	5	2
sec-pentylbenzene	14.6	100	30	36	8	1

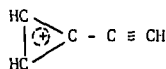
[#] Estimated experimental error for Q_{min} values is less than 0.5 eV.

It can be seen from Table 1 that for $[C_7H_8]^{2+}$ ions formed by hydrogen rearrangement Q_{min} values and intensities of $[C_7H_x]^{2+}$ ions in CID MIKE/spectra are very similar. This must be due to the similar structure since energy-selected ions are being studied. However, this ion structure is different from the structure of ions obtained from C_7H_8 isomers (see Table I). Unimolecular fragmentation reactions of $[C_7H_8]^{2+}$ ions formed in the ion source give further evidence about the structure of these species. The peak due to the reaction

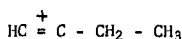


present in the spectra of ions formed by hydrogen rearrangement is absent in the spectra of C_7H_8 isomers studied.

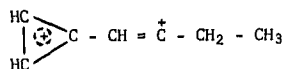
When 92^{2+} ions, formed in the ion source, are transmitted by the magnet, and the electric sector is scanned from E to 2E, two composite charge-separation peaks are observed (Figs. 1 and 2). Peaks (4-4) and (5-5) which are either not present or very weak in the spectra of C_7H_8 isomers are abundant in the spectra of $[C_7H_8]^{2+}$ ions formed by hydrogen rearrangement. The fact that the ions carry two charges and that the interchange distance in the fragmenting ions can be determined,² makes it possible to extract information about the structures of the decomposing ions. The structure of product ions formed in the charge-separation reaction (5-5) is probably the structure A suggested by Lammertsma and von Schleyer³ as the energetically most lively $(C_5H_3)^+$ product.



A



B



C

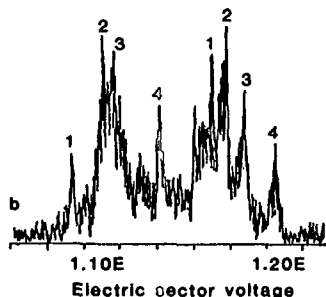
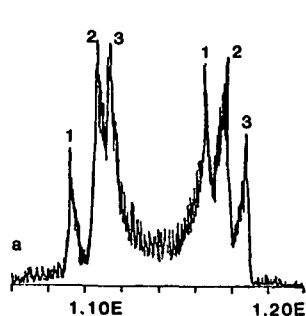
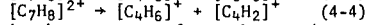
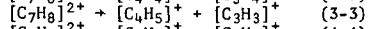
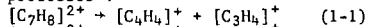


Figure 1

Charge-separation peaks due to the processes :



in the spectrum of cycloheptatriene (a) and phenethylalcohol (b).

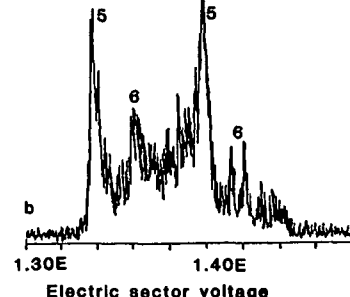
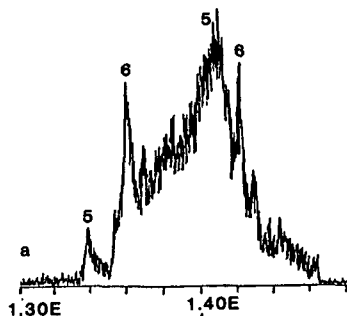
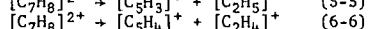
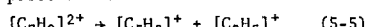


Figure 2

Charge-separation peaks due to the processes :



in the spectrum of cycloheptatriene (a) and n-butylbenzene (b).

Calculated intercharge distance for the reaction (4-4) suggests that the product $C_4H_6^+$ ions have the linear structure B. Therefore, we are suggesting 1-cyclopropenyl-but-1-enyl dication (C) as the possible structure of 92^{2+} ions formed by hydrogen rearrangement. This structure is consistent with all data obtained.

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REARRANGEMENTS IN BICYCLIC ORGANOPHOSPHORUS COMPOUNDS BY ^{13}C AND D LABELLING AND MS/MSH.I. Kenttämä¹¹

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The EI induced fragmentation of small organophosphorus esters is dominated by the formation of fragment ions with several P-OH bonds as shown in Figure 1.¹ A different behaviour would be expected if the usual possibility of stabilizing charge on oxygen via a hydrogen shift and a C-O bond cleavage is retarded for steric reasons. In spite of this, the bicyclic phosphate **1** (Figure 2) produces a peak at m/z 99, most probably due to $P(OH)_4^+$.^{2,3,4} Moreover, the corresponding phosphites, phosphorothionates and phosphor-selenates do not produce the corresponding ions (these bicyclic esters fragment predominantly via the loss of one of the bridges).^{3,4} The aim of this work was to answer the following questions: 1) How does the molecular ion of **1** rearrange, in a specific way, to give rise to ion structures which finally fragment to $P(OH)_4^+$; i.e. are the tentative mechanisms proposed earlier^{2,3,4} for rearrangements of molecular ions of this type correct? 2) Is the formation of P-OH bonds important in these early steps?

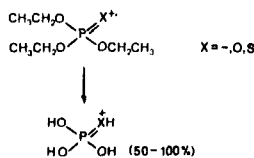


Figure 1



Figure 2

The formation of $[M-C_2H_3]^+$ and $[M-C_2H_4]^+$ ions of **1** was studied by EIMS of the ^{13}C labelled compound **2**, CID of the unlabelled and ^{13}C and D labelled $[M-C_2H_3]^+$ and $[M-C_2H_4]^+$ ions derived from **1**, **2** and **3**, and CID of the analogous ions with different alkyl substituents, derived from **4** and **5** (Figure 2). The EI mass spectra were recorded with a JEOL JMS-01-SG-2 Mattauch-Herzog type instrument. The MS/MS spectra were obtained on a Finnigan triple quadrupole mass spectrometer at Purdue University, USA. Collision energy was 10 eV and Ar was used as the collision gas. The principal breakdown routes of **1** under EI, based on metastable dissociation, and on elemental compositions of ions produced in the ion source, are shown in Figure 3 (expected elemental compositions in parenthesis). The EI and CID mass spectra are shown in Table 1. The CID spectra of the $[M-C_2H_4]^+$ ions of **1**, **2** and **3** are shown in Figure 4 (asterix refers to interfering $[M-C_2H_3]^+$ ion of **2** (m/z 151) which produces this peak under CID).

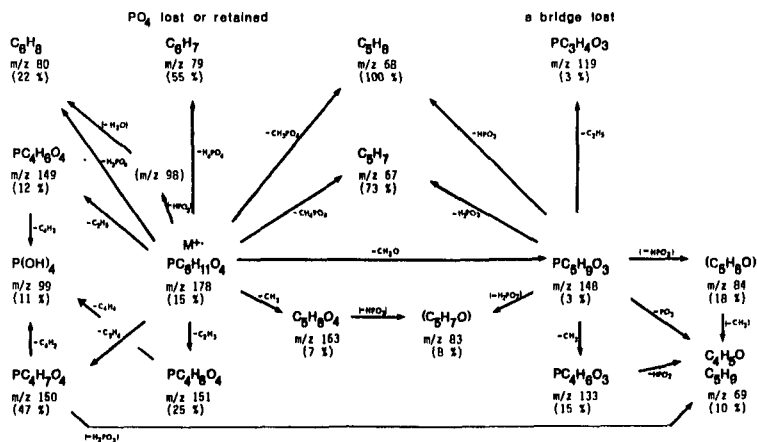


Figure 3

Table 1

compound	E1/C1D	parent ion	fragment ions	m/z (%)
1	E1		27 (3)	25 (15)
			42 (7)	43 (9)
			54 (2)	55 (2)
			68 (1)	69 (1)
			80 (1)	81 (1)
	C1D		102 (1)	103 (1)
			112 (1)	113 (1)
			151 (4)	152 (5)
			169 (1)	170 (1)
			181 (1)	182 (1)
2	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
3	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
4	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
5	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
6	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
7	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
8	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)
9	E1		41 (1)	43 (1)
			55 (1)	57 (1)
			70 (2)	72 (2)
			82 (1)	84 (1)
			94 (1)	96 (1)
	C1D		106 (1)	108 (1)
			120 (1)	122 (1)
			134 (1)	136 (1)
			150 (1)	152 (1)
			166 (1)	168 (1)

ion structures. Under CID, the importance of $\text{P}(\text{OH})_2^+$ product ion increases drastically with the number of hydrogens in the alkyl group. Figure 5 shows the EI induced fragmentation of the molecular ion of **1** to $[\text{M}-\text{C}_2\text{H}_3]^+$ and $[\text{M}-\text{C}_2\text{H}_4]^+$, and further fragmentation of these ions under CID.

The reactions studied demonstrate the extremely strong tendency of organic esters of phosphoric acid to produce fragments with several P-OH bonds via rearrangement: even esters with a rigid bicyclic structure, which are not able to fragment via mechanisms common for organophosphorus esters, still have a significant tendency to reorganize their structure and give rise to products typical for phosphates. In the case studied, this occurs via rearrangement to a monocyclic structure with charge on the hydrocarbon part, a very unusual structure for a non-aromatic organophosphorus ester ion. A further rearrangement of this ion leads to P-OH bond formation, whereas fragmentation results in five-membered ring ions, which tend to isomerize via hydrogen shifts to oxygen. Apparently, P-OH bond formation is important as soon as it is sterically possible.

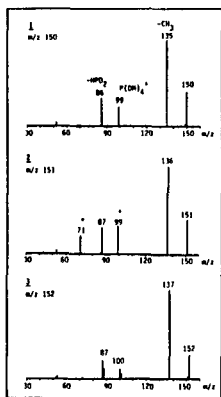


Figure 4

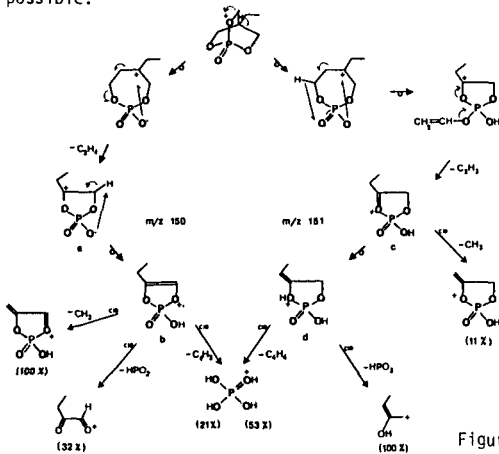


Figure 5

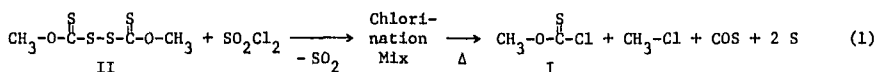
Acknowledgements. Financial support was provided by the Finnish Academy. R.G. Cooks is gratefully acknowledged for instrumental support.

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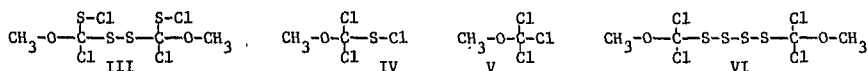
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MASS SPECTROMETRY FOR STRUCTURAL ASSIGNMENTS OF NOVEL POLYCHLORO, POLYSULFUR COMPOUNDS (1)
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Methoxythiocarbonyl chloride (I), a key synthetic intermediate for a new general method to elaborate the dithiocarbonyl functionality (4), is prepared by a process due to Sasse (5) which involves chlorination of bis(methoxythiocarbonyl)disulfane (II, trivially known as dimethyl dixanthogen), followed by a cracking distillation (Equation 1). Chlorination of II

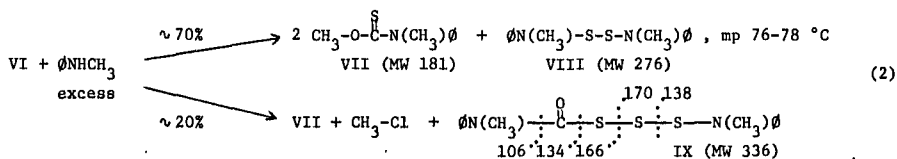


under somewhat different conditions has been described by Shasha *et al.* (6); these authors assigned structure III to the resultant four-chloro adduct. Finally, Douglass and coworkers (7) devised methods whereby chlorination of II could be brought to points from which either methoxydichloromethanesulfonyl chloride (IV) or methyl trichloromethyl ether (V) could be obtained as the major product for preparative isolation.

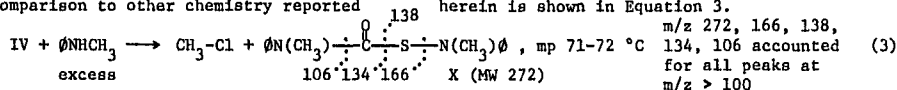


We find that use of the conditions of Sasse with excess (3 equiv.) of sulfuryl chloride provides an initial chlorination product which is a single pure species, analyzing for $\text{C}_4\text{H}_6\text{Cl}_4\text{O}_2\text{S}_4$, and identical to the compound obtained by Shasha. However, the detailed interpretation of the electron impact (EI) and the methane positive chemical ionization (CI) mass spectra led to a proposal that the structure of this four-chloro adduct is VI rather than III. Assignments of ions were aided by the characteristic isotopic clustering ratios, or by observations of appropriate homologues in the ethyl series. Both the EI and CIMS spectra of VI showed base peaks at m/z 113 [$\text{CH}_3\text{OCCl}_2^+$], and further peaks at m/z 145, 177, 209, and 241 [$\text{CH}_3\text{OCCl}_2\text{S}^+$, $n = 1-4$]. The two strongest high mass peaks in the CI spectrum were at m/z 319 [$(M+1)^+ - \text{HCl}$, 13%] and 269 [$(M+1)^+ - \text{HCl} - \text{CH}_2\text{Cl}$, 27%], and these were present in the EI spectrum as well although at much lower relative intensities. Useful EI spectra required gentle conditions, typically source 100 °C, solid probe 50 °C, 20 eV ionizing voltage. In one scan at higher sample pressure, a weak (0.1%) ion at m/z 354 was seen corresponding to M^+ for structure VI. Further evidence that the true structure is VI rather than III came from the H- and ^{13}C -NMR spectra, which were compared to those obtained for a large number of compounds with methoxydichloromethylsulfonyl functionalities available in the laboratory (4).

The linear polysulfide structure of VI is also supported by some of its chemistry. In particular, pure VI was treated with excess *N*-methylaniline, and the mixture of products which resulted was fractionated by preparative high pressure liquid chromatography on a 9.4 mm x 25 cm Zorbax ODS column (DuPont Instruments) eluted with $\text{MeOH}-\text{H}_2\text{O}$ (9:1) at 4 ml/min. The pure *N*-methylanilides which were isolated were subjected to high-resolution EIMS to establish their structures, as further confirmed by alternative syntheses where feasible (for VII and VIII). It was concluded that reaction of VI with *N*-methylaniline followed the course shown in Equation 2.



The mass spectrum of IX contained m/z 336.0157 [calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{OS}_3$, 336.0128, M^+], 304 [$M^+ - \text{S}$], 276 [$M^+ - \text{COS}$], 272 [$M^+ - 2 \text{S}$], 170, 166, 138, 134, and 106 (see diagram). The corresponding compound with one sulfur, namely X, has recently been prepared by us (4) from *N*-methylaniline plus chlorocarbonylsulfonyl chloride; another route of some interest by comparison to other chemistry reported herein is shown in Equation 3.



To fill out the homologous series between X (one sulfur) and IX (three sulfurs), the transformations of Equations 4 and 5 were carried out, and XIII (two sulfurs) was obtained. Contrary to Equation 3, reaction of XI with *N*-methylaniline gave only VII, not XIII.

ENRICHED STABLE ISOTOPES AS INTERNAL STANDARDS

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A variation on the standard isotope dilution method for quantitative assay of bulk and trace elements by thermal mass spectrometry is described. A feature of this method is the elimination of many absolute gravimetric and mass spectrometric measurements. Standards and samples are subjected to identical analytical procedures which results in the cancellation of many systematic errors. The determination of absolute isotopic ratios is not required, eliminating corrections for mass discrimination arising from high-voltage peak switching and multiplier discrimination. A disadvantage is that the approximate concentration of the analyte must be known. Standard solutions of the same isotopic composition as the analyte are prepared to concentrations which closely bracket the concentration of the sample. A spike solution is prepared from an isotopic tracer of the element in question. Standards and unknowns receive identical spikes and isotope-ratio measurements of the spiked standards are made. The concentration of the standards is plotted against the isotopic ratio of the spiked standards. An isotopic measurement is made on a spiked sample and the sample concentrations are extrapolated from the above graph. A standard curve fitting program may also be used to generate an equation relating concentration to the measured isotopic ratio. Both approaches were used in our experiments.

The first experiment was the determination of titanium at approximately 0.7% concentration in a uranium alloy. Four standards from 0.7% to 0.9% Ti were prepared. The samples and standards were spiked and the ratio of the m/z 64 to m/z 63 ions (TiO^+) was measured for each. The unknowns were also analyzed by x-ray emission for comparison.

Method	Lowest Result (g Ti/g)	Highest Result (g Ti/g)	Mean of 29 Samples (g Ti/g)	Mean Difference of Paired Results $\pm$ 2s of Mean Difference (g Ti/g)
Isotopic dilution	0.718	0.837	0.797	0.008 $\pm$ 0.004
X-ray emission	0.730	0.852	0.805	

Group	95% Confidence Limit of Error (g Ti/g)
23 routine samples with good precision	$\pm$ 0.007
4 routine samples with good precision	$\pm$ 0.020
11 control samples	$\pm$ 0.011

The 2s precision of the current x-ray emission method is $\pm$ 0.011 g Ti/g.

The determination of tungsten in uranium at the part-per-million level was performed. In these experiments, five solutions of known tungsten concentration were analyzed to evaluate the accuracy and precision of the idms technique. The known solutions covered the concentration ranges from 80 to 1800 ppm tungsten.

TABLE 1

Isotope Dilution Mass Spectrometer Analysis of Tungsten (W) in Uranium
Dissolved in Solution Containing Known Added Amounts of Tungsten

LE = 95% Confidence Limit of Error

Solution Known $\mu\text{g W/ml}$	Number of Results*	Mean $\pm \text{LE of Mean}$ $\mu\text{g W/ml}$	Standard Deviation $\mu\text{g W/ml}$	LE of a Single Result Relative % $\frac{\text{LE in } ()}{\mu\text{g W/ml} \times 100}$ % in ()	Bias = Mean-Std. Value $\pm \text{LE of the Mean}$ $\mu\text{g W/ml}$
83	8	87 ± 6	4.6	$\pm 13 (\pm 15\%)$	4 ± 6
204	8	202 ± 18	14.7	$\pm 41 (\pm 20\%)$	-2 ± 18
495	8	461 ± 43	34.3	$\pm 95 (\pm 21\%)$	-34 ± 43
621	7	544 ± 50	31.3	$\pm 100 (\pm 18\%)$	$-77 \pm 50^{**}$
925	8	958 ± 82	66.4	$\pm 184 (\pm 19\%)$	-33 ± 82
1804	8	1818 ± 60	48.5	$\pm 135 (\pm 7\%)$	-14 ± 60

*Duplicates were run on the first three days and singles on the last two days.

**Statistically significant - an error in the standard value is suspected.

The idms technique described here has been applied to other elements including yttrium, tellurium, and molybdenum with concentrations from ppm to > 99% levels. Separation of the analyte from its host matrix is not required and simultaneous multielement analysis on a single sample is possible.

ACKNOWLEDGMENT

The Oak Ridge Y-12 Plant is operated by the Union Carbide Corporation's Nuclear Division for the Department of Energy under U.S. Government Contract W-7405-eng-26.

ISOTOPE RATIO MEASUREMENTS BY NEGATIVE THERMAL IONIZATION MASS SPECTROMETRY

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The formation of positive thermal ions has been used for a long time to determine precise isotope ratios of metals. However, it is difficult to produce positive thermal ions from non-metals. In many of these cases the negative thermal ionization (NTI) technique results in high and stable ion beams. The negative thermal ion mode also seems to be an attractive alternative for measuring some transition metals.

In the Periodic Table of the Elements (Figure 1) all elements are marked where a procedure for isotope ratio determinations by negative thermal ionization has been developed or where such ions have been detected up to the present. The following negative thermal ions are measured (the preferred compounds which are used as a sample are given in brackets): BO_2^- (H_3BO_3), NO_2^- (nitron nitrate), X^- for halides (AgX), MoO_3^- (molybdates), WO_3^- (tungstates), TcO_4^- (NH_4TcO_4), ReO_4^- (NH_4ReO_4).

Different techniques are applied for the measurement of negative thermal ions. With a double filament ion source sufficient ion intensities are available for Cl^- and NO_2^- with rhenium filaments whereas carbonized and thoriated tungsten as an ionization filament results in high Br^- and I^- ion yields. With rhenium filaments, a lanthanum nitrate deposition on the ionization filament also increases the ion beam of X^- and BO_2^- . With the single filament mode (Re) the lanthanum addition results in high negative ion intensities of BO_2^- , X^- , MoO_3^- , WO_3^- , TcO_4^- and ReO_4^- .

A selection of isotope ratio measurements for main group elements is given in Table 1, for some transition metal elements it is given in Table 2. The measurements were carried out with a Finnigan MAT mass spectrometer, type CH5-TH or MAT 261. The relative external standard deviation of most of the determined isotope ratios lies between 0.01 and 0.1%.

The advantages using NTI-MS are the selective ionization method, high ion beams for non-metal compounds and some transition metals, only a few problems with interferences in the mass spectrum and in many cases a simple sample preparation. For example, in the positive thermal ionization mode, interferences between Mo and Tc can be a problem, the MoO_3 and TcO_4 ions are well separated in the mass spectrometer by NTI-MS. The isolation of halides as AgX by precipitation with silver nitrate is a simpler method than the conversion into an organo-halide compound which is usually used for the isotope ratio determination.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

- ☐ procedure for isotope ratio determination developed
☐ negative thermal ions detected

Figure 1.
Elements or element compounds measured by negative thermal ionization mass spectrometry (NTI-MS)

Table 1. Precision of isotope ratio measurements
by NTI-MS
(main group elements)

Element	Sample	Isotope ratio	Experimental value	$s_{rel}[\%]$
B	$\text{Na}_2\text{B}_4\text{O}_7$ *	$^{10}\text{BO}_2^- / ^{11}\text{BO}_2^-$	0.2489 ± 0.0002	0.08
N	Nitron nitrate **	$^{14}\text{NO}_2^- / ^{15}\text{NO}_2^-$	1.0768 ± 0.0002	0.02
Cl	AgCl *	$^{35}\text{Cl}^- / ^{37}\text{Cl}^-$	3.133 ± 0.001	0.03
Br	AgBr *	$^{79}\text{Br}^- / ^{81}\text{Br}^-$	1.0261 ± 0.0008	0.08
I	AgI **	$^{127}\text{I}^- / ^{129}\text{I}^-$	0.16180 ± 0.00007	0.04

* Natural isotope abundance ** Synthetic isotope mixture

Table 2. Precision of isotope ratio measurements
by NTI-MS
(transition metal elements)

Element	Sample	Isotope ratio	Experimental value*	$s_{rel}[\%]$
Mo	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ *	$^{92}\text{MoO}_3^- / ^{94}\text{MoO}_3^-$	1.578 ± 0.002	0.13
		$^{97}\text{MoO}_3^- / ^{98}\text{MoO}_3^-$	0.3975 ± 0.0002	0.05
W	metallic W *	$^{182}\text{WO}_3^- / ^{183}\text{WO}_3^-$	1.8483 ± 0.0002	0.01
		$^{184}\text{WO}_3^- / ^{186}\text{WO}_3^-$	1.0778 ± 0.0003	0.03
Tc	NH_4TcO_4 **	$^{97}\text{TcO}_4^- / ^{99}\text{TcO}_4^-$	0.0364 ± 0.0001	0.27
Re	metallic Re *	$^{185}\text{ReO}_4^- / ^{187}\text{ReO}_4^-$	0.5955 ± 0.0003	0.05

* Natural isotope abundance ** Synthetic mixture

* Without corrections for ^{17}O and ^{18}O

COMPUTER CONTROL OF A MOSFET-BASED ELECTROMETER
FOR ISOTOPE RATIO MEASUREMENTS

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In recent years, the disappearance of the Cary 401 vibrating reed electrometer from the marketplace has caused concern among isotope ratio mass spectrometrists. Possible replacements for the Cary were limited and included few, if any, direct substitutions. This paper describes the installation of a Keithley 642 MOSFET-based electrometer on a dual stage Nuclide mass spectrometer used for isotope ratio measurements.

The Keithley electrometer is controlled by a Data General Corporation Nova 4X minicomputer system through an interface which was designed for use with a Cary 401MR electrometer. Additions made to the interface to provide for the use of the Keithley electrometer include an integrated relay system to provide a range changing function and a programmable gain amplifier to condition the input signal.

Standard components of the interface, necessary to the use of either electrometer, include a voltage-to-frequency converter and a programmable 100MHz counter. The end result is a digitized signal, fed to the minicomputer system, that is processed in the same manner as a signal from a Cary 401MR electrometer.

WPC 10 | A NOVEL STABLE ISOTOPE RATIO PROCESSOR AND

ITS PERIPHERALS. H. Rache, H. Rayens, H. Grebe and K. Habfast, Finnigan MAT GmbH, Bremen 14, Federal Republic of Germany.

It has been shown in the past that a precision of better than 0.004 per mil can be obtained for the routine measurement of, for instance, the isotope ratio of $^{13}\text{C}/^{12}\text{C}$, using sample amounts down to 10 micro mol. A relatively expensive mass spectrometer is needed for such measurements.

However, many applications require much less precision as the samples' inhomogeneity is less demanding. Therefore, we have designed a stable isotope ratio mass spectrometer which combines precision and low cost. While costs have been reduced by more than half the traditional cost, precision is still 0.02 per mil at sample amounts of approx. 30 micro mol.

The design of the mass spectrometer has been complemented by the development of sample preparation lines for carbonates, organic carbon, organic nitrogen and oxygen/hydrogen from water samples, most of which are coupled on-line to the mass spectrometer for unattended multi-sample operation. The preparation lines are under control of a microprocessor which, in turn, is coupled to the (host) computer of the mass spectrometer. This distributed processing concept allows completely automated measurements, 24 hours a day, and show an extremely high degree of flexibility and simplicity of operation.

WPC 12

CALIBRATION AND SAMPLE PREPARATION FOR

HIGH FIELD FAST ATOM BOMBARDMENT MASS SPECTROMETRY; Lawrence R. Phillips and Blair A. Fraser; Division of Biochemistry and Biophysics, Office of Biologics, National Center for Drugs and Biologics, Food and Drug Administration, 8800 Rockville Pike, Bethesda, Maryland, 20205.

Employing fast atom bombardment and the high field technologies of the Kratos MS 50 HF/DS system, conditions were established for analyzing as little as 5 pmol of an underivatized peptide delivered in a glycerol sample matrix as a thin film unto a gold-plated copper sample stage and bombarded with xenon fast atoms. Conditions were similarly established for the examination of selected oligosaccharides and oligonucleotides. Calibration of the fast atom bombardment high field mass spectrometer and data system was achieved using cesium iodide in glycerol as a reference, although several other alkali halides were examined and evaluated for this purpose. Several factors were studied for their contributions to the quality of the mass spectrum: components within the glycerol matrix such as various salts, acids, and reducing agents; the nature of the fast atom gas; concentration of the sample delivered to the sample stage; and the effect of the sample stage and sample matrix on sensitivity.

The Use of a High-Resolution/Precision Gas Mass
Spectrometer to Obtain Inert Gas Isotope Ratios

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Inert gas isotope ratios for gases such as Xenon and Krypton are vitally important in the nuclear and fuel-reprocessing industries since the values of the ratios indicate the origin and "burn-up" state of the fuel rods. In addition, other non-natural isotopes such as ^{85}Kr can be monitored to give further information about the fuel, and possible leaks in the containment element.

The Xenon or Krypton gases can be present in a carrier gas such as ^4He or air. When the quantities of inert gases become small in a given sample (perhaps less than 1,000 parts per million), it is found that the residual hydrocarbon background peaks in the mass spectrometer can strongly interfere with the measurement of the low abundance isotope peaks of the inert gases. To overcome this interference it is useful to use a mass resolving power of about 1,000 so ensuring that all hydrocarbon peaks will be fully-resolved even when the inert gas isotopes are at the parts per billion level. The figure shows that natural Xenon isotope peaks in air can be resolved and detected at 1,000 resolution even though the source housing of the mass spectrometer is unbaked and shows hydrocarbon contamination peaks at about the parts per million level.

We have devised a method whereby a high-resolution, single collector mass spectrometer may be readily adapted to yield precision isotope ratios for gases such as Xe, Kr and SO_2 . Using a newly developed fast peak measurement procedure, a typical isotope peak is recorded every 6 to 8 seconds, running through the isotope family of Xe (9 isotopes) in about 63 seconds. This technique produces the best possible performance from a single collector instrument giving ratio reproducibilities of between 0.02% and 0.05% for major peaks.

ISOTOPE RATIO MEASUREMENTS

Xe Isotopes

Two data sets for Xe were taken on consecutive days. The Xe was a 7% component in ^4He carrier gas. Nine repeat measurements were taken on a single sample as it was depleting and depletion corrections were automatically calculated. Precision values (1 sigma) were better than 0.05% on both days for the major components and day-to-day reproducibility was also within this precision value. Precision values for the minor isotopes (^{124}Xe and ^{126}Xe) varied between 0.4% and 0.17%.

Xe and Kr Mixture Analysis

The same gas mixtures were also analysed for total Xe and total Kr using the normal peak jumping and mixture analysis software. Fresh gas samples were taken and the results for two gas mixtures are seen: Mixture A contained 6.172% total Xe and 0.6775% total Kr giving a Xe/Kr ratio of 9.11; Mixture B contained 0.6736% total Xe and 0.0744% total Kr giving a Xe/Kr ratio of 8.87. The actual value of the Xe/Kr ratio was 9.05 showing that the observed ratios were about 2% relative or closer to the actual value.

Kr and SO_2 Isotope Ratios

Further ratio measurements have been taken on Kr and SO_2 with similar precision to those obtained for Xe. Recently ^{85}Kr has also been included in the ratio measurements but no results are available at the time of writing. Identical software routines to those used for the Xe work were adopted for the Kr and SO_2 measurements.

An ion gun with focussing optics for organic SIMS

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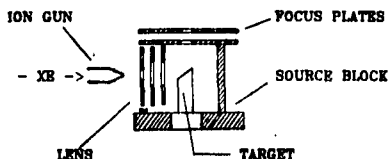
Capillaritron-style ion guns have previously been used for FAB and organic SIMS (1,3). In our hands, sample depletion occurs within 5-10 minutes when employing such a gun at typical operating conditions of 10-20 μA primary ion current. Gun performance has deteriorated after some time, due to enlargement of the orifice from which the gas discharge occurs. To improve the performance of this gun, we investigated the effect of focussing the primary ion beam.

The gun consists of a nozzle with a 25 micron orifice through which the xenon flows. The gun is operated at 6-8kV with respect to the source. The lens consisted of three elements; the outer two at source potential and the inner voltage was adjusted to maximize the secondary ion current. The assembly was fitted onto a modified FD source of a ZAB-2F mass spectrometer.

A fluorescent screen was used to attempt to determine the size of the beam. In the absence of accelerating voltage, a spot of approximately 0.3mm diameter was observed. However, with 6kV source potential, the fluorescent pattern appeared as a band, approximately 2mm wide, aligned in the direction of the entrance slit of the mass spectrometer.

A sample of methionine-enkephalin ($\text{mw}=573$) was used as a test compound. A 100ng sample mixed with glycerol/oxalic acid gave a signal for MH^+ that was at least $\text{S/N} > 4$ for 25 minutes with a focussed beam at 1 μA primary ion current. To achieve an equivalent MH^+ signal required 9 μA primary ion current with the unfocussed gun, and the MH^+ signal only lasted for 2-3 minutes. The collected secondary ion yield for MH^+ was approximately 7X higher for the focussed primary beam.

No deterioration of gun performance has been observed over several weeks operation. There is less back-sputtering when operating at lower primary ion currents, thus the isolation ceramics do not require frequent cleaning. The present design requires an extra power supply for the focus plate, but should be operable using a voltage divider with the ion gun power supply. The source is small and inexpensive and could be added to many mass spectrometers without the necessity for vacuum housing modifications.



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ANALYSIS OF METASTABLE IONS IN FAB SPECTRA

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The FAB technique has shown its merits in the field of peptide analysis in terms of molecular weight determinations as well as yielding sequence information. Especially the negative mode of ionization has been very successful in sequencing, since the majority of significant fragmentation processes yield terminal fragment ions. However, the matrix (mostly glycerol is used) may give also a significant contribution to the mass spectrum, making the attribution of substance ions difficult in the lower mass range.

To overcome this type of background metastable's analysis is an ideal tool. By means of the newly developed linked scan processor, featuring a 16 bit microprocessor, the Finnigan MAT 8200 mass spectrometer is able to perform all the hitherto available metastable ion scans on a routine basis.

Of special interest in the field of peptide analysis are the $B/E = \text{const.}$ and the neutral loss capabilities. With the $B/E = \text{const.}$ all the daughter ions of a given precursor are measured, preferably with collisional activation. The neutral loss scan is valuable in obtaining information on fragmentation processes with a constant nominal mass difference throughout the spectrum. This could possibly indicate the loss of amino acids or other groups of the molecule.

Linked scans of the first and second field free region will be demonstrated in peptide analysis.

APPEARANCE OF DOUBLY-CHARGED IONS AS A MEASURE OF FIELD EFFECTS IN DESORPTION MASS SPECTROMETRY

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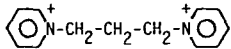
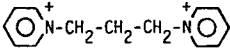
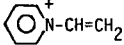
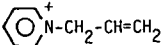
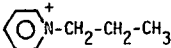
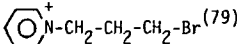
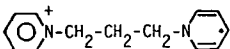
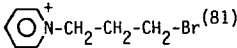
Doubly-charged ions are rarely encountered in desorption methods (1-3). For divalent ions, the production of singly-charged species by clustering with counter ions, decomposition, and one-electron reduction are generally observed. Doubly-charged ions are however more frequently observed in field desorption than in the other desorption methods, indicating the importance of the field in their extraction from the surface. The observation of doubly-charged ions might therefore be useful as a probe of field effects in desorption mass spectrometry.

The mass spectra of diphosphonium and bis(pyridinium) salts, using field desorption, laser desorption and fast atom bombardment were compared. Doubly-charged ions were observed with greatest intensity in the field desorption spectra. In the fast atom bombardment and laser desorption spectra, trimethylene bis(pyridinium bromide) shows a singly-charged cation (m/z 200) corresponding to reduction of the intact dication (TABLE I.), as confirmed by high resolution FAB. Ions corresponding to clusters of cations and anions, i.e. C_2Br^+ and $C_2Br_2^+$, are also singly-charged and result from reduction of the dications.

These observations can generally be explained by the greater activation energy for removal of doubly-charged ions from a surface, interpreted as a larger induced "image potential", which is easily overcome in the presence of a high field.

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This work was supported by grants from the National Science Foundation, PCM-820-9954 and CHE-80-16440, and carried out at the Middle Atlantic Mass Spectrometry Facility.

TABLE I Trimethylene bis(pyridinium bromide)  2Br^-				
m/z	FAB % R.A.	FD % R.A.	accurate mass	probable structure
100	-	18		
106	38	-		
120	100	100		
122	31	-		
200	45	16	200.0075	
			200.1308	
202	20	12	202.0052	
279, 281	20, 19	20, 18		$\text{C}^{+2} \text{Br}^{-1}$
479, 481	4, 3.5	-		C_2Br^+
558, 560 562	0.3, 1, 0.4	-		C_2Br_2^+

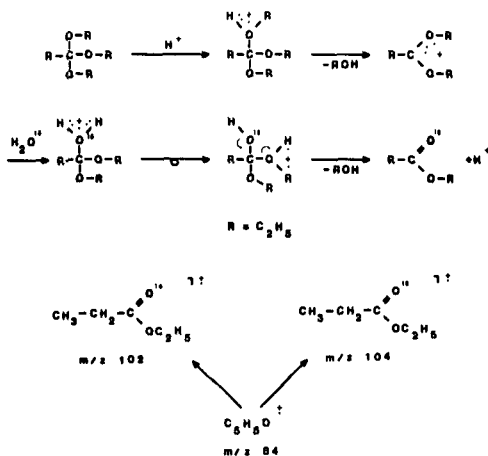
USE OF METASTABLE IONS FOR ACCURATE DETERMINATION OF OXYGEN-18 IN WATER

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Oxygen-18 is widely used in chemistry as well as in biochemistry. Nevertheless, oxygen-18 quantification at ppm level from natural abundance is still the attribute of expensive mass spectrometers (MS) dedicated for isotope measurement.

Taking advantage of the capability of double focusing MS to trace back parent ions of a given metastable ion, we have developed a two step method, usable for routine analysis, to quantify oxygen-18 at ppm level in water samples.

First, oxygen from water is selectively introduced in the carbonyl group of ethyl propanoate by hydrolysis of its ortho ester. The reaction is fast and reproducible. Second, the obtained mixture is injected through the septum inlet into the source of a double focusing MS and parent ions from ion 84^+ (loss of water from molecular ion) are retraced by scanning up the accelerating voltage.



The ratio of the intensities between transitions $84^+ \rightarrow 102^+$ and $84^+ \rightarrow 104^+$ yields, after calibration, a direct measurement of the oxygen-18 content in water sample.

The calibration curve is linear between 0.0 and 0.5% of oxygen-18 with R-square = 0.99994. At higher concentration the obtained curve responds to a polynomial equation of degree 2 with similar coefficient of correlation. The curves are reproducible within a variation of less than 0.3% whatever the concentration of oxygen-18 in the sample.

The method developed is rapid and usable for routine analysis with a great number of samples.

CHARACTERISTICS OF A SURFACE IONIZATION SOURCE WITH A POINT EMITTER

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In recent years the formation of a fine ion beam has been demanded in the fields of microsurface analysis by secondary ion mass spectrometry (SIMS), microfabrication, etc. Especially, the use of a cesium ion source with a high brightness is very important in the high sensitivity SIMS using the negative secondary ion emission to improve its spatial resolution over conventional Cs ion sources operated in the surface ionization mode by means of a porous W flat emitter. Thus, we developed a new type surface ionization source with additional mode of electrohydrodynamic(EHD) operation.

Surface Ionization Source

Figure 1 shows the construction of this surface ionization source. It is composed of a reservoir for the source material, an emitter tip and a tungsten filament. Electrons from the filament bombard the tip to raise its temperature. Melted source material is supplied to the tip by surface migration, or from gas phase, and surface-ionized at the area of the highest temperature, leading to the source size to be very small. The use of a sharp tip allows to form a much higher electric field near the tip compared with usual surface ionization sources with a porous W flat emitter. This reduces a space charge limitation of the emission current density. The extraction field in front of the tip with near concentric shape makes the virtual source size small. Thus, we can expect this source to be operated with a high brightness.

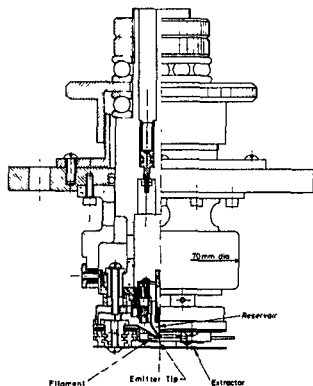


Fig. 1 Construction of the ion source.

We investigated the characteristics of this gun using CsCl as the source material. Figure 2 shows the relationship between the intensity of extracted ions within the acceptance half angle of 20 mrad and the electron current for heating. The ion intensity increases with the temperature of the tip. Current density of a few microampere per unit solid angle can easily be obtained.

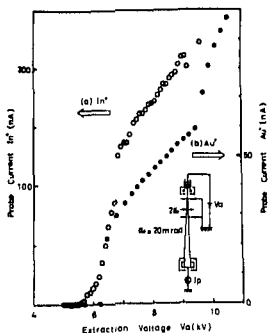
As this ion source employs the electron bombardment, some gas ions originated from CsCl vapor may be produced due to the electron bombardment. Thus, we performed energy analysis of the ions. The result is shown in Fig.3 as a chain line. To compare with the energy spectrum of ions caused purely by surface ionization, we produced Cs ions by resistive heating of

Figure 1 is a scatter plot showing the relationship between Electron Emission Current (mA) on the x-axis and Cs Ion Probe Current (nA) on the y-axis. The x-axis ranges from 0 to 15 mA with major ticks every 5 units. The y-axis ranges from 0 to 5 nA with major ticks every 1 unit. There are seven data points plotted, showing a non-linear increase in probe current with emission current. An inset diagram shows a schematic of the probe structure with labels: CsO, 2 M, and V0. Text in the top right corner specifies V0 = 8 kV and Ie = 20 mrad.

Electron Emission Current (mA)	Cs Ion Probe Current (nA)
0.5	0.2
0.6	0.3
0.7	0.6
0.8	1.1
1.0	1.8
1.2	3.2
1.4	4.8

Electrohydrodynamic Ion Source

The ion gun was operated in the EHD mode using Ga, In, Au, Zn, etc. as the source material. Figure 4 shows curves of ion probe current I vs extraction voltage V for In and Au, respectively. Current density of several tens microampere per unit solid angle can easily be obtained in both cases. Figure 5 illustrates the relationship between the energy broadening of Ga ions and the extracted current, showing typical EHD characteristics. These results prove that this gun functions also in the mode of EHD ion source with new features.



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FAST HEAVY ION INDUCED DESORPTION OF BIOMOLECULES - MECHANISM STUDIES

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In 1974 Macfarlane and coworkers discovered (1) that fission fragments from a ^{252}Cf source can desorb and ionize nonvolatile and thermally labile biomolecules from a surface. They used this ionization process in a new type of ion source for time-of-flight mass spectrometry. The technique was given the name PDMS (Plasma Desorption Mass Spectroscopy) by the authors and with this method spectacular results have been presented for large molecules. Our group has recently obtained a spectrum with a quasi-molecular ion peak for an Australian tiger snake venom protein with a mass of 13309 u. (2). It is remarkable that such a large molecule can be desorbed and ionized as one unit and knowledge about the underlying desorption mechanism is of course important in order to fully exploit this technique in future applications in mass spectrometry. It is however difficult to study the desorption process with fission fragments because they have a distribution in mass, energy and charge-state. We have therefore used beams of heavy ions from the Uppsala EN-tandem accelerator so that all parameters can be changed one at a time. The yields of molecular ions have been measured for different molecules (CsI, valine, glycylglycine, ergosterol, two protected oligonucleotides and bovine insulin) as a function of primary ion parameters like mass, velocity, angle of incidence and charge-state. In summary the experimental results are:

Yield vs primary ion mass. The heavier the mass of the primary ion the higher is the yield for a constant primary ion velocity. This holds for all molecules studied so far but the scaling with the primary ion mass is different for different molecules. No major differences in the fragmentation pattern for different primary ion masses have been found (3).

Yields vs primary ion velocity. The shapes of the yield functions are the same for different molecules but the absolute yields are different. The yield function has a maximum located at a slightly lower velocity than the maximum in the corresponding electronic part of the stopping power function. The relation to the stopping power (amount of energy deposited by the primary ion per path length) is however not obvious (3).

Yield vs primary ion stopping power. The yield of Cs^+ -ions scales roughly as the electronic stopping power to the fourth power and the yield of ergosterol to the second power. However the yield of other investigated molecules (valine, two protected oligonucleotides and bovine insulin) scales linearly with the stopping power above a certain stopping power threshold (4) but with different powers at low energy deposition rates. There seems to be no universal scaling with electronic stopping power indicating that this is not a well-defined parameter in the desorption process.

Yield vs primary ion angle of incidence. All measurements have given yield functions of the approximate shape $1/\cos^n\theta$ with $n \leq 1$ for bovine insulin, $n \geq 1$ for Cs^+ -ions and glycylglycine and $n \approx 2$ for ergosterol (5).

Yield vs primary ion charge-state. With a beam of constant energy (20 MeV ^{16}O) the charge-state dependence has been investigated for the yield of Cs^+ -, glycylglycine- and ergosterol-ions. All samples gave a q^4 charge-state dependence for charge-states above the equilibrium charge-state (6). The result for glycylglycine can be interpreted as an effective interaction depth of $\approx 60 \text{ \AA}$ of the primary ion in the desorption process (7).

We have also investigated damage cross sections for valine and bovine insulin when bombarded with 90 MeV $^{12}\text{I}^{14+}$ ions. The preliminary results indicate that each iodine ion destroys an area with a radius of about 40 $\text{\AA}$ for valine and 80 $\text{\AA}$ for bovine insulin. These areas are considerably larger than those reported on for keV ions (8) in similar systems.

This large body of experimental information has been discussed in terms of various models proposed for the desorption process like Coulomb explosion- and thermal-models. The general features of the data resemble those found for other systems which sputter electronically like condensed gases and alkali-halides.

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ION FORMATION FROM SOLIDS DUE TO FAST NON-EQUILIBRIUM PHASE TRANSITIONS: TWO NEW TECHNIQUES AND COMMON FEATURES OF THEM ALL

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There are several techniques already known to form molecular ions from organic and inorganic solids by a very rapid deposition of energy in a condensed phase surface regime. The energy may be primarily deposited either into the lattice or into the electronic system of the bulk. Primary lattice excitation is achieved by the bombardment with keV ions or atoms (SIMS, FAB-MS) or by pulse irradiation with far-infrared laser light. Primary electronic excitation is achieved by the bombardment with MeV ions (252-Cf-FFID, HIID, "PD-MS") or by pulse irradiation with ultraviolet laser light. Anyhow, the qualitative behaviour of molecular ion formation is very much alike, independent on the detailed path of primary excitation, if comparable amounts of energy are deposited, namely, 2 - 5 kJ/cm³ typically within a very short time interval. This time period should not exceed nanoseconds, better picoseconds only as done with, e.g., ion bombardment or picosecond lasers, in order to achieve also high molecular weight information. The physical and chemical properties of the secondary ions are consistent with a general description of all these processes by a far-from-equilibrium transition caused by a high-frequency perturbation of the electric environment of the surface molecules. The kinetic energy distributions of the secondary ions are in the eV regime, whereas the internal energy is in most cases well below 100 meV/degree of freedom, as vibrational excitation is strongly hindered. The ions are electronically cold and reflect the chemical distribution within the condensed phase at room temperature, especially its acidity and basicity scale concerning the distribution of anions and cations.

The only prerequisite of this kind of ion formation is to deposit sufficient energy into a certain surface regime within not more than nanoseconds. So one may think of simpler methods to do this: How this is done by lattice excitation easily is shown in another paper by F.R. Krueger (this volume) using 1 km/sec fast dust particles impinging on the surface to be analyzed (PaID; Particulate Induced Desorption).

Even more simply an electronic primary excitation is possible by conducting an electric current pulse over a wire or into an organics-coated metal target of about 100 Å or more within 5 nanoseconds or less. This pulse can also be used as trigger for starting time-of-flight mass spectrometric detection of the instantaneously produced ions (EPID; Electric Pulse Induced Desorption). Two related ion sources are shown in fig.1.

The TOF-mass spectra of tetrabutylammonium iodide as an example taken by LAMMA^R, PaID, and EPID are shown to be very much comparable. In the next figures some mass spectra taken with the new techniques, esp. EPID, are given.

One is able to say that with the new EPID-technique mass spectra of comparable information content as with laser pulses are produced, if a similar detection system as with LAMMA is used, however, avoiding on one hand the huge costs of the laser system and on the other hand the very complicated beam pass system.

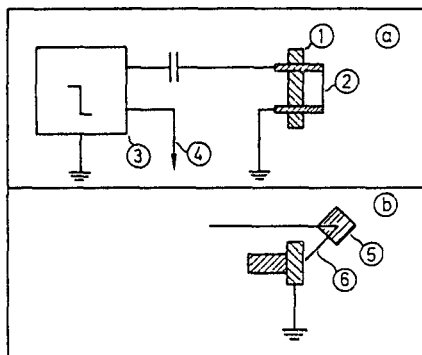


Fig.1: Electric pulse ion source arrangements;
a) (1) insulator, (2) tungsten wire, (3) fast HV-switch, (4) trigger for TOF
b) (5) insulator, (6) tungsten tip, movable on the target surface

It has been measured that the ion emission with EPID (but also with PaID and LAMMA) is directly related to the current pulse (or the excitation, respectively) in length, i.e. it does not last longer than 10 nsec. However, the wire is hot after the electric pulse for milliseconds; so, a small thermionic emission, preferably of Na^+ and K^+ , seems to occur, whereas the prompt molecular ion "rate" is several orders of magnitude larger although only for nanoseconds. So we cannot exclude that "thermally accessible" molecular ions as quaternary kations may even be produced with a higher yield over the milliseconds time interval than instantaneously with the pulse. However, due to the very high "rate" in the short time interval of nanoseconds, time-of-flight mass spectrometry is possible. Quasimolecular ions of sugar-derivatives as nucleosides, however, cannot be produced "thermally", but are easily accessible with these pulse techniques.

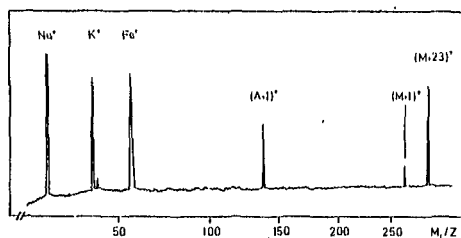


Fig.2: Original EPID-spectrum of adenosine (M) (A: adenine base). Linear intensity scale.

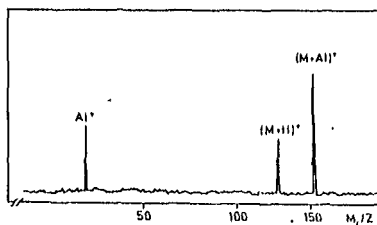


Fig.3: Original LAMMA1000-spectrum of 10 pg thymine on an aluminium foil. Linear intensity scale

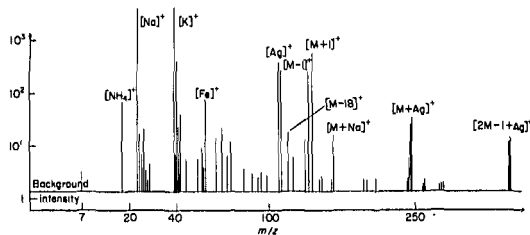


Fig.4: PaID-spectrum of adenine (M) on a silver foil. Logarithmic intensity scale.

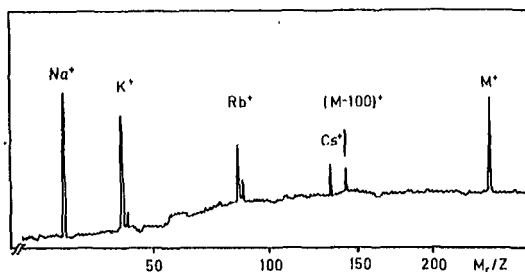


Fig.5: Original EPID-spectrum of Na-,K-,Rb-,Cs-,tetrabutyl-ammonium-iodide. Linear intensity scale.

TIME RESOLVED LASER DESORPTION MASS SPECTROMETRY

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A time-resolved laser desorption mass spectrometer has been described (1,2). This system introduced a time delay between the laser pulse which impinges upon the sample/surface and the pulse which draws the ions from the source for mass analysis. A thermal model was used to explain the delayed emission of alkali metal and organic species. The time dependence of the ion emission followed an order of decreasing ionization potentials and/or lattice energies, as the temperature resulting from the high energy laser pulse was dissipated by the substrate. In this way the competition between ion and neutral desorption, intact species and fragments, could be observed and distinguished.

This paper is concerned with two other aspects of laser desorption:

1. the kinetic energy spread of laser desorbed ions and its possible time-dependence
2. the analysis of compounds of high molecular weight, and the effects of energy spread on focussing, resolution and sensitivity for large ions.

The energy spread for K^+ does in fact vary from well above 10 e.v. immediately following the laser pulse to tenths of an electron volt within a few microseconds (3).

Time delayed mass analysis therefore has two effects upon the mass spectra of large compounds (Figure 1.). 1. Analysis of low energy ions at the appropriate delay time improves focussing of heavy ions, and hence the mass resolution. 2. The % total ion current of the heavier ions is enhanced, since the lighter ions have a shorter residence time in the source. Sensitivity for high mass is increased, because of the buildup of heavy ions in the source, during the long desorption time.

- (1) Cotter, R.J.; Van Breemen, R.; Snow, M. 30th Meeting of the American Society for Mass Spectrometry, Honolulu (1982).
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This work was funded by a grant, CHE 80-16440, from the National Science Foundation.

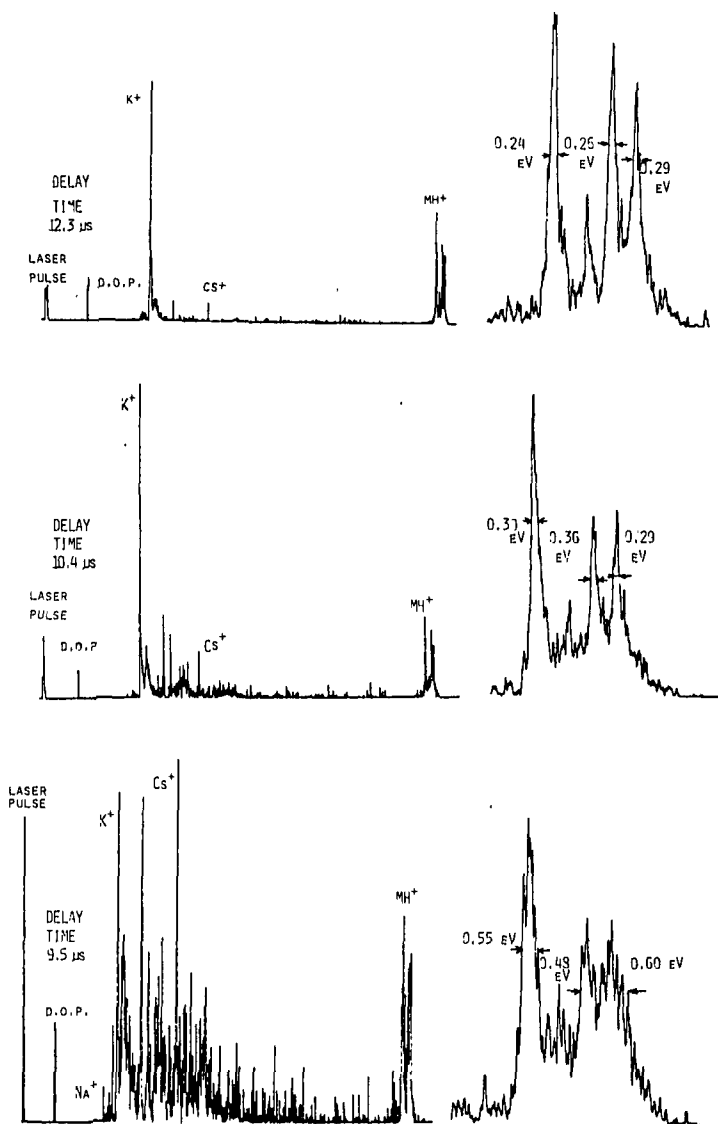


Figure 1. Laser desorption mass spectra of CYCLOSPORIN (M.W. 1201) at three different delay times between laser and drawout pulses.

THE USE OF THERMIONIC EMISSION GLASSES IN CHEMICAL IONIZATION AND FAST ATOM BOMBARDMENT MASS SPECTROMETRY; B. Ackermann, J. Allison, B. Musselman, D. Pinkston, D. Bombick, G. Dolnikowski, A. Tsarbopoulos, J.T. Watson, Departments of Chemistry and Biochemistry, Michigan State University, East Lansing, MI 48824

Certain materials produce ions when heated and positively biased. These are called "thermionic emitters". Frequently these compounds are glasses. For example, a mixture of SiO_2 , Al_2O_3 and K_2O forms a glass which can be used as a source of gaseous K^+ ions. A number of uses of these glasses is presented here.

Chemical Ionization (CI)

A technique is reported here in which a K^+ emitter is inserted into the source of an HP 5985 GC/MS via the solids probe inlet. In this way, the CI reagent ions K^+ can be generated. (Note, K^+ ions are generated, not K atoms. This contrasts with other CI reagents such as CH_5^+ , which require high pressures of CH_4 which can lead to pressure dependent CI spectra). The kinetic energy of these ions in the source can be easily varied - a unique new option for CI. By varying this energy, CI products can be "turned on and off", with different energy ranges being useful for sampling different types of molecules. For example, K^+ forms adducts with many molecules at low kinetic energies, but not at higher energies. In contrast, K^+ reacts with compounds having low IP's by charge transfer. These CI products increase with increasing kinetic energy of the K^+ . The spectrum in Figure 1 is representative of a GC/MS/ K^+ CI spectrum.

Fast Atom Bombardment

When thermionic glasses are subjected to FAB (without heating), ions are generated in abundance. When glycerol is placed on " K^+ glass", K^+ ions and $(\text{K}+\text{Glycerol})^+$ adducts are observed with some suppression of the usual glycerol FAB spectrum. Advantages of conducting FAB on glycerol/analyte mixtures placed on " K^+ glass" as opposed to metal supports are presented. $(\text{K}+\text{Analyte})^+$ adducts are observed. Detection limits are lowered by 2-3 orders of magnitude for compounds such as fructose (in glycerol) on K^+ glass tips rather than on stainless steel tips. A typical spectrum is shown in Figure 2.

An ongoing project in this group is the isotopic analysis of Pb from archeological artifacts for determination of geographical origins. A mixture of NBS standard lead as lead oxide, when mixed with Al_2O_3 and SiO_2 and subjected to FAB, provided a useful, stable source of Pb^+ . These results were sufficiently encouraging, such

that direct FAB on archeological glazes was attempted. This also yielded Pb^+ and other metal ions representative of those metals present in the glaze. Thus, direct analysis of some "solids", such as glass, by FAB may replace the much more time consuming procedures presently used.

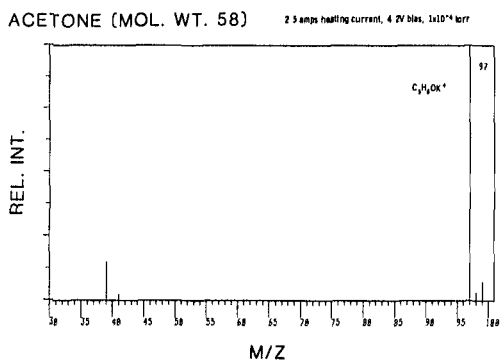


Figure 1: K^+ CI spectrum of acetone. Pressure is measured at throat of diffusion pump.

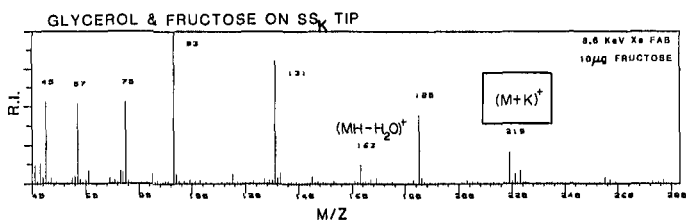


Figure 2: FAB spectrum of 10µg fructose in glycerol on " SS_K " ("K glass" coated over stainless steel) tip.

A LIQUID METAL ION SOURCE DESIGN FOR ORGANIC SIMS

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Recently we reported on the use of liquid metal ion (LMI) emitters as primary sources for molecular SIMS (1-3). Their small size, high vacuum compatibility, and lack of auxiliary vacuum or gas supply facilities are striking practical features in view of application to organic SIMS.

A simple, nonfocusing LMI source, suitable for installation within the ion source housing of most existing mass spectrometers has been developed. The ion gun can be operated with different metals or alloys and generates ion currents of 5 to 100 μ A. The design of the LMI source is depicted in Figure 1. The arrangement for the new SIMS source is shown schematically in Figure 2.

The ion gun consists of an emitter assembly, the counter electrode, a spacing ring, a collimating aperture, and the housing tube. A MAT field desorption emitter holder supports the heating filament for the LMI tip. Preparation and wetting procedure are described elsewhere (3). The emitter lifetime strongly depends on backspattering from the counterelectrode and additional apertures within the first 10 mm of the ion beam.

By placement of a conical-shaped collimator at an appropriate distance from the tip, metal deposition on the source insulators due to the widely divergent beam can be reduced. With this design we were able to operate the LMI gun even under poor vacuum conditions (10^{-2} Pa) for more than 10 hours before replacement of the tip was necessary. Examples of secondary ion mass spectra of different organic compounds obtained with the new ion gun have been described in preceding publications (1-3).

¹ D. F. Barofsky, U. Giessmann, L. W. Swanson, & A. E. Bell, in the "Proceedings of the 29th International Field Emission Symposium," H-O. Andren, and H. Norden, Eds., Almqvist & Wiksell Int., Stockholm, Sweden, 1982, p. 425.

² D. F. Barofsky, U. Giessmann, L. W. Swanson, & A. E. Bell, Int. J. Mass Spectrom. Ion Phys. 46, 1983, 495.

³ D. F. Barofsky, U. Giessmann, A. E. Bell, & L. W. Swanson, Anal. Chem., in press.

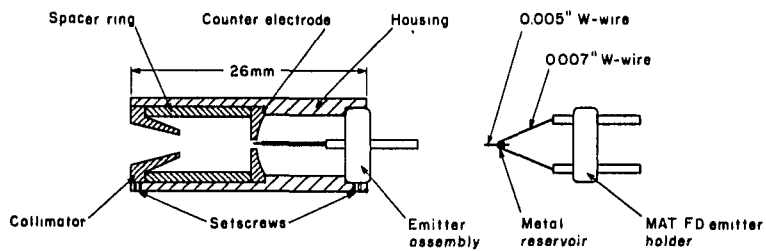


Figure 1

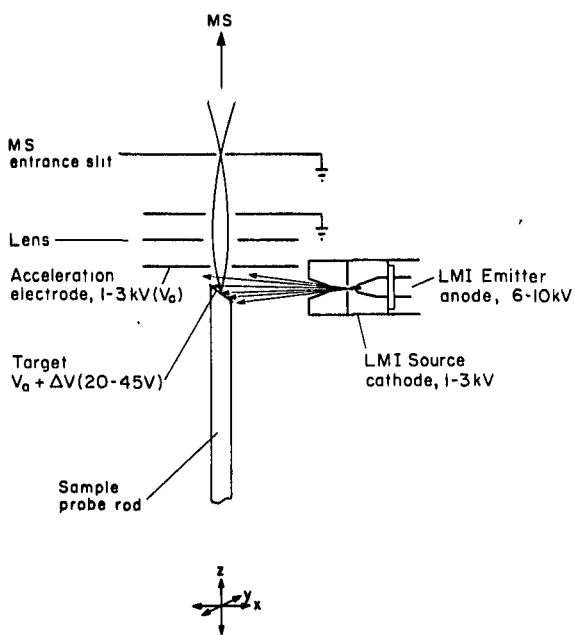


Figure 2

THE EFFECT OF METAL IONS ON THE SECONDARY ION EMISSION FROM GLYCEROL: Mike Connolly, Frank Hardcastle, and Robert Orth, Dept. of Chemistry, Montana State University, Bozeman, Mt. 59717.

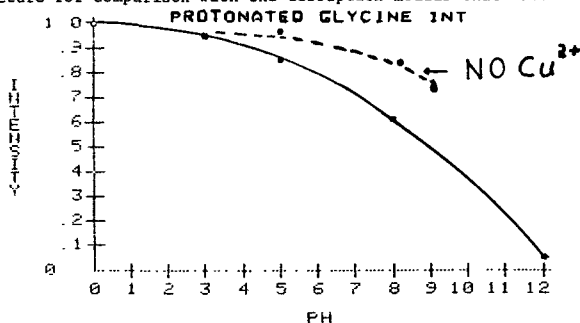
In the desorption of ions from solids and liquids it is recognized that the matrix plays an important role in the desorption of particular ions. For instance, in secondary ion mass spectrometry cationization is useful in obtaining spectra of various peptides and amino acids. In liquid substrates alkali metal ions are added which assist in observing the molecule of interest as the cationized pair. This study examines the influence of metal ions in liquid substrates such as glycerol on the desorption of glycine and adenine from the glycerol. These readily complex with such metals as Cu(II) and Co(II) forming complexes which can be monitored by UV-visible spectrometry. The amount of complexation can be controlled by adjusting the pH of the glycerol solution.

The Cu(II) complexes with the glycinate ion and the amount of glycinate ion available is controlled by adjusting the pH such that at high pH values all of the available glycine will be complexed with the copper. As the pH is lowered the amount of complexed glycine decreases until at a pH of zero essentially no copper-glycinate complex exists. The secondary ion emission indeed reflects this change. As shown in the figure below the relative intensity of mass 76, protonated glycine, decreases in the secondary ion mass spectra as the pH of the solution is increased. The (Cu-glycinate) complex is not observed in the intermediate pH range, 5 to 8, as one might expect. Indeed no complex of Cu and the glycine was observed in the positive secondary ion mass spectra.

It is clear that the loss of the desorbed ions arising from glycine is due to the complexing with the Cu(II). The lack of a cationized (Cu-glycinate) ion in the mass spectra suggests that direct desorption does not occur. However, the existence of the complex clearly influences the desorption of the glycine. The adjustment of the pH also has the effect of decreasing the intensities of the secondary ions due to glycine, but the effect is not as pronounced as when the Cu(II) is present. The effect of the Cu(II) on the glycine emission can be further observed when an excess of Cu(II) is added to the glycine-glycerol solution. At a ratio of Cu(II) to glycine of 20:1 the amount of protonated glycine observed in the secondary emission has decreased by a factor of five when compared with a solution having a ratio of 1:1.

Metals that do not complex well with the glycinate ion such as Ni(II) show no loss of intensity when compared with the solution of glycine and glycerol containing no Ni(II). Adenine and para-aminobenzoic acid were found to yield similar results. The para-aminobenzoic acid does not complex as extensively with the Cu(II) as the adenine and the glycine. This was also observed in the secondary ion mass spectra in that the secondary ions arising from para-aminobenzoic acid did not decrease as rapidly with pH change as the glycine. Ni(II) and Ag(I) were found to have little effect on the secondary ion emission of the three compounds although with Ag(I) present an enhancement of some of the secondary ions appeared to occur. Co(II) yields similar results as the copper.

In conclusion, metal ions can inhibit secondary ion emission without the production of cationized secondary ions. The results suggest that the matrix effect that affects the emission is complexation. At this time the observed results are being formulated into a picture for comparison with the desorption models that have been suggested.



An Improved Xenon Atom Source
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Developments of the Ion Tech saddle-field ion source have been widely adopted as sources of energetic neutral particles for Fast Atom Bombardment mass spectrometry (FAB MS). The neutral output of the ion source is increased (1), to an atom flux equivalent to 500uA at 3-10 KeV by operating at high pressure and by using a hollow shielded cathode. The conventional cathode has a hollow top-hat cross-section which with a 1.5mm diameter exit aperture produces a 3.5° angular spread of the emergent atom beam.

Typically a source will run for ~ 1000 watt hours, usually 1mA total beam current at 7 KeV, before servicing becomes necessary. During this period the beam erodes the aperture which increases in diameter by x2 and often becomes grossly distorted. This causes an increased angular spread and irregular collimation which results in reduced beam intensity at the sample which can lead to a loss (x4) in ultimate mass spectrometric sensitivity in the FAB mode. Additionally the gas transmission of the front aperture increases by x4 causing the pressure in the ion source region to rise by this factor.

Replacement of the standard hollow top-hat cathode with a solid version made in the same metal changes the performance characteristics and leads to a number of worthwhile improvements viz:

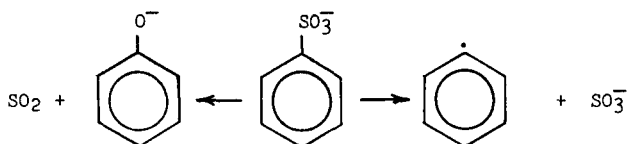
1. Reduced inert gas flow through the atom gun results in more economical operation. However the reduced gas flow demands more precise gas flow control to maintain a stable discharge.
2. Reduced inert gas flow through the atom gun and into the mass spectrometer source housing results in a significant lowering of the pressure in the latter. On a V.G. Analytical ZAB mass spectrometer the source ion gauge reading was reduced from 8×10^{-6} mm Hg to 2×10^{-6} mm by this modification.
3. The modified gun gives an initial FAB-MS sensitivity and other operating characteristics, such as dependence of atom flux on power, as the standard version.
4. Erosion of the exit aperture, which now has a very thick walled tubular shape, is much reduced. Severe erosion at the saddle-field ion source end does not penetrate through the length of the electrode which maintains its final circular profile for very much longer periods. Distortion of the final aperture profile has not been observed but there is some increase in its diameter with time but at a much slower rate than before modification. We have experienced a lengthening of the period between necessary servicing of the gun by at least x 2 i.e., we can routinely expect more than 2000 watt hours of use. We also note that the cathode is still serviceable after this time with whisker growth in the anode being the most common cause of gun failure.
5. The fixed geometry of the system results in the gun maintaining its initial characteristics for most of its useful life thus maintaining mass spectrometric sensitivity at an optimum.

The modified atom gun has been installed for several months on a V.G. Analytical ZAB-HF, reverse geometry, high resolution mass spectrometer. The high field magnet allows operation up to an upper mass limit of m/z 3000 at 8 KeV ion energy i.e., full sensitivity/mass resolving power specification. It has EI, CI, GCMS and FAB-MS capability and is coupled to a V.G. Analytical 2050 data system. It is used for general chemical analysis problem solving with FAB, high mass or high sensitivity applications predominating as illustrated in the following example.

Example: A small quantity of an ionic impurity in an organic product was detected by HPLC but could only be isolated in the presence of a large excess of an ion pair reagent (IPR) which was tetrabutyl ammonium phosphate. Excellent stable FAB mass spectra resulted from bombarding the sample in glycerol with 7 KeV Xe atoms. The low mass part of the positive ion spectrum is dominated by the cation, Bu_4N^+ from the excess of IPR. At higher mass we see species with a single positive charge resulting from aggregation of two tetrabutyl ammonium cations with any singly negatively charged species available. Thus IPR was shown to contain the anions, Cl^- , H_2PO_4^- and BuOP_3H^- while the sample contained an additional peak at 641^+ , indicating the presence of an anion with $m/z = 157^-$. This ion was observed to be enhanced in the negative ion spectrum of the sample relative to IPR alone which was dominated by phosphate ions.

High resolution mass measurement of this negative ion relative to a fragment ion from the glycerol dimer showed the major component of the multiplet to be $\text{C}_6\text{H}_5\text{SO}_3^-$, consistent with the impurity being benzene sulphonic acid.

This assignment was confirmed by comparison of metastable scans on the sample with that from IPR alone. These spectra were obtained by CID of 157 by collision with air in the first field-free region of the ZAB. Daughter ions of lower mass m_2 , formed with lower energy $E_{\text{cm}2}/157$, are transmitted to the collector during a B/E linear scan. Both showed the transitions, confirming the structural assignment.



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Acknowledgment

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Desorption chemical ionization(D/CI) mass spectrometry has become a popular technique for the analysis of involatile and thermally labile compounds, because of its potential usefulness for its relatively low cost.¹⁾ Evaluation of the analytical potential is imposed on the problems of how to enhance the desorption of "intact neutral molecules" and how to reduce the competitive process of "thermal decompositions".²⁾ It is considered that various parameters(e.g. features and heating-current rate of emitter wire and chamber conditions) are significantly concerned with the desorption rate of "intact neutral molecules".

We have now carried out the experiments to determine the optimum D/CI conditions in order to extend further the usefulness of the method. We have selected sucrose as a model compound and restricted reagent gas to ammonia. A typical D/CI(NH_3) mass spectrum of sucrose contains a QM^+ ($[\text{M}\cdot\text{NH}_4]^+$; m/z 360) and fragment ions due to the glycosidic bond cleavages(e.g. $[\text{S}\cdot\text{NH}_3]^+$; m/z 180).

1. EMITTERS

1-1. Features Our D/CI emitter consists of coiled wire (tungsten, $\phi 50\mu\text{m}$) combined with FD-like holder.

To determine the optimum emitter features for productions of $[\text{M}\cdot\text{NH}_4]^+$, we examined three kinds of wires with different

pitch(0.2, 0.5, 0.7mm), however no appreciable spectral changes were observed.

1-2. Surface When the wire surface was covered with "silicon-microneedles", a pronounced enhancement of $[\text{M}\cdot\text{NH}_4]^+$ ion production was observed; the ratios of $[\text{M}\cdot\text{NH}_4]^+ / [\text{S}\cdot\text{NH}_3]^+$ as well as $[\text{M}\cdot\text{NH}_4]^+ / \text{total ion intensity}$ were increased by the use of silicon activated wire in comparison with those in the examination of naked wire(Table 1).

1-3. Heating rate The effect of emitter heating-current rates(1, 2, 4, 8, 16 mA/s) on the ratios of $[\text{M}\cdot\text{NH}_4]^+ / \text{total ion intensity}$ was also examined. When a faster heating rate is used, larger total ion percentage of $[\text{M}\cdot\text{NH}_4]^+$ is generally obtained, but the life time becomes shorter.

2. CHAMBER CONDITIONS

2-1. Temperature When chamber temperature is raised, more extensive fragmentation occurs and $[\text{M}\cdot\text{NH}_4]^+$ intensity decreases. For instance, the ratios of $[\text{M}\cdot\text{NH}_4]^+ / [\text{S}\cdot\text{NH}_3]^+$ were found to be drastically dependent on chamber temperature(Fig.1). Other temperature effects are summarized in Table 2.

2-2. Reagent gas pressure The most outstanding effect at different gas pressures was a dependence in sensitivity. The maximum absolute sensitivity was achieved at housing pressure in the range of $4\text{--}5 \times 10^{-5} \text{ Torr}$ (Table 3). In contrast, the ratios of $[\text{M}\cdot\text{NH}_4]^+ / [\text{S}\cdot\text{NH}_3]^+$ were independent of gas pressures. In this examination, "best" spectrum for qualitative analysis was recorded at the lowest pressure($2 \times 10^{-5} \text{ Torr}$), where interfering peaks in low mass region were very low intensity as shown by the highest total percentage of $[\text{M}\cdot\text{NH}_4]^+$ (Table 3).

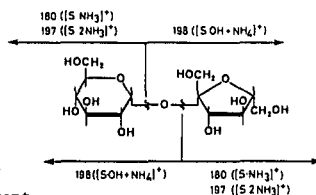
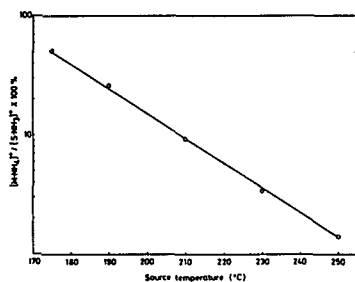


Table 1.. The ratios of characteristic peak intensities of sucrose desorbed from naked wire vs. silicone activated wire^a

	Normalized ratios of [M-NH ₄] ⁺ int./[S-NH ₃] ⁺ int. ^b	Maximum ion int. (counts) Total int.	[M-NH ₄] ⁺	Normalized ratios of [M-NH ₄] ⁺ int./total int. ^c
Naked wire	1.0	0.52 X 10 ⁷	5890	1.0
Activated wire				
1st	2.3	0.52 X 10 ⁷	82900	14.5
2nd	3.1	0.61 X 10 ⁷	110500	16.5
3rd	1.9	0.50 X 10 ⁷	40300	7.4
4th	3.3	0.33 X 10 ⁷	63500	17.5
5th	1.7	0.35 X 10 ⁷	51300	13.5
6th	1.7	0.48 X 10 ⁷	24640	4.6

^a Conditions: source temp.; 170°C. housing pressure; 2 X 10⁻⁵ Torr, sample size, 4µg, heating current rate; 2 mA/s. ^b Data obtained at the BET spectra. ^c Data obtained at the point of maximum intensities of total ion intensity profiles and [M-NH₄]⁺ mass chromatograms.



Temperature dependence of the relative intensity of characteristic ions for sucrose, m/z 360/m/z 180

Fig. 1

Table 2. Source temperature effects on D/CI(NH₃) mass spectra of sucrose

	lower source temp.	higher source temp.
sensitivity	low	high
fragmentations	reduced	enhanced
shape of total ion current profiles	sharp	dull
best emitter temp. (BET)*	high	low

* the current affords the highest abundance of ions characteristic of sample weight.

Table 3. The spectral change of sucrose at various source pressure*

Housing pressures (Torr)	Maximum ion intensities (counts)			BET(mA)	[M-NH ₄] ⁺ int. under BET conditions (total ΣI ₁₀₀)
	Total int.	[M-NH ₄] ⁺	[S-NH ₃] ⁺		
2 X 10 ⁻⁵	0.13 X 10 ⁷	16500	16710	145	17.5
3 X 10 ⁻⁵	0.48 X 10 ⁷	99600	54100	149	5.0
4 X 10 ⁻⁵	0.60 X 10 ⁷	156300	58700	162	5.1
5 X 10 ⁻⁵	0.57 X 10 ⁷	137000	62900	176	6.0
6 X 10 ⁻⁵	0.40 X 10 ⁷	70200	41800	178	4.1
7 X 10 ⁻⁵	0.12 X 10 ⁷	73000	16500	188	7.6

* Conditions: sample size; 4µg, heating current rate; 2 mA/s, source temp., 170°C

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MECHANISM FOR PRODUCING PSEUDOMOLECULAR IONS FROM NONVOLATILE MOLECULES

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A number of apparently dissimilar techniques have been developed for producing intact pseudomolecular ions from nonvolatile samples. A common feature of all these methods appears to be "direct" production of ions from a condensed phase without formation of neutral gas phase molecules as intermediates. Also, these new techniques produce relatively stable, even-electron species similar to those present in solution rather than the odd-electron species produced initially by gas-phase electron ionization. Work related to the mechanism of the processes involved has been summarized in recent reviews.^{1,2}

Recently, a technique was discovered for producing ions from liquid solutions which required neither electrical field nor an external source of ionization. In this "thermospray" technique, electrolytic solutions are vaporized by forcing them through a small diameter- (ca. 0.015 mm) capillary tube which is heated sufficiently to vaporize a substantial fraction of the liquid and produce a supersonic jet of vapor in which liquid droplets and/or solid particles are entrained. Samples whose volatility is less than that of the solvent are preferentially contained in these droplets or particles. It appears that ions are produced by field-assisted vaporization of molecular ions or small clusters from charged droplets.

The objectives of the present studies are to further elucidate the mechanism of thermospray vaporization and ionization and to relate this mechanism to other techniques for producing pseudomolecular ions from nonvolatile molecules. The initial emphasis in these studies is placed on fundamental studies of the vaporization process with the goal of obtaining quantitative data on the kinetics of ion evaporation from liquid droplets. The experimental techniques being developed include mass spectrometry, light scattering, high-speed flash photography, time-of-flight analysis, mobility analysis, and microscopic and photographic observation of charged droplets in electrical fields.

This paper presents the first results from high-speed flash photography of the thermospray jets. Also, a new experimental vaporizer is described in which the energy required to vaporize the liquid is provided by direct Joule heating of the stainless steel capillary. This technique allows the power coupled into the liquid to be more precisely monitored and allows mass spectrometric observation to be correlated with the fraction of the liquid initially vaporized. These studies together with theoretical analyses have led to a much better understanding of the primary energy deposition and vaporization processes occurring in the thermospray technique, but considerably more work will be required to obtain needed data on the kinetics of ion evaporation.

From these studies a conceptual "unified" model has been developed which may be applicable to other processes for producing ions from nonvolatile samples. The steps in this model include: (1) energy deposition at a sufficiently high rate to produce a locally superheated fluid, (2) nonequilibrium boiling of the fluid, (3) spraying of charged and neutral clusters and droplets along with vapor molecules and ions, and (4) production of molecular ions by either field-assisted ion evaporation from charged droplets or by reactions of ion with

neutral clusters.

Many aspects of this model remain to be studied in detail; but we tentatively conclude that *ion evaporation from charged droplets or particles is the primary ionization mechanism* in thermospray ionization and that this mechanism may be important in other "soft" ionization techniques. The critical variables are the size, charge, and effective temperature of the clusters and droplets initially produced. Quantitative data are needed on the rates of ion production as functions of particle radius, charge, and temperature. We suggest that other ionization techniques (e. g. FAB, SIMS, ^{252}Cf , and laser desorption) may involve production of localized, transient "thermosprays" at the point of interaction with the surface.

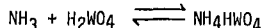
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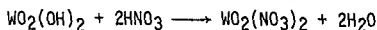
AN INVESTIGATION OF THE ADSORPTION AND THERMAL DESORPTION
PROPERTIES OF SOME SURFACES BY MASS SPECTROMETRY"

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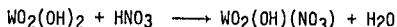
The adsorption of gaseous NH_3 and HNO_3 onto the tungstic acid coated surface of a hollow tube followed by the thermal desorption of these substances forms the basis of an established analytical technique for the determination of these two gases at very low concentrations in atmospheric samples¹. The chemistry of the adsorption and desorption of ammonia on the tungstic acid surface is not entirely clear. However, tungstic acid is known to be a weak acid and the adsorption is thought most likely to proceed through an acid-base type reaction:



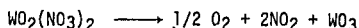
This reaction is reversed by heating the tungstic acid surface to approximately 350°C , at which temperature the adsorbed NH_3 gas is released. The mechanism of nitric acid adsorption is also not clearly understood, but experimental work indicates that irreversible chemisorption is involved. Adsorption may take place due to the formation of an inorganic acyl nitrate:



or



Thermal decomposition of this type of compound would be expected to occur in a manner similar to that of other nitrates:



Experimental evidence indicates that the adsorbed nitric acid is released as NO_2 gas on heating of the tungstic acid tube to approximately 320°C . Prior to this work detection of the desorbed gases was confined to the identification of nitrogen containing substances using a commercial model Bendix NO_x analyzer.

This study was undertaken to investigate further the adsorption and thermal desorption properties of the tungstic acid surface, and also to investigate these properties for some other surfaces; the long term goal of the project being the development of analytical methods for other environmentally important substances.

The surfaces chosen for study in addition to tungstic acid were: silver, copper, gold, platinum and carbon. Hollow quartz tubes with interiors coated with these substances were prepared.

Experimental - To accommodate the small sample size of the desorbed gases (typically less than 500 ng) a special inlet system was constructed which allows the gases to leak directly into the source region of a Time-of-Flight Mass Spectrometer (CVC - MA2). The sample tubes can be attached and detached readily from the inlet system using Ultratorr fittings. A system of valves allows the atmosphere in contact with the surface being studied to be pumped away at the start of each run. The temperature of the surface being studied was increased by passing a current (controlled by a Variac) through a coil of wire wrapped around the tube. The tube temperature was calibrated using the Variac setting in conjunction with a thermocouple positioned inside a blank sample tube during a dummy run.

Sample tubes were prepared in one of two ways:

1. Atmospheric air (usually from the roof of the Physics building) was drawn through the tube using a small pump for a period of several hours.
2. Known substances, e.g. NH_3 , HNO_3 , SO_2 , HCN were loaded onto the tubes, usually by allowing the gas to diffuse through the tubes.

Typically mass spectra were obtained as follows:

- (i) Mass Spectrometer background
- (ii) Atmosphere in contact with tube surface
- (iii) Tube surface background
- (iv) Gases desorbed at a series of increasing temperatures.

Results and Discussion - As the temperature of the surface under study was increased the intensity of the $M/e = 44$ peak was observed to increase and the intensity of the $M/e = 32$ peak was observed to decrease. This phenomenon was observed for all of the surfaces studied, whether the sample tubes were 'loaded' or 'blank'. In several cases the intensity of the $M/e = 44$ peak was two to three times greater than the intensity of the $M/e = 32$ peak at the higher temperatures studied. The conclusion drawn from these observations is that all of

these surfaces appear to catalyze a reaction which converts oxygen to carbon dioxide. This reaction may involve carbon deposited in the production of the surface or residual hydrocarbons adsorbed onto the surfaces.

In addition to this general finding specific results were obtained for the different surfaces.

Tungstic Acid.

(i) Roof Air (70eV) A very intense peak at $M/e = 18$ was observed, which increased as the temperature was increased. This indicated a large amount of water had been adsorbed by the surface. Two peaks were observed at $M/e = 64$ (SO_2^+) and $M/e = 48$ (SO^+) which exhibited a definite desorption temperature profile. This indicates the adsorption and thermal desorption of SO_2 .

(ii) Loaded with NH_3 (15eV) Ions were observed at $M/e = 17$ (NH_3^+) and $M/e = 30$ (NO^+). This indicates that some of the adsorbed NH_3 is desorbed as NO under these conditions.

(iii) Loaded with HNO_3 (70eV) An intense peak at $M/e = 30$ (NO^+) and a much smaller peak at $M/e = 46$ (NO_2^+) were observed. Both of these exhibited a definite desorption temperature profile, indicating that under these conditions most of the adsorbed HNO_3 is desorbed as NO and a small amount is desorbed as NO_2 .

Silver

(i) Roof Air (70 eV) No particular increase in $M/e = 18$ was observed, indicating that not much water was adsorbed onto this surface. However, intense peaks at $M/e = 64$ (SO_2^+) and $M/e = 48$ (SO^+) with a definite desorption temperature profile were observed, indicating the adsorption of SO_2 . A medium sized peak at $M/e = 27$ and smaller one at $M/e = 26$ were observed, both with a definite desorption temperature profile. This was suggestive of HCN and was tested by (ii) below.

(ii) Loaded with HCN (70eV) An intense peak at $M/e = 27$ (HCN^+) and a smaller one at $M/e = 26$ (CN^+) were observed, with a similar temperature desorption profile to that observed in (i). From this it was concluded that HCN is adsorbed and desorbed in that form.

(iii) Loaded with NH_3 (15eV) Peaks were observed at $M/e = 17$ and $M/e = 30$, indicating that ammonia is adsorbed onto the silver surface and is thermally desorbed, some of it as NH_3 and some of it as NO.

(iv) Loaded with HNO_3 (70eV) A very small peak at $M/e = 30$ was observed and no peak at $M/e = 46$. In contrast to the tungstic acid surface, the adsorption of HNO_3 onto the silver surface appears to be a minor process.

The other four surfaces have not been studied as extensively. Conclusions for these are summarized below.

Copper: This surface adsorbs water to a small extent. It adsorbs HNO_3 which is thermally desorbed as NO. It also adsorbs and thermally desorbs SO_2 .

Platinum: This surface adsorbs and thermally desorbs water to an appreciable extent. It also adsorbs HNO_3 to a smaller extent than the copper surface and it is thermally desorbed as NO. SO_2 is adsorbed and thermally desorbed and there is some evidence to indicate that HCN will also adsorb and thermally desorb on this surface.

Gold: This surface adsorbs and thermally desorbs large amounts of water. Some HNO_3 is desorbed as NO and SO_2 is adsorbed and thermally desorbed.

Carbon: This surface adsorbs and thermally desorbs large amounts of water. It appears to adsorb HNO_3 very efficiently and this is thermally desorbed as NO and again there is some evidence that this surface may adsorb and thermally desorb HCN.

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MASS SPECTRA OF IONS FORMED BY DUST PARTICLE IMPACT FOR EXPLORATION OF THE COMETARY DUST COMPOSITION

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In 1986 the comet Halley will meet again the inner solar system and get his perihelion distance in April. During this period two space missions are dedicated, i.e., to measure the composition of the dust forming the comet's tail by means of mass spectrometry. The European Space Agency (ESA) will have its GIOTTO mission which spacecraft will carry several instruments among them a Particle Impact Analyzer (PIA). A similar Soviet mission will measure the dust composition by means of an analogous instrument, named PUMA.

The mass spectrometric method makes use of the fact, that during an impact of a fast dust particle on the surface of a solid material, instantaneously atomic and molecular ions from the dust particulate and the target material are emitted. These ions are detected by time-of-flight mass spectrometry. The start signal can be achieved either by the light flash which accompanies the impact or by the charge pulse at the target. The time-dependent ion current at the multiplier is sampled electronically. The sample intervals can be equidistant as being used in previous simulation experiments with a Biomation[®] transient recorder. In the mission experiments the samples are taken according to intervals given by a t^2 -generator in order to achieve a linear mass scale.

The simulation experiments have been performed with the Heidelberg Dust Accelerator, which accelerates dust particulates of .2 to 20. μm diameter to velocities of 1. to about 60. km/sec. Smaller particles refer to higher velocities and v.v. The arrangement is depicted in fig.1.

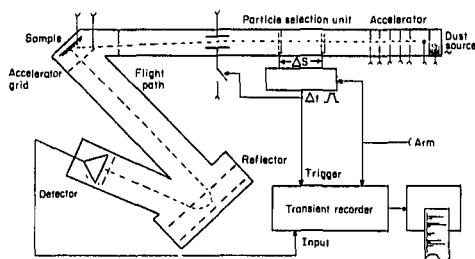


Fig.1: Dust accelerator and TOF mass spectrometer for dust impact simulation and dust particle induced desorption.

The composition of the mass spectra is also dependent on the velocity of the dust particulates. In fig.2 Fe-particulates of 5, 32, and 64 km/sec, respectively, are shot on to a LiBr-coated (by electrospray) Ag-metal surface. Best molecular spectra especially from organics are produced with lower velocities. However, with the GIOTTO comet mission a relative velocity of 68.7 km/sec between the spacecraft and the dust is fixed due to astronomic reasons. So, one is interested especially in the ion formation behaviour in this velocity regime, although the dust accelerator is not capable to produce so fast particulates.

As most particulates in the comet's dust are expected to contain mainly anorganics like water, ammonia, silicates of various composition like chondrites, the behaviour of these compounds in ion formation has to be evaluated. Glasses are the most similar products we have on earth. Thus in fig.3 a spectrum is shown produced by a 28 km/sec bombardment with Fe-particulates on a barium silicate glass. On the other hand, in fig.4 a Ca-, Mg-, Al-glass particulate itself of similar velocity is shot on to a silver foil. The mass range 1 ... 116 amu, as shown there, will also be available with the PIA instrument. One sees the contribution of several molecular mass lines, although some authors did predict, that with these velocities only atomic ions should be emitted. However, as given in another paper by P.K.D. Feigl et al. (this volume) molecular information from the solid is conserved by all rapid dissipation techniques, as for instance, this technique or FAB-MS, SIMS, Laser pulse desorption, or EPID (see Feigl). Namely, all these techniques deal with non-equilibrium surface-gas phase transitions due to rapid deposition of energy in a certain surface regime. With all these techniques molecular ion formation even from very involatile materials is a common explainable feature.

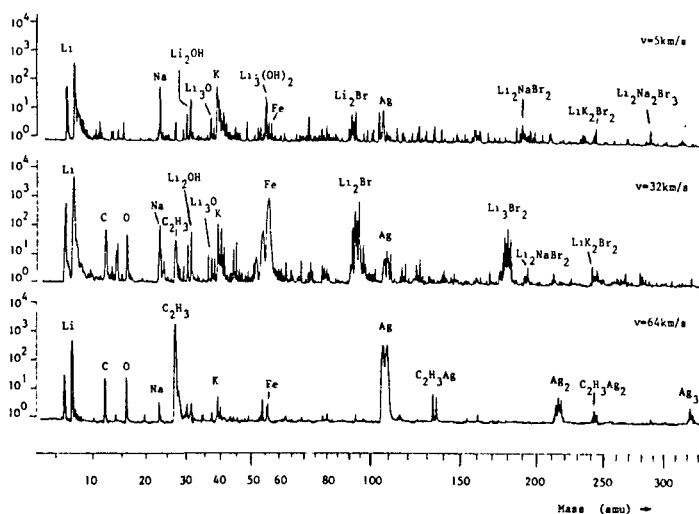


Fig.2: TOF mass spectra of a Li-Br coated silver foil produced by bombardment of 5, 32, and 64 km/sec fast Fe-particles, respectively.

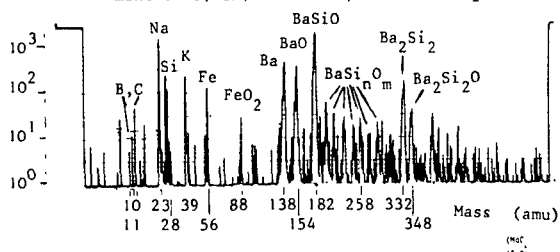


Fig. 3: TOF-spectrum of Fe-bombardment on to-(FeSi)-Si₂ barium silicate glass.

As mentioned above, mass spectra of organic molecules deposited on the target, are best produced with only 1 km/sec particulates. With those so slow, no melting or heating above 1000 K of the material is induced, however, typically 10^6 ions are produced within 10 nsec. This is explained only by non-equilibrium thermodynamics.

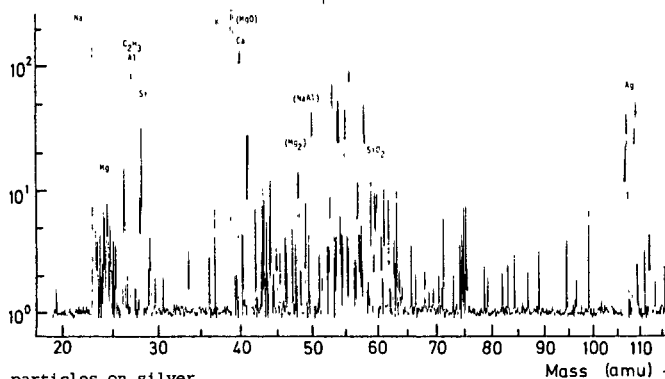


Fig.4: Glass particles on silver.

The Optimisation of Mass Spectrometer Design to Permit the Analysis of High Molecular Weight Molecules.

Taylor L.C.E., Evans S., Compson K.R., Ryan P.A., Kratos Analytical Instruments Limited

The design of an instrument for high molecular weight analysis, involves three distinct areas:- the production of ions; the transmission of these ions through the analyser; the detection of the analysed ion beam.

To design an efficient ion source, the principle that Brightness is Invariant must be considered. That is, $W \cdot \sin \alpha \cdot \sqrt{V}$ is a constant. (fig 1). In the case of FAB, the only viable technique at present for high molecular weight ionization, the ions are produced from a relatively large area, with a large initial angular distribution. Secondary ion energy spread is small probably of the order of 1 or 2 eV. This means that, provided the source does produce an image of the origin at the resolving slit, source optics do not have a first order effect on sensitivity, that is, over a wide range of source magnifications, the resolving slit and angular acceptance (α) slits of the analyser will be uniformly filled. Sensitivity is however linearly dependent on accelerating voltage, including the effect of angular spread in the Z plane, and on the efficiency of the atom bombardment process.

Efficient detector systems are now generally available, based on post acceleration. (Fig 2) By accelerating the ion beam through an additional 8 to 20kV after passing through the collector slit, then impacting the accelerated beam onto an aluminium target, the effect of the "velocity threshold" for secondary emission is much reduced, enabling masses in excess of 10000 to be efficiently detected. The source accelerating voltage should be high in order to give both maximum secondary ion extraction from the source and maximum ion impact velocity at the detector.

The analyser, therefore, should be capable of transmitting high molecular weight ions at high energy, in order to obtain maximum efficiency from the ion source and the detector. Additionally, path length through the analyser should be as short as possible, the angular acceptance in both the y and z planes high, with aberration coefficients minimised. One effective approach is to use an inhomogeneous field magnet of large radius. Kratos are now manufacturing a 27" radius, 60° magnet for MS50, which fulfills these criteria, giving a mass range in excess of 7500 at 8kV. Path length is increased to 103 inches compared to the standard instruments 80 inches, and all second order aberration coefficients approach zero (Fig 3). Inhomogeneous (wedge) fields which are strong focussing in y are inevitably defocussing in Z. To overcome this, and to increase the angular acceptance in the Z plane (α_z), a Z focussing lens is included prior to the magnet. A second Z lens can be included after the magnet to further reduce image size (fig 4). Aberrations are such that resolution in excess of 100000 will be obtained, and because of the fully laminated construction scan rates in the low mass range, i.e. up to m/z 3500, will be of the order of 0.1 s/d.

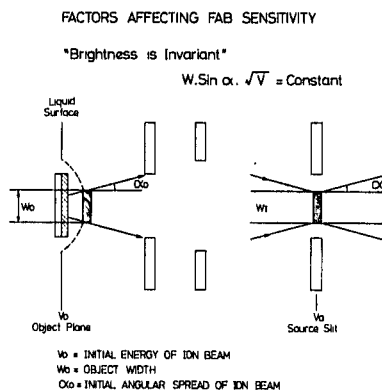


Figure 1.

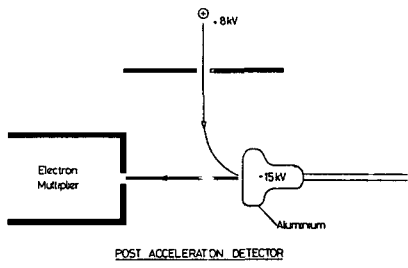


Figure 2

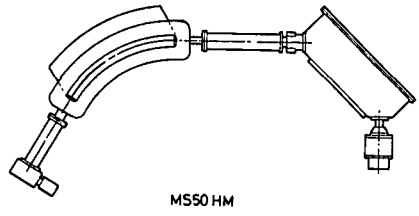


Figure 3

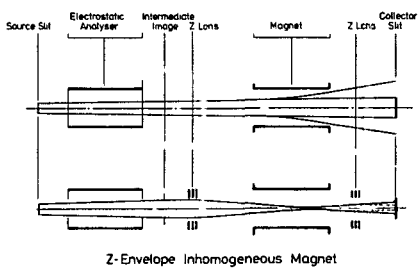


Figure 4

M550HM Provisional Specification

Magnet Radius	27 inches
Deflection Angle	60°
Mass Range at 8kV	
Low Mass Range	3500
High Mass Range	7500
Scan Rate	
Low Mass Range	0.1 s/d
Flyback	0.2 s
High Mass Range	0.5 s/d
Flyback	1 s
Resolution	> 100,000
Sensitivity	Standard M550 Specification
Detector	Post Acceleration

Figure 5

AN INVESTIGATION OF SOME UNDERLYING PHYSICAL PRINCIPLES IN FAB SOURCE OPERATION, T. A. Wittstruck and D. J. Marshall, Nuclide Corp., 916 Main St., Acton, MA 01720

Since the introduction of Fast Atom Bombardment mass spectrometry in 1981, several FAB source designs have been reported. Nuclide has developed and extensively tested a complete FAB system for the 12-90G series of magnetic deflection mass spectrometers. The design is also suitable as a retrofit to the Hitachi Perkin-Elmer RMU and other instruments.

Some performance was evaluated on a 12-90G instrument but most work was done on an older RMU spectrometer, modified with a Nuclide 6" I.D. source housing, pumped by a 4" diffusion pump system. The RMU has a mass range of ca. 500 amu at 3.5 kV ion energy and rather low resolution, but this was sufficient for evaluation of most of the characteristics of the FAB system.

The FAB gun is a modification of the electron gun used in the Nuclide Luminoscope[®]. The cathode in the Luminoscope[®] gun always shows a small, well defined, well centered spot where ions from the discharge impinge upon its surface. Over a period of weeks or months of normal use these ions will actually etch a hole through the cathode. This suggested that a well focused source of ions for FAB could also be obtained with this gun. A cylindrical exit channel was added to the cathode, of such dimensions that a relatively high pressure is maintained in this region, allowing charge exchange between ions and neutrals of the discharge gas, and thereby producing fast atoms.

The gun is mounted inside the source housing pumping arm, with the gas and beam exit 3" from the center of the ionization chamber. Its presence does not interfere with the use of other ionization sources such as EI or FI/FD and it can be left in place even when it is not in use. The reduction of the pumping speed of the 4" system, with the FAB gun in place, is less than 15%.

The alignment was determined by mounting a ZnS coated glass slide at the center of the source housing. The beam from the gun produces a circular luminescent spot on the slide. This was used to determine the exact positioning of the gun in the pumping arm. Thereafter, a docking mechanism assures maintenance of alignment.

With energetic electrons, some concern must be taken for the possibility of x-ray emission. This is of most concern when a viewing window is directly opposite the beam exit. This is not the case in our system. We made a special test fixture to measure the x-rays as viewed through a pyrex window. At 7.5 kV, in an Argon discharge, x-rays were absent. At higher energies, or with other cathode materials, one should still check for them in straight through arrangements.

The sample probe is a modification of the Nuclide Direct Insertion Probe. A 1/8" diameter threaded stainless steel rod is used. It is bevelled on the sample end at an angle appropriate for a 20 deg. interception between the FAB beam and the sample surface. The temperature of this probe tip was measured, using a thermocouple while operating the gun at 7 kV, 1 mA for 45 minutes, and did not increase more than 2 deg. C.

Gas flow into the gun, and hence pressure within the gun, is controlled by a needle valve. For a steady discharge, the Argon pressure in the gun must be maintained within a small pressure range. Flow rates of Argon were calculated, using various combinations of dimensions for gas entry and exit to the gun, from the complete Knudsen equation, in order to determine those dimensions which would provide the needed gun pressures while not overloading the source housing pressure, and not requiring excessively long equilibration times. In the final design source pressure increase did not exceed 4×10^{-2} Torr above base pressure when the gun was

operated at 5 kV, 3.5 mA, or 3×10^{-5} Torr when operated at 7 kV, 3mA. At a typical operating condition of 6 kV and 1 mA, 1.6×10^{-5} Torr is the increase.

System performance was evaluated using glycerol coated probe tips. When the probe was inserted into the ionization chamber with the gun already operating, the total ion current produced by Argon bombardment was found to increase for ca 5-10 minutes and then essentially to level off. This current level persists for ca 45-60 minutes, irrespective of the operating conditions of the gun, and then abruptly drops.

The total ion current from glycerol increases with increasing discharge current in a linear manner at low discharge currents. At higher discharge currents a leveling off (or perhaps even a decrease) in intensity was seen, perhaps due to sputtering of the sample at these higher power levels. For a fixed discharge current, the total ion current from glycerol increases with discharge voltage, at least up to 7 kV energy.

The ion source potential was held at 3.5 kV for most of the experiments while the FAB gun voltage was in the range of 5 to 7 kV. In this situation, any positive ions in the FAB beam with energies greater than 3.5 kV would be able to reach the sample. We tried to estimate the ionic content of the beam by monitoring sample ion beam currents as a deflection field was applied perpendicular to the exiting beam direction. The field was established by imposing a deflection voltage between two rods. The ion currents (total, m/e 93 from glycerol, and m/e 232 from alanylalanylalanine) at a discharge voltage of 6 kV were recorded while increasing the deflector voltage up to 6 kV. Up to about 3 kV deflector voltage, very little change in beam current was noted, indicating that the ionic content of the gun beam was very small. In all cases a dropoff was seen at higher deflection voltages. The magnitude of the drop-off varied with mass. The total ion current showed the least amount of dropoff. The magnitude of the dropoff increased with mass for the few masses looked at. This dependence on mass suggests that the effect is due to a subtle influence of deflector voltage upon beam focusing, in the ion source region where ion energies are low, rather than an indication of the presence of ions in the FAB beam. Calculations show that at a deflector voltage of only 600V, ions of 6 kV energy in the FAB beam should be deflected by ca. 0.35° . This deflection is sufficient to completely prevent their entry into the ion chamber. No appreciable change (less than 5%) in the sample ion beam intensity was noted, even at voltages several times higher than this. Hence we conclude that the ionic content of the FAB beam is quite low.

Spectra of a number of different types of compounds were obtained from glycerol solutions, including steroids, di-, tri-peptides, and sugars. In the early scans the intensity of glycerol peaks were weak relative to peaks from the solute. With time the former increased in intensity. Compounds which are insoluble in glycerol were investigated as glycerol slurries, but did not produce spectra. When such compounds were dissolved in an appropriate solvent, producing a true solution, spectra could be obtained. Thus, for instance, Cholestane dissolved in hexadecane produces an excellent FAB spectrum whereas a slurry of cholestane in glycerol produces either a very weak or no FAB spectrum. This observation suggests that selecting the appropriate solvent is highly crucial for the success of a FAB analysis.

*Luminoscope is a registered trademark of Nuclide Corporation.

ROA 3 | EXACT MASS DETERMINATIONS WITH ALTERNATIVE
IONIZATION TECHNIQUES - APPLICATIONS OF ACCELERATING
VOLTAGE SCAN

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Data system integration opens up new analytical opportunities in mass spectral applications. Magnetic field scanning is the usual way to acquire mass spectra. However, it may be advisable to use other scan techniques such as accelerating voltage scans. It offers a very precise linear mass scale, ensuring exact mass attributions within the range scanned. Only few (1-3) reference masses are needed to calculate the masses of interest.

All these features make the accelerating voltage scan (ESCAN, a newly introduced software module of the Finnigan MAT SS 300) ideally suited for exact mass determinations when using alternative ionization techniques such as FAB.

During FAB measurements glycerol together with its cluster ions is nearly always present. When the pure glycerol is fading out in the higher mass range a saturated KI/glycerol solution may be used as reference instead. The ion series $(KI)_n^+$ shows cluster ions up to mass 3000 which are conveniently usable as reference.

Some selected examples such as peptides and natural products will be presented, where the exact mass determinations do not exceed few millimass units. The accuracies obtained under different resolution values such as 1000 and 3000 are shown and discussed.

ROA 7 | FISSION FRAGMENT IONIZATION MASS SPECTROMETRY
OF POLYETHERS, B.T. Chait and F.H. Field; The Rockefeller
University, New York, N.Y. 10021

The fission fragment ionization mass spectra of three polyether oligomer samples have been measured. The samples consist of methoxypolyethylene glycols with nominal average molecular weights of 750 and 2000 and lauryloxypolyethylene glycol with nominal average molecular weight of 1200. 6LiCl , 7LiCl , and $NaCl$ were added to the samples to help effect the ionization. The spectra consist of a series of quasi-molecular ions, four series of ions produced by identified fragmentation processes, and low mass fragment ions produced by unidentified processes. The quasi-molecular ion series can be used to obtain average molecular weights for the samples. The identified fragmentation processes can be used to provide information about the identities of the polymers. Evidence is presented that the attachment of Li^+ to polyethers occurs more readily and extensively than the attachment of Na^+ , but more fragmentation occurs as a result of the Li^+ attachment. The relative amount of quasi-molecular ions declines with increasing average molecular weight of the polymer sample. Thus the quasi-molecular ion intensity in the methoxypolyethylene glycol with average molecular weight 2000 is only about 2% of the total ionization.

DIRECT EXAMINATION OF TLC SPOTS AND HIGH
MASS ASSIGNMENT BY FAB MASS SPECTROMETRY

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In this communication we present: (1) a procedure for the direct examination of thin layer chromatography (TLC) spots by fast atom bombardment (FAB) mass spectrometry, (2) a FAB probe capable of producing FAB spectra of sample and reference compounds consecutively, and (3) a procedure for the assignment of masses higher than those available with the commonly used reference compounds.

Although TLC is a simple and effective separation method, positive identification of unknown components after separation is generally a laborious procedure which is subject to problems. Usually the TLC spot is physically removed from the plate and the adsorbent extracted with a suitable solvent. The solute is then examined using various appropriate analytical methods. The use of mass spectrometry for this purpose has certain limitations, in spite of the good sensitivity of the method. Conventional mass spectrometric ionization techniques (e.g., EI or CI) require vaporization of sample, whereas TLC is typically used for non-volatile or thermally labile materials for which gas chromatography is not feasible. Although field desorption (FD) is suitable for such materials, the undesirable extraction procedure is still necessary in order to transfer the sample to the FD emitter. However, it was felt that the recently developed FAB ionization technique is ideally suited for the examination of TLC spots because: (a) extraction of the TLC spot is not required, and (b) sample vaporization prior to the mass spectrometric ionization is also not required. We have demonstrated the applicability of FAB mass spectrometry for the direct examination of TLC spots. To facilitate the procedure, a simple method was developed for transferring TLC spots to a FAB probe.

The proposed procedure involves the following basic steps: (1) after TLC separation, the spots are located, e.g., by ultraviolet fluorescence; (2) the FAB probe tip is covered with a strip of double-faced masking tape and pressed against the TLC spot of interest to transfer the adsorbent; (3) about 5 μ L of a suitable solvent and 5-10 μ L of FAB matrix liquid are added to the TLC adsorbent adhering to the probe tip; and (4) the probe is then introduced into the mass spectrometer to get FAB spectra in the usual manner.

Many of the components separated by TLC are not only thermally labile but are also high molecular weight compounds, frequently exceeding m/z 1,000. Although FAB is well suited for the direct analysis of TLC spots, the accurate assignment of nominal masses for the acquired signals is not trivial. Commonly used procedures are manual counting,

an extremely tedious operation, and peak matching versus an appropriate reference compound. Due to the problem of selective ionization, it is difficult to calibrate FAB spectra using a mixture of the sample and a reference compound. Therefore, we have designed a "roof-shaped" probe tip which allows loading the sample on one side and the reference compound on the other without cross-contamination. Rotating the probe 180° around its axis will produce FAB spectra of the sample and the reference compound consecutively and facilitate the assignment of the correct masses. In addition, high resolution exact mass measurement can be obtained by peak matching and applying an appropriate correction for differences in geometry.

However, this procedure is only applicable up to approximately m/z 1,500 for routine operation, which is the maximum mass range for commonly available reference compounds. Difficulties unique to the high mass range such as isotope effect, mass defect, insufficient resolution, etc., further complicate the correct nominal mass assignment for the high mass ions. We have therefore devised a calibration procedure for high masses, in principle up to m/z 12,000 for the 8KV magnetic-type instrument, with an accuracy of at least unit mass or 100 ppm.

Based on the well-known fundamental formula for magnetic-type instruments,

$$\frac{m}{z} = \frac{H^2 R^2}{2V}$$

the observed mass is inversely proportional to the accelerating voltage, V , at constant magnetic field strength. For instance, m/z 2,000 at 4KV will superimpose on m/z 1,000 at 8KV. Therefore, if one calibrates at 8KV and then switches to 4KV, the apparent mass values at 4KV will be one-half those assigned at 8KV. On the other hand, if the reference masses are assigned twice their true values at 8KV, then the calibration should assign the correct mass values at 4KV. Since it is fairly common to calibrate at 8KV up to m/z 1,500 using commercially available reference compounds such as perfluoroalkyltriazone, this procedure provides a reliable calibration range up to m/z 3,000 for 4KV operation. In principle, the calibration range could be extended to m/z 6,000 for 2KV and m/z 12,000 for 1KV operation. Our experiments showed an error of less than 100 ppm in mass assignment, which by extrapolation corresponds to one dalton at m/z 10,000. The assumption of even multiples for the ratio of various accelerating voltages was checked by peak matching several reference peaks. The deviation was found to be the same for all reference masses at the same voltage ratio and remained constant over an indefinite period (assuming no alteration of the electronics).

A major advantage of this calibration procedure is that the mass range of the commonly used reference compounds is, in effect, extended. In addition, this principle is applicable to FD and other mass spectrometric methods requiring the accurate assignment of nominal mass at high molecular weight.

Particle-Induced Desorption Mass Spectrometry With A Quadrupole GC/MS Instrument. K. F. Faull, H. Sim and J. D. Barchas, Dept. Psychiatry, Stanford University, Stanford, CA 94305 and I. Massey, Syntex Research, Palo Alto, CA

Organic mass spectrometry has recently been revolutionized by the introduction of the Particle Induced Desorption (PID) technique in which a beam of fast atoms or a mixture of fast atoms and fast ions is used to desorb charged sample molecules and charged fragments of molecules from a surface. Although the most spectacular applications of this technique have been with high molecular weight multifunctional compounds of biological origin which in many cases have proven refractory with conventional ionization methods, the technique can also be used to obtain mass spectra of low molecular weight polar molecules. In fact, the success with which the technique is being used for the direct analysis of a wide variety of organic compounds gives reason to believe that it will become a valuable ionization method which will find frequent use as a complementary technique to the electron impact and chemical ionization approaches conventionally used in mass spectrometry. One feature which has contributed to the rapid development of this technique is the relative ease with which most mass spectrometers can be inexpensively field retrofitted with a suitable source capable of supplying a beam of fast atoms and/or ions. Here we describe the performance of a quadrupole GC/MS instrument modified for operation in the PID mode by installation of a Capillaritron ion gun. The results demonstrate that quadrupole mass spectrometers can be used to obtain desorption ionization spectra of a wide variety of polar, involatile and thermally labile organic molecules.

Source Design and Operation

A Fast Atom Capillaritron Source (FACS) equipped with an ion deflector, power supply and gas control unit (constructed by Phrasor Scientific, Duarte, CA¹) was installed with minor modifications to the vacuum manifold and EI/CI source of a Hewlett Packard 5985B quadrupole GC/MS instrument². The power supply and gas control unit provided independent control of the ion beam energy and ion beam current. The installation allowed for rapid interconversion between the EI/CI and PID ionization modes without venting the mass spectrometer.

Characteristics of the FACS Gun and Ion Deflector

Under the conditions employed here, the FACS gun produced a mixture of fast xenon atoms and ions in approximately equal abundance¹. The xenon ions were readily detected by the mass spectrometer which in the absence of any sample in the ionization chamber showed ions ranging from m/z 128-136 corresponding to the 7 most abundant naturally occurring xenon isotopes.

A feature of the FACS gun is the ability to apply a variable positive voltage to the deflector plate built in the gun, and thus deflect away a proportion of the ions in the beam. A maximum deflection voltage (2200 V) produced a beam of exclusively fast xenon atoms as shown by the absence of xenon ions in the mass spectrometer.

Angle of Incidence

The angle between the axis of the primary ionizing beam and the plane of the sample surface dramatically influenced secondary ion intensity. The angle was conveniently optimized by rotating the direct insertion probe during SIM data collection of desorbed sample ions. The optimal angle for detection of molecular ions and fragment ions from compounds dissolved in glycerol matrices was found to be close to the 26° reported in the literature for magnetic sector mass spectrometers equipped with Saddle Field ion guns.

Characteristics of the Primary Ionizing Beam

The combined effects of ion beam current and ion beam energy on secondary ion intensity were studied using hexadecyltrimethylammonium para-toluene sulphonate. This was done using a beam of fast ions and fast atoms (1:1 ratio, zero deflection voltage) and a beam of exclusively fast atoms (maximum deflection voltage). With ion beam currents of 20, 40, 60 and 80 μ A and ion beam energies between 4 and 9 KV, the signal for the molecular ion was more intense with the ion and atom beam. Background noise was greater with the ion and atom beam. Consequently, signal-to-noise ratios were greater with the atom beam.

The ion and atom beam produced maximal signal intensities for the molecular ion at 8, 6, 7 and 8 KV for the 20, 40, 60 and 80 μ A beams, respectively. The intensity of the molecular ion produced by the atom beam steadily increased as the energy of the beam was increased. While the intensity maxima obtained for the molecular ion with the 20, 60 and

80 μ A beams were similar, the intensity maxima obtained with the 40 μ A beam was notably higher. These data extend and confirm our earlier conclusions that the primary difference between the ion and atom and exclusively atom beams was an enhanced signal intensity from the former attributable to its 2-fold greater power density³.

Spectrum Averaging

One obvious feature of spectra produced by the FACS gun-quadrupole mass spectrometer combination was the high level of noise in the spectra. A large part of the noise was random, suggesting that it was the result of either fast neutral species and/or fast ions, which escaped mass filtering, striking the electron multiplier. Spectrum averaging took advantage of the long-lived and stable secondary ion currents produced by the source and the rapid scanning capabilities of the quadrupole mass spectrometer and resulted in considerable improvement in spectral quality and enhancement of signal-to-noise ratios, which was particularly dramatic at the high mass regions of the spectra (Figure 1).

Mass Axis Calibration and Optimization of Source Parameters

Initial experiments were carried out using tuning parameters which were the same as those used in the EI ionization mode. This procedure produced positive ion PID spectra of a variety of organic compounds, including small peptides, nucleic acid bases, nucleosides, nucleotides, sugar phosphates, and quaternary ammonium salts, which were qualitatively identical to PID-spectra published in the literature for the same classes of compounds.

For more precise work it was necessary to calibrate the mass axis and tune the source parameters in the PID mode. Nanogram quantities of quaternary ammonium salts in glycerol matrix were useful for this purpose because they produced intense signals for the molecular ions. Judicious choice of the chain length provided standards which were suitable for calibration of any area of the mass range. The signals derived from the molecular ions of hydrocarbon containing quaternary ammonium salts were sufficiently intense, stable and long-lived to allow tuning of the instrument and calibration of the mass axis. Although the signals derived for the molecular ions of fluorocarbon containing quaternary ammonium salts were similar in intensity, their shorter life, presumably attributable to increased volatility, precluded their use as convenient calibration standards.

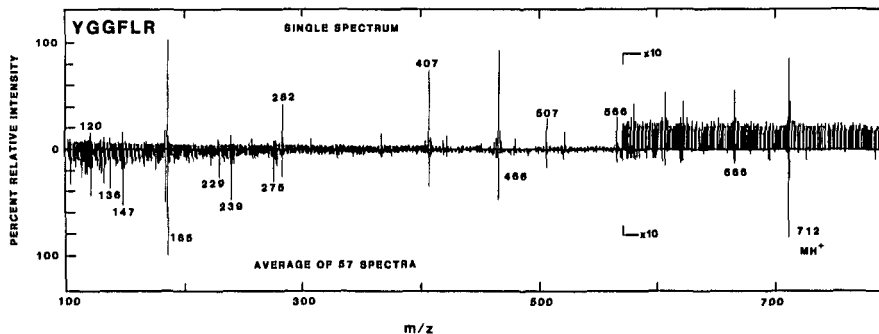


Figure 1. Positive ion PID spectra of the hexapeptide Tyr-Gly-Gly-Phe-Leu-Arg (2 μ g in 1 μ l 6N HCl with glycerol matrix)

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Acknowledgements

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PARTICLE INDUCED MASS SPECTROMETRY USING A CONVENTIONAL HP 598X QUADRUPOLE MS WITH A CESIUM PRIMARY ION BEAM

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The technique of using fast atom and/or fast ion bombardment in a glycerol assisted matrix for secondary ion mass spectrometry is a rapidly growing method for studying compounds which are thermally-labile or nonvolatile, particularly compounds of biological or biomedical importance. Since the introduction of Fast Atom Bombardment (FAB), other high energy primary sources using ions have been found to produce similar results to those obtained using fast atom sources. A variety of these primary ion sources are now available for Particle Induced Desorption Mass Spectrometry (PID). One of the primary ion sources which is available for PID experiments is the Cesium primary ion source. The objectives of this paper are to: describe, in perspective, the Cesium primary ion source and the interfacing of this source and the Capillaritron gas-fed primary source to the Hewlett-Packard 598X quadrupole GC/MS/DS; demonstrate the feasibility of use of the Cesium primary ion source mounted on the HP 598X for particle induced desorption experiments; and compare mass spectra obtained using the Cesium primary ion source with those obtained using the Xenon gas-fed Capillaritron.

In the Cesium primary ion source used for these experiments, Cesium aluminosilicate is embedded in a tungsten matrix through which the Cesium ions generated at high temperature (ca. 1000°C) diffuse. The ions are accelerated out of the source by an accelerating lens. A modified Pierce lens provides some focusing for the ions emitted by the Cs source without the need for external lens electrodes. Only fast ions are produced by the Cs source.

We have compared the results obtained with the Cs primary ion source mounted on the HP 598X with those previously obtained using the Capillaritron Xe-fed source. The Capillaritron and its interfacing to this system has been previously described in a number of papers (1,2,3). Because of the similarity in mass of Xe to Cs, it is appropriate to compare results obtained with the Xenon gas-fed source to those obtained with the Cs primary ion source as we do in this paper. Glycerol is the most commonly used matrix for PID experiments. Often ions corresponding to the monomer, dimer, trimer, tetramer of glycerol are predominant ions in the mass spectra obtained by this technique. For the mass spectra shown in this paper, when the glycerol m/z 's were prominent, they were deleted from the spectra shown.

In Figure 1 is shown the Cs PID mass spectrum of a quaternary ammonium compound, dimethyldioctadecyl ammonium chloride. There are clearly m/z 's available in the regions of m/z 300 and m/z 550 which could be used for tuning the instrument in the PID mode of operation. In addition, there are hydrocarbon m/z 's (14 amu apart) which also could be used for instrument tuning. The mass spectra of leucine-enkephalin obtained by the two techniques are shown in Figure 2. A clear $[M+1]^+$ is apparent as well as other fragments which can be used to establish the sequence of the peptide. Phe and Tyr fragments at m/z 120 and m/z 136 are also obtained in addition to the di- and tripeptide fragments which are labeled. The spectra are obviously quite similar although the noise in the higher mass range shown appears to be somewhat lower in the Cs PID experiment. In Figure 3 are shown the comparative results for the antibiotic kanamycin. In this example, the difference in signal to noise when using the two sources of bombarding species is even more apparent than in Figure 2. Both spectra in this Figure are quite weak, but the only significant ions in the Cs PID spectrum are the protonated molecule and the [protonated molecule - hexose] $^+$.

In conclusion, the types of ionization methods used for PID, the instrumentation necessary for each and the advantages of each these methods were described. The successful interfacing of a Cs+ primary ion source to a Hewlett-Packard 598X GC/MS/DS has been performed as well as the previously described interfacing of a Xe gas-fed source for PID. The feasibility of using the Cesium primary ion source for particle induced desorption experiments has been demonstrated by obtaining mass spectra of selected compounds. The compounds included dimethyldioctadecyl ammonium chloride, leucine- and methionine-enkephalin, kanamycin and AMP. These mass spectra have been compared to those obtained using a Xenon gas-fed source. It appears that the signal/noise obtained is enhanced when the Cesium primary ion source is used as compared to the signal/noise when the Xenon gas-fed source is used. Further rigorous experiments comparing the two techniques may lend support to this preliminary observation.

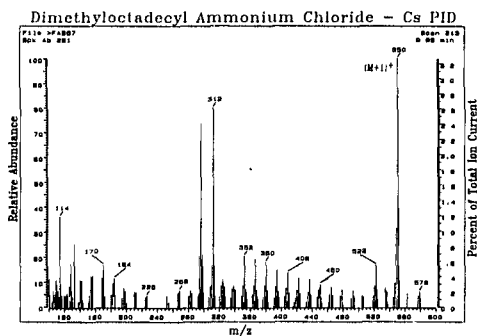


Figure 1. Cs PID mass spectrum of dimethyloctadecyl ammonium chloride.

Figure 2. Cs and Xe PID mass spectra of leucine-enkephalin. Cs PID-6kV (6uA); Xe PID-7.4kV(10uA); sample in glycerol + TEAA buffer pH 3.02.

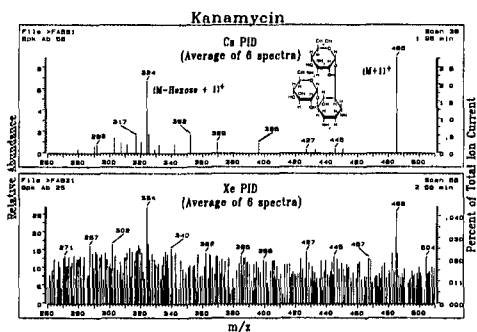
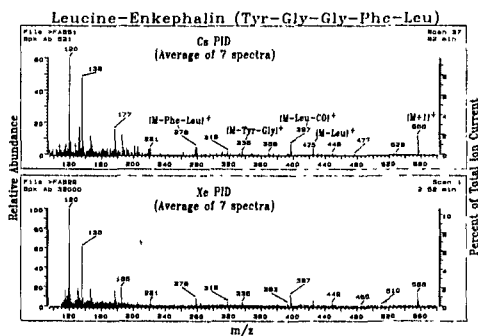


Figure 3. Cs and Xe PID mass spectra of kanamycin. Cs PID-8kV (17uA), sample in glycerol + NH_4Cl ; Xe PID-7.4 kV (40uA), sample in glycerol + NaCl .

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PERFORMANCE OF A Cs^+ LIQUID SIMS SOURCE OPERATING IN 90° AND IN-LINE CONFIGURATIONS

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We have recently demonstrated the efficacy of using a cesium ion primary beam for producing secondary ions from bioorganic compounds dissolved in a liquid matrix target (Cs^+ LSIMS) (1). The main advantages of a Cs^+ primary beam are: 1) no additional gas burden to the ion source chamber; 2) compactness of Cs^+ gun simplifies retrofitting ion sources for LSIMS operation; 3) more precise control over primary beam energy and intensity.

The redesigned ion gun, equipped with a cesium alumina silicate source (2) is capable of producing a sharply focused beam of 0.5 mm FWHM at a distance of 2.5 cm from its exit aperture and can deliver beam energies of over 10 keV. Figure 1 shows the amount of total Cs^+ current obtained at 8 keV ion energy as a function of source heater power. The wide range of current (from 0 to 20 μamp) and energy (1-10 keV) provides the flexibility of operating conditions often necessary to obtain structurally useful spectra. This is demonstrated by the observation that as the primary beam flux density and energy increase so also does the relative amount of molecular fragmentation (see paper WOF4, this conference). This effect may be due to a depletion of sample material on the target surface caused by limited resupply via bulk diffusion which results in observing greater amounts of sample molecular ions and fragments produced from the bulk rather than the surface.

In a further effort to control the quality of sample spectra, we have explored the effects of aiming the primary beam close to the direction of the extracted secondary beam. It was anticipated that the similarity in momentum directions between primary and secondary beams in this in-line geometry (see Fig. 2A) would provide more effective extraction of the parent molecular ion compared to the commonly used 90° geometry (see Fig. 2B). The 5° offset of the primary beam shown in Fig. 2A is to prevent sputter damage of the variable source slit by the cesium beam. The probe tip for the in-line source consists of a conical hollow in which the liquid sample matrix is placed. The slope of this cone is 15° and the smaller end is 1 mm in diameter. Spectra from cyanocobalmin (vitamin B_{12}) produced by the two geometries is shown in Fig. 3. A comparison of these spectra shows that both have the characteristic fragment structure previously observed (3). However, in the 90° geometry, the relative fragment amplitudes are generally somewhat larger but are also more obscured by the presence of a greater amount of background peaks. It is also observed that the level of background signal increases more rapidly below about mass 700 for the in-line geometry and becomes several times larger than that of the 90° arrangement at low masses. This increase in lower mass background peaks reflects the larger cross section for small angle knock-on type collisions which the in-line geometry transmits. It thus appears that the in-line arrangement produces spectra which reflect soft ionization (low fragmentation) in the higher mass parent molecular ion region and hard ionization (high fragmentation) in the lower mass region. These characteristics should prove useful for the analysis of specific bioorganic compounds.

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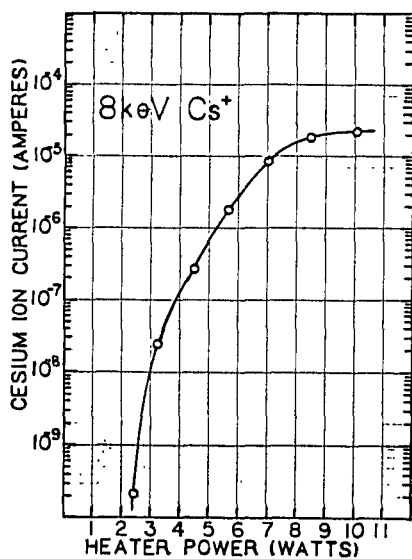


Figure 1

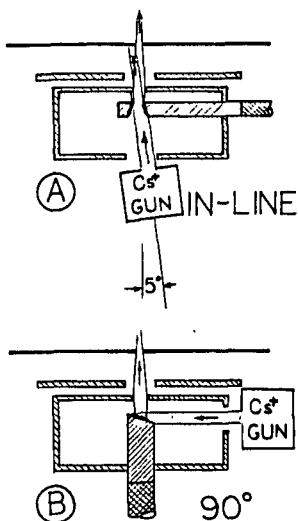


Figure 2

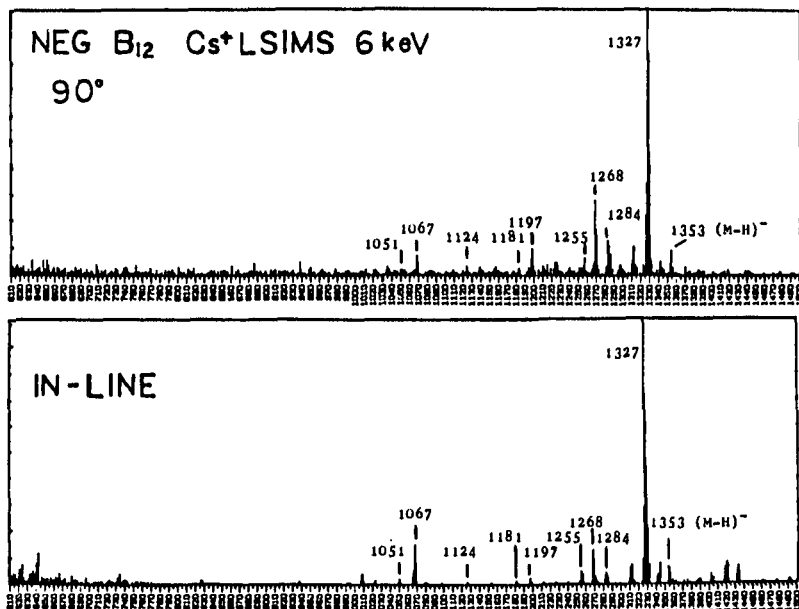


Figure 3

THE CHEMICAL STATE OF SOME ION-IMPLANTED TRANSITION METALS AND STEELS

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The chemical states of Cr, Fe, and Ni in nitrogen implanted 304 stainless steel (SS) are determined by secondary ion mass spectrometry (SIMS) using the depth profiling capability of the CAMRECA IMS 300 ion microscope (5.5 keV Ar^+ ions at 30-70 $\mu\text{A}/\text{cm}^2$). Both the SIMS and recent Auger electron spectroscopy (AES) results (1) indicate the formation of CrN , but not that of Fe or Ni nitride as the result of nitrogen implantation. The SIMS results also show that the implanted nitrogen affects the secondary ion yields of Cr, Fe, and Ni in that enhanced secondary ion yields for Cr, Fe, and Ni are observed as the concentration of implanted nitrogen is increased. The enhancement appears to follow the rules established by Yu and Reuter (2) for binary alloys which state that for alloy A-B where A forms a stronger oxide bond than B, the presence of A enhances the emission of B^+ and the presence of B suppresses the emission of A.

Chemically nitrided 304 SS is known to contain CrN precipitates which improve the relative wear resistance of the steel along with certain other properties such as hardness and corrosion resistance. Ion implanting nitrogen into 304 SS at doses ranging from $2 \times 10^{16}/\text{cm}^2$ to $1 \times 10^{18}/\text{cm}^2$ also results in the formation of CrN as evident by the emission of CrN^+ ions. Figure 1 shows the SIMS depth profile of nitrogen implanted 304 SS with an implant dose of $2.3 \times 10^{17}/\text{cm}^2$. The CrN^+ ions are detected even at the lowest implant dose (i.e., $2 \times 10^{16}/\text{cm}^2$) and their distribution showed the same profile as the N^+ ions in all cases.

The secondary ion yields of Cr^+ , Fe^+ , and Ni^+ began to increase as the nitrogen implant dose exceeded $1 \times 10^{17}/\text{cm}^2$. Significant enhancements (20-50%) are apparent at implant doses of $2.3 \times 10^{17}/\text{cm}^2$ and $1 \times 10^{18}/\text{cm}^2$. A plot of the enhanced ion intensity (peak signal divided by bulk signal) for Cr^+ , Fe^+ , and Ni^+ versus nitrogen implant dose is shown in Figure 2. The plot shows a strong increase in the Fe^+ and Ni^+ ion intensities relative to the Cr^+ for implant doses up to the saturation dose (atomic percent of N equals the atomic percent of Cr) of $2.3 \times 10^{17}/\text{cm}^2$. These results are interpreted according to the secondary ion emission rules (developed by Yu and Reuter (2) for binary alloys) and appear to indicate the formation of a strong CrN bond which in turn enhances the emission of Fe^+ and Ni^+ .

Cr is known to form a more stable nitride than Fe (i.e., $\Delta H_f(\text{CrN}) = -29.8$ kcal/mole; $\Delta H_f(\text{Cr}_2\text{N}) = -23.4$ kcal/mole; $\Delta H_f(\text{Fe}_3\text{N}) = -2.55$ kcal/mole; $\Delta H_f(\text{Fe}_2\text{N}) = -0.9$ kcal/mole), and we expected that CrN would form preferentially during implantation or at least until the atomic percent of N is equal to the atomic percent of Cr (i.e., at $2.3 \times 10^{17}\text{N}/\text{cm}^2$). Beyond this nitrogen concentration, the formation of Fe nitride is expected. However, even at the highest implant dose of $1 \times 10^{18}/\text{cm}^2$ where the atomic percent of N was 4 times that of Cr, no FeN^+ ions are detected by SIMS. The Auger results did however seem to indicate that the Fe may have become nitrided at the highest implant dose.

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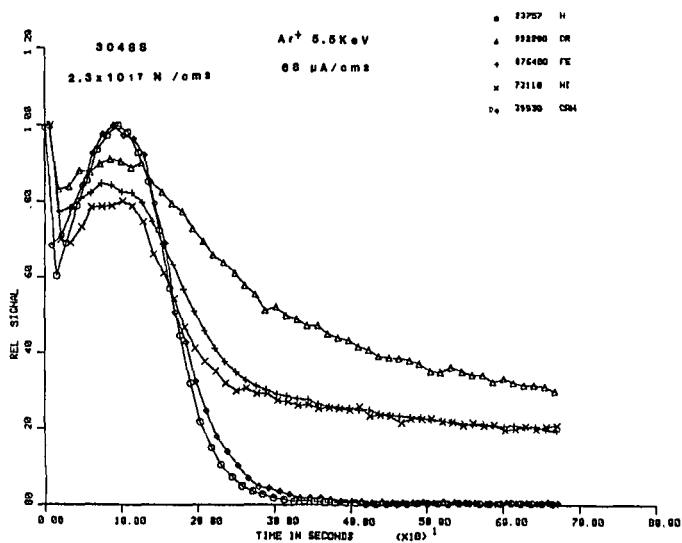


Figure 1. SIMS Depth Profile of Nitrogen Implanted 304SS

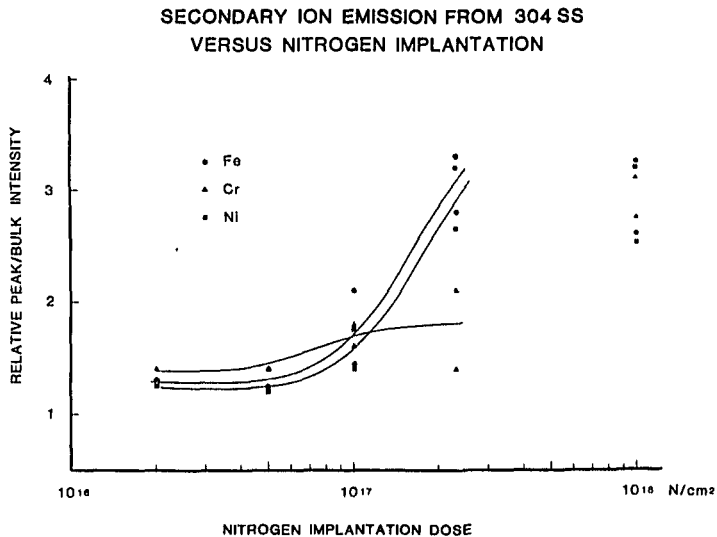


Figure 2. Cr, Fe and Ni Enhanced Ion Intensity vs. Nitrogen Implant Dose

ROA 10

MATRIX CALIBRATION OF GROUP III AND V
COMPOUND MATRICES FOR QUANTITATIVE
SECONDARY ION MASS SPECTROMETRY

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The sputtering and ionization of surface atoms by ion bombardment is the basis of secondary ion mass spectrometry (SIMS). High sensitivity for all elements, and good depth resolution have made SIMS an attractive method for concentration depth profiling. Although the technique has been applied successfully to a variety of materials, the complexity of the sputtering event has made quantitative analysis difficult. Secondary ion yields can vary over several orders of magnitude due to the influence of a variety of factors including primary and secondary ion identities, sample matrix, sputtering rate, and residual gas pressure.

Ordinarily, ion intensities from unknown samples can be calibrated using external standards. Although this approach yields an accuracy of about 10%, it only works when standards match the sample composition. This composition match between standard and unknown is not always feasible. However, quantification can sometimes be achieved by calibrating matrix effects. This type of empirical calibration for Group III and V compound matrices will be presented and discussed.

1. Metastable Decomposition: Large secondary cluster ions ejected from CsI by 8 or 28 keV Cs^+ ion bombardment have been observed in the Manitoba time-of-flight mass spectrometer¹. Positive ions $[(\text{CsI})_n\text{Cs}]^+$ up to $n=40$ and negative ions $[(\text{CsI})_n\text{I}]^-$ up to $n=20$ were detected². The yield of clusters was observed to decrease smoothly with n , in contrast to results obtained with a sector-field mass spectrometer^{3,4}, where striking anomalies were observed in the neighbourhood of particularly symmetrical cluster geometries; e.g. the $3 \times 3 \times 3$ structure at $n=13$.

The apparent disagreement may be explained by the difference in methods of measurement if the clusters are metastable on a μs time scale. The time required to accelerate heavy ions in the sector-field instrument is several tens of μs ; e.g. $\sim 50 \mu\text{s}$ for $n=13$. If they decay early in the acceleration region, then the measured distribution represents the distribution of the charged daughters. By contrast, the time-of-flight spectrometer measures the mass distribution at an effective time $\ll 1 \mu\text{s}$. Thus it appears that there are no anomalies in the intensity distribution as a result of the emission process.

In order to examine the mass spectrum at much later times we installed retarding grids in front of the detector at the end of the flight tube. Above $n=7$, the clusters were found to be predominantly metastable, decaying mainly by the emission of one or more CsI molecules. The distribution of decay products exhibits a striking anomaly; product clusters at $n=13$ are favored, and clusters at $n=14$ and $n=15$ are suppressed.

2. Sample Irradiation: We have observed a marked increase in the yield of cations, anions and all cluster ions after irradiating alkali halide samples with $\sim 10^{14}$ alkali ions/ cm^2 . Figure 1 shows the spectrum of CsI before and after irradiation with $\sim 10^{16}$ Cs^+ / cm^2 . The scale on the left spectrum is magnified by 100, i.e. the cation yield is increased by a factor of 100 by irradiation. Our initial interpretation was surface cleaning. However, while the yield does decrease somewhat in time after irradiation, it never returns to its original value, even when the target is exposed to air for more than one week. We therefore suspect that the irradiation causes some other changes in the sample properties which enhance the ion yield.

Our observations with Cs^+ irradiation of CsI have been found to result from at least two effects. These may be observed separately by irradiating KI with Cs^+ . Figure 2 shows the yield of KI clusters as a function of Cs^+ irradiation. The initial increase takes place with the first $\sim 10^{14}$ ions/ cm^2 . After this the yield remains essentially constant up to at least 10^{17} ions/ cm^2 . This increased yield does not vary significantly in time.

A second effect is the appearance of clusters containing the incident alkali atoms. This is illustrated in figure 3, where irradiation of KI with Cs^+ results in significant yields of $(\text{KI})\text{Cs}^+$ and $(\text{CsI})\text{Cs}^+$. The behavior of these yields with irradiation (figure 4) is quite different from the KI cluster yields. The initial increase is more gradual and it continues for much larger doses. The appearance of these mixed clusters indicates that in fact the sample properties are significantly modified by irradiation.

Similar results have been obtained with several other alkali iodides using K^+ and Cs^+ irradiation.

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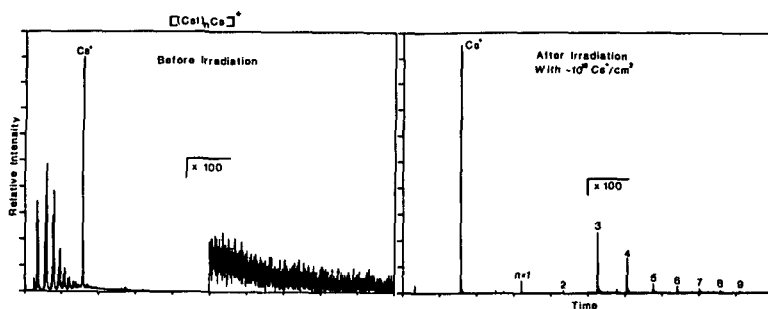


Figure 1. Positive ion mass spectra of CsI before and after irradiation with 10^{16} Cs^+/cm^2 . The scale on the left is magnified by 100; i.e. the Cs^+ peak is 100 times as intense after irradiation.

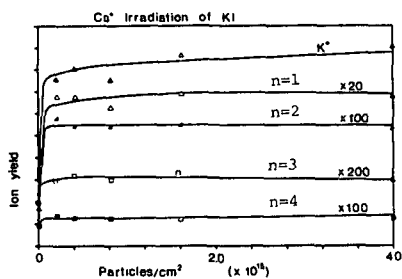


Figure 2. $(\text{KI})_n\text{K}^+$ cluster yields as a function of Cs^+ irradiation. The curves remain flat up to at least 10^{17} Cs^+/cm^2 .

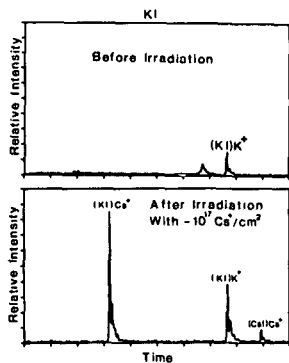


Figure 3. Positive ion spectra of KI before and after irradiation with 10^{17} Cs^+/cm^2 .

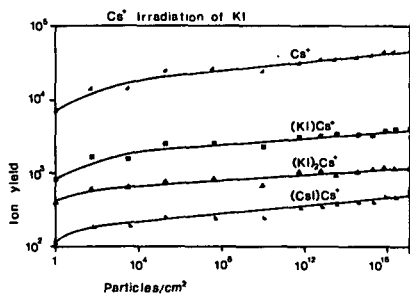


Figure 4. Yields of mixed clusters from a KI target as a function of Cs^+ irradiation.

Of all the procedures available for biopolymer structure determination those employed for polysaccharides are perhaps the most archaic and arduous. The analytical techniques for sequencing oligosaccharides have changed little, while those for other biopolymers have undergone tremendous evolutions in sensitivity and specificity. Until recently, the only significant contribution attributed to mass spectrometry has been to confirm the structures of sugar monomer units following total hydrolysis of the parent oligomer. However, recent instrumental developments provide new approaches to today's problems and mass spectrometry is now far more capable of addressing problems of polysaccharide structure (1). Therefore, it is quite appropriate that a society of mass spectroscopists include this topic in a symposium on biopolymer sequencing.

A discussion of polysaccharide structure is additionally appropriate because of our increased awareness of the physiological significance of glycosylated proteins and lipids, and, most opportune because it provides a forum to discuss our most recent analytical progress in the development of new methodology for the study of these structures.

The study of protein glycosylation and processing has only recently, and with great difficulty, been pulled from the depths of scientific curiosity. We now know that the function of the carbohydrate portion of the molecule can be ascribed to at least three very broad and important categories:

- 1.) to provide molecular stability to the product molecules;
- 2.) to serve as a site directing label, (carbohydrates serve as the zip code);
- 3.) to impart the product molecule with totally different properties; (e.g., adhesion, solubility, ionic character).

Numerous examples further suggest that carbohydrate sequence recognition is the key to the specificity of these processes. Binding is based on a detailed structural sequence, not only in terms of which sugars are involved, but also in terms of the anomeric configuration of the glycosidic linkage, the linkage position, and the branching. Each of these factors impart additional specificity. For example, our immune system can differentiate between Man $\alpha(1-2)$ Man-X, Man $\alpha(1-3)$ Man-X, and Man $\alpha(1-6)$ Man-X residues attached to red blood cells, and many glycosyl transferases are sensitive to the total composition and sequence of their substrates. If biological information is communicated via oligosaccharide structures, then an incredibly varied and complex "language" can be coded from a relatively simple "alphabet" consisting of the few monosaccharide constituents found in glycoproteins and glycolipids.

POLYSACCHARIDE STRUCTURAL CONSIDERATIONS

The identification of each residue and its linear array, for most biopolymers, would suffice as a complete structural characterization. Unfortunately, this is far from the case for an understanding of polysaccharide structure. This complexity of language accounts for much of the analytical difficulty in oligosaccharide structural studies and it is the reason why comparatively few glycoconjugate structures have been elucidated.

I will briefly define some of the major concerns and problems encountered when trying to resolve a polysaccharide structure and, where appropriate, introduce techniques we have developed which greatly improve the analytical chemistry of carbohydrate structural determination (2).

HETEROGENEITY

A structural assignment cannot begin to be approached until the sample has been adequately purified. Many glycosylated molecules and oligosaccharides isolated from biological tissues have a precisely defined internal sequence but lack the same precision on chain termination. The consequence is a series of molecular entities differing by single saccharide residues. High performance liquid chromatography (HPLC) has been shown to be ideally suited for resolving this heterogeneity. For example, HPLC separation of the mannose oligosaccharides obtained from the urine of patients suffering from mannosidosis or the phosphorylated high mannose-type oligosaccharides obtained from whole cell glycoproteins of mouse macrophage cells exhibit a complex pattern of cleanly resolved peaks which differ by single residues. Thus, molecular heterogeneity is frequently a fact of life for many carbohydrate oligomers and glycosylated molecules. Definitive structural assessment must contend with this complication and sample evaluation and purification by HPLC is a necessary analytical adjunct prior to analysis by mass spectrometry.

Our approach to all polysaccharide structural problems is first to develop a chromatographic procedure for purification at maximal sensitivity. An understanding of molecular heterogeneity is frequently a product of sample purification and the HPLC approaches we have developed are discussed below.

SENSITIVITY

Many of the physiological important glycoconjugates are present in trace amounts in a very complex biological matrix. Extensive demands must be placed on chromatographic separations to resolve the sample of interest from extraneous materials as well as define any molecular heterogeneity as discussed above. HPLC is excellently suited for these problems, but since carbohydrates show only end absorption in the UV, and refractive index detectors are hopelessly insensitive, pre- or post-column derivatization is mandatory.

We have approached these problems by sample precolumn derivatization with the fluorescent groups 2-amino coumarin and 2-amino pyridine followed by chromatography on an amine column. The attachment of a fluorescent label to the reducing-end not only amplifies our sensitivity some 1000- to 10,000-fold, but, in addition, the derivatives show improved chromatographic properties and induce a basic character to the sample which provides an opportunity for further purification by ion exchange HPLC. Moreover, the pyridinyl amine labeled samples can be subsequently permethylated which produces a lipophilic product amenable to reverse-phase HPLC. These purified permethylated derivatives are excellently suited for DCI-MS, partial depolymerization by reductive cleavage, and linkage studies which will be discussed separately below.

DEPOLYMERIZATION

The second important analytical concern is sample depolymerization. High molecular weight biopolymers must be brought within the mass range of the instrument by enzymatic or chemical degradation. Protein sequence studies have relied on a series of enzymatic and chemical cleavages that produce families of overlapping peptides from which the structure of the intact polymer can be deduced. There is no comparable methodology for polysaccharide materials. Endo-carbohydrases are essentially non-existent and no selective chemical cleavages are yet available. Acetolysis, or acetic acid hydrolysis, has been the method of choice for polysaccharide degradation. However, the stability of underivatized sugars to acid hydrolysis varies widely and a large fraction of the very important polysaccharides, particularly those containing sialic acid, may be irreversibly degraded. In addition, acid hydrolysis exposes a reactive aldehydic reducing-end fragment which causes the loss of the original pyran or furan ring form. These procedures are wholly unsuccessful for trace amounts of material.

We have avoided many problems encountered with hydrolytic procedures by a hydride reduction of the glycosidic linkage controlled to provide a smaller family of oligomers. The products of this partial reductive cleavage are excellently suited for further analysis and are covered in greater detail below.

LINKAGE ANALYSIS

The position of attachment between monomer units of an oligosaccharide is usually approached by sample permethylation, hydrolysis, reduction of the hemiacetal, acetylation of the free hydroxyls and determination of the point of acetylation after chromatographic separation. This later step frequently relies on GC-MS using electron ionization. This alditol acetate procedure was established over fifty years ago and represents a classical approach to understanding linkage position and those residues involved in branching of a polysaccharide chain. Limitations of the method are not related to the structural assignment of linkage by GC-MS but to the chemical manipulations required to prepare the derivatives, (e.g., hydrolysis, reduction). Anomeric concerns of the glycosidic linkage are most frequently resolved enzymatically.

SEQUENCE ANALYSIS AND SOFT IONIZATION TECHNIQUES

Structural detail relating to polymer molecular weight, sequence and its branching has, over the years, been the most troublesome analytical data to obtain. The development of "soft" ionization techniques has provided the tools required to address some of these difficult problems. Field desorption can provide molecular weight information on many oligomers, however, the technique provides very limited sequence information. Attempts to induce greater structural detail, e.g., sequence information, by heating the sample more rapidly is counter-productive. Samples frequently exhibit a narrow desorption range and it is not uncommon to lose the total sample in one brief spark during the heating profile.

Analysis of polysaccharides (under 3000 u) by fast atom bombardment (FAB) mass spectrometry has provided an excellent combination of fragment and molecular weight-related ions for sequence studies. The ability of FAB to generate, and modern instrumentation to transmit, these high molecular weight ions is most encouraging. However, many oligosaccharides provide only MW information and few sequence informative fragments by this technique. Derivatization (acetylation, methylation) readily extends the size of the oligomer for which MW information may be obtained, but the effect of derivatization on structural information is less clearly understood. The ion distribution of molecular vs sequence ions for biopolymers of high mass will continue to be a function of the energy distributed to the desorbing molecular species and it may be expecting too much to manipulate this process within the ion source. Perhaps we must look to MS/MS employing collision activation or other adjunct techniques to more precisely control

these processes.

An alternative soft ionization technique that we have found very suitable for the analysis of permethylated oligosaccharides has been direct chemical ionization (DCI) using ammonia as the reagent gas. Our initial interest in ammonia DCI is related to its specific ability to provide detailed sequence information. For small oligomers (5-10 residues) DCI provides considerable structural information and unusual stability at higher mass; $(M+18)^+$ is often the base ion for compounds under 1000 μ . Desorption is initiated by supplying heat to the sample deposited on a specially coated wire placed in the ion plasma of the CI chamber (3). The technique produces an array of spectra within each total-ion envelope in which early scans provide molecular weight information followed by considerable structural detail at later scans. This flexibility cannot be matched by other ionization techniques and, moreover, possesses sensitivity that far exceeds FAB analysis.

SULFATED AND PHOSPHORYLATED GLYCOCONJUGATES

Up to the present I have not discussed a class of polysaccharides that has baffled many analysts. These are the sulfated sugars characterized as glycosaminoglycans, chondroitin sulfates, heparin, and a host of acidic polysaccharides frequently having structural roles in body tissues. Since acid hydrolysis and other chemical manipulations cause the loss of the sulfates, gentle and direct analytical methods are required. Preliminary analysis of these materials by FAB-MS has recently been undertaken and is described in this volume (TPA#23). This study indicates that FAB-MS is a powerful tool for the structural analysis of these difficult biopolymers.

POLYSACCHARIDE PERMETHYLATION

Protection of carbohydrate materials by permethylation has long been an experimental approach to avoid pyrolysis during MS analysis. The same derivative can also provide linkage information using the well-established alditol acetate procedures. An equally important aspect is that permethylated oligosaccharides are easily purified on reverse phase HPLC columns. Using permethylation numerous structures of high molecular weight glycolipids have been determined. This "in beam" EI-MS approach is the subject of the next paper. We have had an interest in similar materials but have observed considerable enhancement in structural detail using ammonia DCI-MS when compared with EI-MS.

Thus, for a complete structural assessment, sample permethylation remains the derivatization procedure of choice although these factors vary somewhat with the lower molecular weight samples. Linkage analysis, DCI, reductive hydrosilylation, reverse phase HPLC, and glycolipid EI all have proven highly successful and dependent on this derivative. Molecular weight determination at high mass by FAB (5000 μ) would probably profit by this approach, although only the peracetylated derivatives, at a relatively higher mass, have been investigated. Clearly, permethylation will be with us for some time.

NEW APPROACHES TO POLYSACCHARIDE STRUCTURAL ANALYSIS

The different procedures discussed above have made very significant contributions to several aspects of polysaccharide structure. But a complete solution is multifaceted and one analytical technique is inadequate to resolve all the details of fine structure. Unfortunately, because of sample limitations, one is frequently limited to the number of chemical manipulations or instrumental approaches that can be brought to bear on a structural problem and it is important to consolidate all experimental procedures. It is with these considerations that we have developed a series of new experimental approaches, some aspects of which have been described in the extended abstract FPA #9.

Whenever possible, samples are tagged with a fluorescent group to maintain sensitivity during purification by HPLC. Subsequently, these fractions are permethylated and, when appropriate, rechromatographed on reverse phase HPLC. To approach biopolymer depolymerization while still maintaining structural intensity we partially reduce glycosidic linkages to yield a series of smaller overlapping oligomers. Reductive cleavage, or hydrosilylation, of the glycosidic bond relies on the successful hydride transfer from electropositive silicon to an activated carbon center. Under the conditions chosen, glycosidic linkages which undergo cleavage yield an anhydroalditol on the terminal side of the oligomer chain and a free hydroxyl group on the reducing side.

In combination with this unique degradation approach we have amplified the sensitivity for detection of these smaller oligomers by again introducing a fluorescent derivative at this newly exposed hydroxyl group. HPLC of this reaction mixture allows separation of individual oligomers for direct sequence determination by DCI and an assessment of linkage by complete reductive cleavage, acetylation, and GC-MS.

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SEQUENCING OF OLIGOSACCHARIDES BY DIRECT INLET MASS SPECTROMETRY. APPLICATION TO CELL SURFACE GLYCOLIPIDS

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Glycosphingolipids, which are essential components of the surface of animal cells (1,2) have been shown to display high structural specificity in the binding of ligands of two types, of particular biomedical interest. One is poly- or monoclonal antibodies with specificities for blood group substances (1) and for tumor antigens (3). The second type is a group of ligands of microbial origin including cholera and shigella toxins and bacteria and viruses (2, Karlsson et al. unpublished). However, a physiological function of a cell surface oligosaccharide has not yet been established.

In receptor studies of the kind mentioned above direct inlet mass spectrometry using EI and chemical derivatization is now an indispensable technique for the structural characterization of glycolipids (see 3), as isolated substances or in complex mixtures. Attempts in recent time to apply FAB on unprotected or derivatized glycolipids have been less successful, at least on larger species (4,5, Karlsson et al. unpublished), in contrast to highly informative spectra recorded for unprotected oligosaccharides (6).

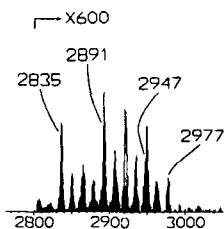


Fig. 1. Top endions of a LiAlH_4 -reduced permethylated 12-sugar glycolipid with blood group A activity (ref. 7). MS 902 instrument, electron energy 29 eV, acceleration voltage 2.9 kV, and ion source temperature 305°C (in-beam).

A reliable determination after EI of saccharide sequence and lipophilic components is possible using a combined interpretation of spectra of three derivatives, permethylated, LiAlH_4 -reduced permethylated, and silylated LiAlH_4 -reduced permethylated derivatives (the latter in case of reduced sialic ester). The limit in size for a complete sequence is at present 12 sugars (7) but partial information is obtained from still larger species (4). Fig. 1 shows the series of peaks close to m/z 3000 recorded for the 12-sugar glycolipid (7) representing the complete oligosaccharide and the mixture of fatty acids from 16:0 at 2835 and to 2-hydroxy-24:0 at 2977.

The specific ions produced at EI of separate saccharide sequences (see Table 1 for masses of actual terminal and internal derivatized sugars) allow the interpretation even of novel species present in a mixture of glycolipids. By a programmable fractional distillation of the sample in the ion source many species may separate completely,

Table 1
Masses of terminal and internal sugars found as constituents of glycolipids.

	Terminal Sugar			Internal Sugar		
	Met	Met-red	Met-red-TMS	Met	Met-red	Met-red-TMS
Pent	175			160		
Fuc	189			174		
Hex	219			204		
NAcHex	260	246		245	231	
NeuAc	376	334	406	361	319	391
NeuGc	406	364	436	391	339	421

mainly based on the number of sugars (8). An illustrative example is the recent characterization of an interesting tumor antigen, present in very small amounts in human pancreas and colon tumor (0.5 mg from 750 g dry tumor) and representing a novel combination of sugars (3). After advanced purification the active glycolipid was homogenous in 4 different solvents at thin-layer chromatography. However, mass spectrometry clearly showed at least 4 different glycolipid oligosaccharides, which guided further purification. The mass spectrum of Fig. 2 shows sequence ions obtained from the permethylated

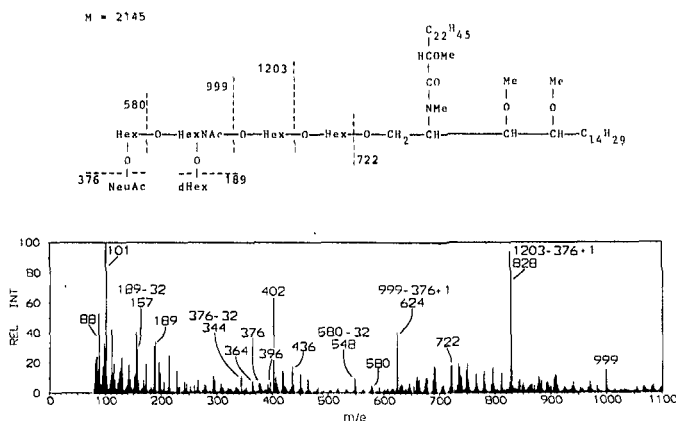


Fig. 2. Mass spectrum and simplified formula of the permethylated derivative of a human tumor antigen representing a novel glycolipid species of clinical interest (ref. 3). ZAB-HF instrument, electron energy 44 eV, acceleration voltage 8 kV, and ion source temperature 330°C (in-beam).

purified glycolipid. The other two derivatives gave important supplements including molecular size (3). The rather strong peak at m/z 402 is diagnostic for a Hex1-3HexNAc linkage and is not obtained for Hex1-4HexNAc and represents one of the few cases where position of linkage is also obtained from this technique.

As a conclusion we have found that direct inlet EI mass spectrometry of derivatized glycolipids is superior to other available techniques as concerns a more detailed information from isolated species or mixtures. The limit in size to 12 sugars is well within range of biomedically interesting glycolipids of animal cells.

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PEPTIDE MIXTURE ANALYSIS BY MOLECULAR ION MASS SPECTROMETRY

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With the progress made in the techniques of molecular ion production, the molecular weight of a peptide now can be easily determined by Molecular Ion Mass Spectrometry (MIMS). We have developed new methods of polypeptide sequencing. The project has proceeded along the following four steps. Firstly we have analyzed various kind of known single peptides and learned how to determine their molecular weights. Secondly, the molecular weights of several unknown single peptides can be determined using established techniques. MIMS method has gradually been recognized as an important tool in the weight determination of a peptide by many protein chemists. Thirdly, on the process of analyzing a peptide mixture sample, we obtained, unexpectedly, clear mass spectra of the components of the peptide. We realized immediately that the most powerful use of MIMS is the capability of molecular weight determination of the components of the mixture without pre-separation. Though it is often said that FD-MS is not effective for the structural elucidation of a peptide because it gives only few useful fragment ions, we believe that this point (only protonated molecular ions are observed) is an advantage over various kind of ionization techniques. This is especially important for mixture analysis. Fourthly, we performed the following three kinds of investigation of polypeptide sequencing.

(1) Combination of MIMS with Edman degradation^{1),2)}

One of the most effective methods to determine amino acid sequence is to use the Edman degradation. The sequence can be determined subsequently from N-terminals by identifying the PTH amino acid. When peptide mixture samples obtained from a protein by specific chemical or enzymatic cleavage, laborious prior separation processes are necessary and small peptide fragments might be lost. We determine the molecular weights by MIMS of residual component peptides before and after each cycle of Edman degradation. Released PTH amino acids are also identified by HPLC. From the MIMS and the HPLC results, the partial sequence can be elucidated simultaneously.

(2) Combination of MIMS with collision induced dissociation³⁾

It is clear that metastable sequence ions are produced from protonated molecular ions of a peptide (precursor ion) by the process of collision induced dissociation. A linked scan technique is useful for detecting metastable daughter ions. We analyzed an artificial peptide mixture in the normal scan mode and in linked scan mode after choosing one precursor ion. By the help of computer program "PAAS", we can elucidate the amino acid sequence of the component peptide. It became clear that useful negative metastable peaks are also produced when all measurement are executed in negative mode.

(3) Combination of MIMS with standard known amino acid sequence

An important application of MIMS is the characterization of a polypeptide containing amino acid substitution. The principle and the application for the diagnosis of hemoglobin variant are now explained. Let us assume we have a known polypeptide $P_S(A_1-A_2-A_3-A_4 \dots A_n)$ and an unknown polypeptide $P_R(A_1-A_2-B_3-A_4 \dots A_n)$. The substitution (A_3+B_3) occurs in this sample and we intend to determine the position "3" and the kind "B" by MIMS. All probable pairs of substitution which give the mass difference (P_S-P_R) can be simply calculated. There are two kinds of ambiguities that still remain in this method:

(a) An known peptide P_S may contain the same amino acid A_3 at a different position (for example A_{23}). The substitution (A_3+B_3) , $(A_{23}+B_3)$, can not be distinguished.

(b) If the mass differences of different pairs (A_3+B_3) and (A_5+B_5) are equal, they can not be distinguished from each other.

However, we can solve these ambiguities by the following techniques.

For (a), the original polypeptide and the standard polypeptide should be digested by the same enzyme (trypsin) under the same condition in order to cleave between A_3 and A_{23} . After comparing these two kinds of mass spectra, the probable substitution can be determined. For (b), various non-mass

spectrometric informations: e.g. base sequence of a gene, chemical properties such as the electrical charge state and the amino acid analysis, are introduced. Then the most "probable" pair of substitution can be suggested in most cases. In order to confirm the "probable" substitution, the original polypeptide should be digested by other enzymes, for example, chymotrypsin and staphylococcal protease. If the expected mass peak containing the amino acid substitution suggested by tryptic digestion is clearly observed, we conclude that the "probable" substitution is the "actual" substitution. The sequences of fragments obtained from two model polypeptides P_S and P_R after digestion are listed in Fig. 1. The expected mass spectrum is also shown in Fig. 2.

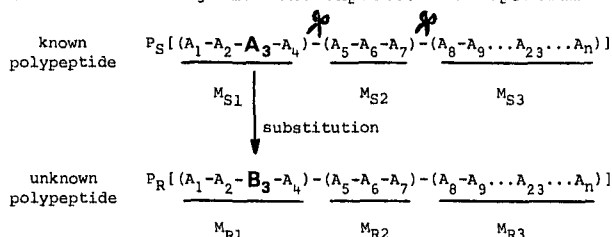


Fig. 1 Sequences of known and unknown polypeptide

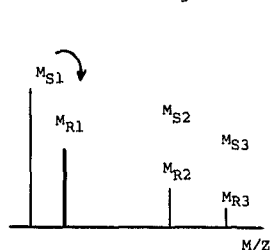


Fig. 2 Mass Spectrum of peptide mixture

The most useful application of this method is the diagnosis of hemoglobin variant. Firstly we take FD mass spectra of tryptic peptides of normal human α , β globins. Protonated molecular ions of most tryptic peptides have been observed.⁴⁾ Then, known variants Hb-S ($\beta 6$ Glu $\rightarrow$ Val), Hb-Boston ($\alpha 58$ His $\rightarrow$ Tyr) are analyzed and the position and the type of amino acid substitution are identified from FD mass spectra.⁵⁾ We take also M-SIMS and FAB mass spectra of digested peptide mixture of globins, and obtain useful mass spectra. M-SIMS and FAB is more suitable for the peptides heavier than 1500 dalton or contaminated by impurities. Recently we succeeded in characterizing two unknown hemoglobin variants (Hb- β x, Hb- γ y) which could not be identified by the conventional peptide mapping method.⁶⁾

The merits of the MIMS method are summarized as:

- (1) Polypeptides heavier than 10000 dalton can be characterized by Molecular Ion Mass Spectrometry
- (2) The ability to characterize hemoglobin variants which can not be separated from normal hemoglobin by any conventional techniques.
- (3) The ability of quick determination (usually in a few day) with small sample requirement (nano mol order).

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Recent advances in DNA sequencing techniques have made it possible to sequence entire genes that code for particular proteins. Thus, by simply decoding the gene sequence one can deduce the amino acid sequence of the corresponding protein in a rapid and reliable manner, making this the method of choice in the sequencing of large proteins. However, errors can be made that will result in an incorrect amino acid sequence. For example, a deletion or insertion error will shift the reading frame (RF) into a different one resulting in the improper decoding of the gene. Or if a single base is misidentified, an incorrect amino acid will be substituted into the protein sequence. Posttranslational modifications can also greatly effect the final protein structure.

Over the past two years, we have developed a new and sensitive method that identifies these modifications and errors and helps to correct the latter as well (1,2). This method involves the mixture analysis of underivatized peptide mixtures by FABMS. First, 10-20 nmole of protein is specifically hydrolyzed, in our case with trypsin which cleaves only at Lys and Arg, to yield a mixture of peptides. This is then partially separated by HPLC into 5-8 fractions (Fig. 1). Each fraction is analyzed by FABMS giving primarily MH^+ ions, from which their molecular weights are readily deduced. These results are then compared with the molecular weights of the peptides predicted on the basis of the proposed amino acid sequence. In some cases, FABMS of the mixture is carried out after one Edman degradation step to confirm the identities of the peptides. In the proteins studied so far, the majority of the peptides matched in a single RF. However, some peptides have been found to match those in another RF or do not match any predicted peptide, indicating that one or more errors of the type previously mentioned had occurred.

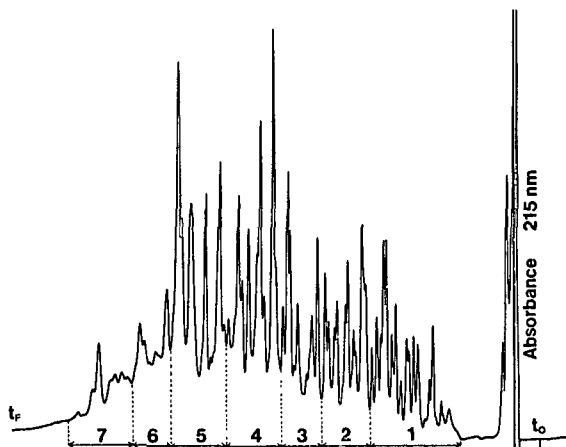


Figure 1. The tryptic digest of methionyl-tRNA synthetase chromatographed on a μ -Bondapak C-18 HPLC column. The hydrolyzate was separated into 7 fractions and analyzed by FABMS. The UV absorbance was monitored at 215 nm. The starting solvent was 0.05% TFA/ H_2O with the modifier, 0.035% TFA/ CH_3N , added linearly to 60% over 45 min with a total flow rate of 1.3 ml/min.

During the analysis of the two unseparated subunits of glycyl-tRNA synthetase GlyRS from *E. coli*. (3), $M_r(\alpha) = 35000$ and $M_r(\beta) = 65000$, we encountered both types of problems. This protein was hydrolyzed with trypsin and separated into seven fractions and analyzed by FABMS. In one such fraction, fraction 2 (Fig. 2), 17 peptides were identified, 14 of which could be readily matched to RF 1. However, 2 peptides fit RF 3 and another one could not be matched (see Table 1). After 2 cycles of Edman degradation of this mixture, almost all assignments were confirmed with the exception of the MH^+ of m/z 936 which showed its N-terminal amino acids to be Met-Val and not Gly-Glu, indicating that it is not the predicted peptide of mass 936. The other fractions gave results similar to fraction 2.

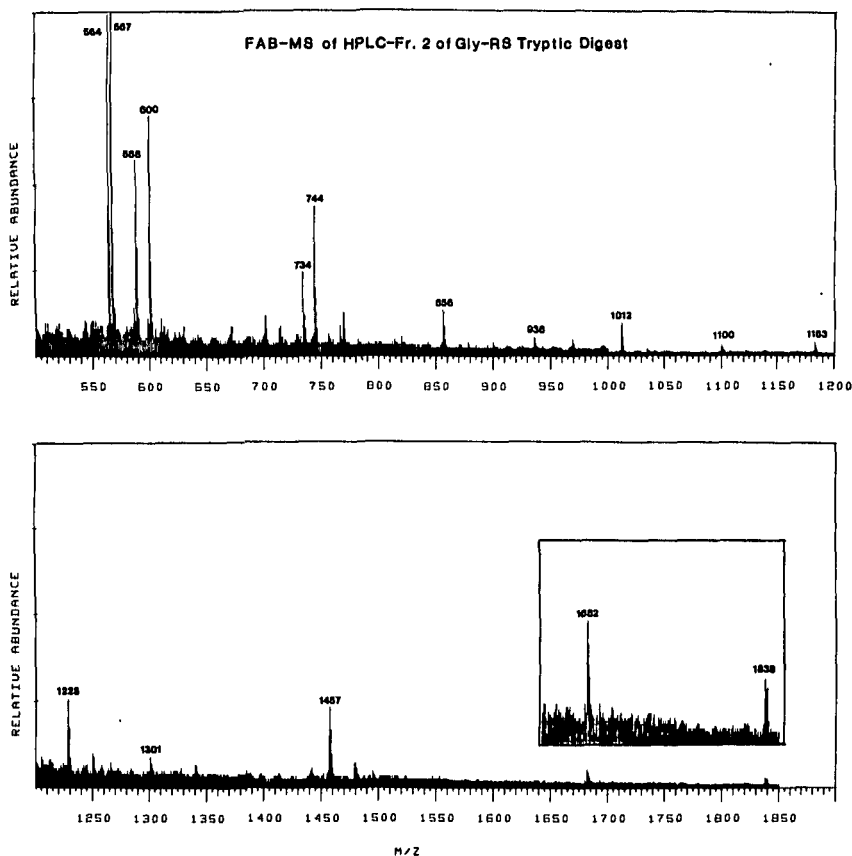


Figure 2. Partial FAB/MS spectrum of HPLC fraction 2 from the tryptic hydrolyzate of glycyl-tRNA synthetase. The protonated molecular ion for the identified peptides are numbered and listed in Table 1.

A total of 3 peptides matching RF 3 covering positions 339-360 were bordered by peptides belonging to RF1, with a gap of 41 bases and 79 bases separating them on both sides. Clearly a deletion and insertion error had occurred in these gaps. For example, the gap of 41 bases has one base too few and must contain the deletion error. To identify the actual site and base, we considered errors where poorly resolved bases on a sequencing gel are counted as one too few, the most common error. Thus, we inserted one base at a time in each of the 42 positions of the same type as the neighboring base and translated their base sequences into amino acids and predicted their tryptic peptides (Fig. 3). Four of the 42 possibilities, 6, 7, 16, and 17, gave peptides that matched an experimental one, the unmatched peptide with MW = 1444. Three cycles of Edman degradation of this peptide showed the N-terminus to be xLeu-Ala-Asp, thus eliminating possibilities 16 and 17, and indicating that the missing base must be either a C at position 6 or a G at position 7 counting from the last unambiguously correct base. Reinspection of the gels showed the error to be the latter.

MH ⁺	Subunit Read.Fr.	Position	1st Edman Cycle MH ⁺	Position	am.acid removed	2nd Edman Cycle MH ⁺	Position	am.acid removed
444	β 1	52-55	466	53-55*	Leu			
564	α 1	272-275	401	273-275	Tyr	288	274-275	Ile
567	β 1	140-144	589	141-144*	Leu	492	142-144*	Pro
588	β 1	387-392	652 ^a	388-392*	Ala	595	389-392*	Gly
600	β 1	51-55	579 ^a	52-55*	Arg			
734		no match	756		Leu(Lys*)	655		Thr
744	β 1	584-590	780 ^a	585-590	Val	693	586-590	Ser
	β 1	606-611 (Glu-Pro- . . .)						
856	β 3	340-346	743	341-346	Leu			
936	β 1	111-117 (Gly-Glu- . . .)						
		no match	972		Val(Lys*)	841		Met
1012	β 3	339-346	856	340-346	Arg	743	341-346	Leu
1100	α 1	282-291	1029	283-291	Ala	930	284-291	Val
1183	β 1	56-66	1084	57-66	Val	1013	58-66	Ala
1228	β 1	309-319	1248	310-319*	Asp	1151	311-319*	Pro
1301	β 1	551-561	1145	552-561	Arg	1048	553-561	Pro
1457	β 1	599-611	1628 ^a	600-611**	Val			
1682	β 1	56-70	1718 ^a	57-70*	Val			
1838	β 1	56-71						

Table 1. Peptide identifications for fraction 2 of GlyRS. (a) accompanied by peak 135 mass units lower which has E-NH₂ of Lys not reacted with Ph-NCS (*) contains a Lys which reacted with Ph-NCS, adding 135 amu.

RF1---	Glu	Lys	Val	Val	Arg	Pro	Arg	Leu	Ala	Met	Pro	Ser	Ser	Ser	Ser	Thr	Pro	Thr	Val	Lys	Asn	---
	GAG	AAA	GTC	GTT	CGT	CCG	CGT	CTC	CGC	ATG	CCG	AGT	TCT	TCT	TCT	TCA	ACA	CCG	ACC	GTA	AAA	AAC
RF3---								Ser	Gly	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys	Arg
								Ser	Gly	Asp	Ala	Glu	Phe	Phe	Phe	Asn	Thr	Asp	Arg	Lys	Lys	Arg
Base Inserted at Position	1																					

At this point we had yet to identify the C-terminal tryptic peptide from the β -subunit and began to suspect a premature stop codon (a). After translating the region beyond the stop codons into all RF's, 2 were found that matched RF 2 which also gave the correct N-terminal residues (b). Additional DNA sequencing in this gene region discovered two errors, an inserted guanine before the stop codons and a misidentified thymine. After these corrections were made almost all of this extended C-terminal region was found to match peptide data that was previously unmatched e.g. peptide ions in Table 1 of m/z 734 and 936 now matched this region (c).

```

631      a
CGT TAC CAG GAT GCG CTG GTC GAA CTG GCT GAG CTG CGT GAA CCG GTT GAT GGT TTC TTC GAT GAA AGT GAT GGT CAT GGT TGA TGA
Arg|Tyr Gln Asp Ala Leu Val Glu Leu Ala Glu Leu Arg|Glu Pro Val Asp Ala Phe Phe Asp Glu Ser Asp Gly His Gly STOPSTOP
657

631      b
CGT TAC CAG GAT GCG CTG GTC GAA CTG GCT GAG CTG CGT GAA CCG GTT GAT GGT TTC TTC GAT GAA AGT GAT GGT CAT GGT TGA TGA CAA
Arg|Tyr Gln Asp Ala Leu Val Glu Leu Ala Glu Leu Arg|Glu Pro Val Asp Ala Phe Phe Asp Lys|Val Met Val Met Val Asp Asp Lys|
660
RF 2 → Asn Arg Leu Met Leu Ser Ser Met Lys|Val Met Val Met Val Asp Asp Lys|

AGA ATT GCG TAT CAA CCG TCA GAC CAT GCT GGA CAA ACT GCG CG. . . . .
Glu Leu Arg|Ile Asn Arg Gln Thr Met Leu Glu Lys Leu Arg. . . . .

631      c
CGT TAC CAG GAT GCG CTG GTC GAA CTG GCT GAG CTG CGT GAA CCG GTT GAT GGT TTC TTC GAT GAA AGT GAT GGT CAT GGT TGA TGA CAA
Arg|Tyr Gln Asp Ala Leu Val Glu Leu Ala Glu Leu Arg|Glu Pro Val Asp Ala Phe Phe Asp Lys|Val Met Val Met Val Asp Asp Lys|
660

GAA TTG CGT ATC AAC CGT CTG ACC ATG CTG GAG AAA CTG CCG GAA CTG TTC CTG CCG GTT GCG GAT ATT TCG CTG TTG CAA TAA TAA
Glu Leu Arg|Ile Asn Arg|Leu Thr Met Leu Glu Lys|Leu Arg|Glu Leu Phe Leu Arg|Val Ala Asp Ile Ser Leu Leu Gln|STOPSTOP
687

```

Another protein we are working on is methionyl-tRNA synthetase from yeast. It was not susceptible to Edman degradation indicating a blocked N-terminus. In prokaryotes, the Met codon signifies the initiation of translation which was in this case ambiguous since there were three closely spaced Met codons at the beginning of the gene. After tryptic hydrolysis and FAB/MS analysis, we found numerous peptides that matched this N-terminal region with some originating between the first and second possible Met residues. Thus, the translation must have begun at the first Met. In addition, preliminary evidence indicates that an unmatched MH^+ ion at m/z 998 corresponds to the blocked N-terminus with a posttranslational removal of Met and N-acetylation of the neighboring Ser residue. This assignment was supported by a high resolution measurement to within 1.5 ppm of that predicted for this peptide.

A last example of this strategy involves the prediction of single base errors in the DNA sequence. In work on endoglycosidase H, we found a MH^+ of m/z 1374 that could not be matched to the protein sequence. After a computer search of all single base errors in each gene region corresponding to a tryptic peptide, a match was found for a peptide with an uncorrected MH^+ of 1402. In this case only a change of Val/GTG to Ala/GCG was possible for the 28 amu difference. This was subsequently confirmed by reinspection of the sequencing gels and Edman degradation which gave N-terminal xLeu.

```

ATC ATC AGC CTC TAC AAC ATC GGC CCG GCC GTG TCC CCG
Ile Ile Ser Leu Tyr Asn Ile Gly Pro Ala Val Ser Arg  MH+ = 1402

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ATC ATC AGC CTC TAC AAC ATC GGC CCG GCC GTG TCC CCG
Ile Ile Ser Leu Tyr Asn Ile Gly Pro Ala Ala Ser Arg  MH+ = 1374

```

In conclusion, it is important to understand the interactive nature of this strategy. It not only saves time and expense by eliminating the need to double check DNA sequences that have peptide matches, but helps to identify any errors and pinpoint their locations. In addition, the correctness of the resulting protein sequence is supported by the fact that a large fraction of it is directly covered by the data obtained from all regions of the protein, not just from the N- and C-terminal.

Acknowledgement: Work supported by grants from the National Institutes of Health (GM05472 and RR00317).

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SEQUENCING OF OLIGONUCLEOTIDES BY FAB - MS

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ABSTRACT

Negative ion fast atom bombardment mass spectrometry (FAB-MS) (ref. 1) is a powerful tool for sequencing deprotected and protected oligonucleotides. In the case of fully protected oligonucleotides it is necessary to have present a free unprotected phosphate group.

INTRODUCTION

Chemists have made considerable progress in the synthesis of DNA fragments. Such molecules serve as "Linkers and Adaptors" in recombinant DNA techniques and allow a vast number of new combinations of gene material to be produced. Other synthetic DNA fragments are used to perform site directed mutagenesis of natural DNA. The synthesis of these small DNA-fragments can be performed in a matter of days and as a consequence the longer part of the work is now the careful analysis of the deprotected oligonucleotides. Negative ion FAB-MS is a quick and reliable method for control of DNA-synthesis either for the starting materials and for the deprotected end product up to 10 base units in length.

METHODS

Oligodeoxyribonucleotides were synthesized following, in general, established phosphate triester procedures (ref. 2).

The mass spectra were recorded on a Kratos MS 50 S with a Kratos FAB source. The atom gun used xenon and produced a beam of neutral atoms at 8 - 9 kV. A solution of the triethylammonium salt of each oligodeoxyribonucleotide (4 - 5 μ l, containing 1 - 1,5 OD₂₆₀, ca. 10 nmol) was injected into the glycerol matrix (ca. 2 μ l) present on the copper probe tip. The water was removed in the direct insertion lock and the spectra recorded at a magnet scan rate of 300 s/dec.

RESULTS

Figure 1. shows the negative ion FAB mass spectrum of the oligodeoxyribonucleotide d(G-A-A-G-A-T-C-T-T-C) and figure 2. the corresponding short-hand structure. The highest mass is the (M-H)⁻ ion. The corresponding doubly charged ion is found at half mass. The mass difference between two markers is indicative of a particular nucleotide. Main fragmentation occurs only at bonds involving an O-atom at phosphate ester and a C-atom of a sugar moiety. This results in the formation of sequence ions with either 5'- or 3'-terminal phosphates (5'- and 3'-P sequence ions). 5'-P sequence ions are always more intense than 3'-P sequence ions with the same number of nucleotide units. This simple fragmentation behaviour allows an independent sequencing of the oligonucleotide chain either from the 5'- or from the 3'-end (bidirectional sequence analysis).

All fragments with the same base composition have the same mass for 5'-P and the 3'-P sequence ions. In such oligonucleotides masses and peak intensities of all sequence ions clearly allow the bidirectional analysis.

When oligodeoxyribonucleotides are protected with a monomethoxytrityl-group at the 5' end the fragmentation behaviour is different (ref. 3): in this case the 3'-P sequence ions from the 5'-end are in general more intense than the corresponding 5'-P sequence ions; of course, the 3'-P sequence ions are higher in mass by 272 d. This makes the sequence analysis easier and it is now also independent of the intensities of the 3'- and 5'-P sequence ions.

Figure 3, shows an example of a 5'-monomethoxytrityl protected octanucleotide, and as previously described, the sequence can be read from both ends.

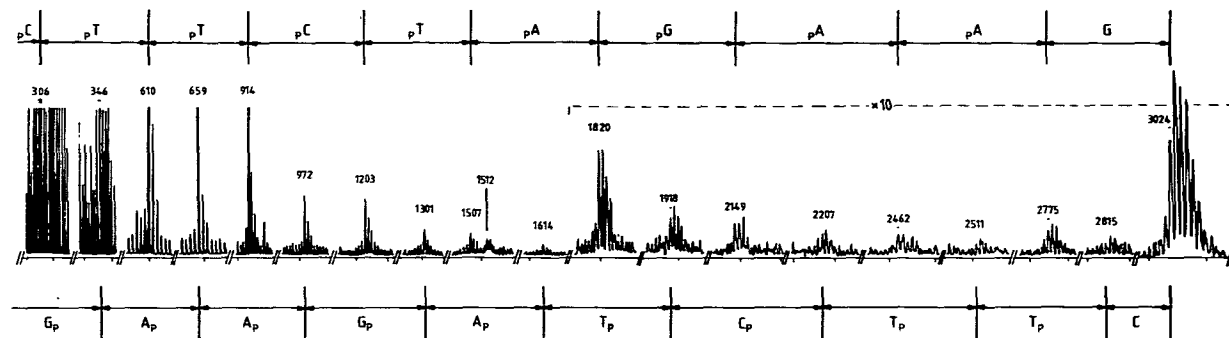
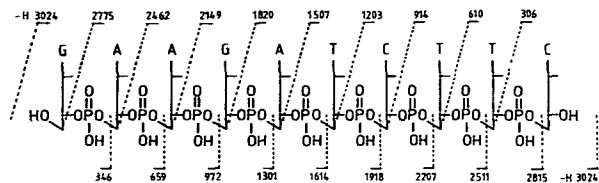


Fig. 1. Relevant regions of the negative ion FAB mass spectrum of d(G-A-A-G-A-T-C-T-T-C)

Fig. 2. Shorthand structure of d(G-A-A-G-A-T-C-T-T-C)



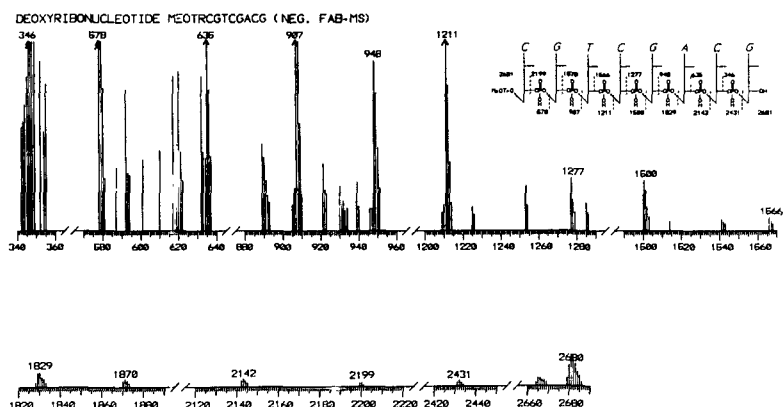


Fig. 3. Negative ion FAB mass spectrum of d[(MMTr)C-G-T-C-G-A-C-G]

X\Y	A	C	G	T
A	M 1373	M 1349	M 1355	M 1260
	pAp 734	pCp 710	pGp 716	pTp 621
	Ap 544	Cp 520	Gp 526	Tp 431
	DMTrAp 846	DMTrAp 846	DMTrAp 846	DMTrAp 846
C	M 1349	M 1325	M 1331	M 1236
	pAp 734	pCp 710	pGp 716	pTp 621
	Ap 544	Cp 520	Gp 526	Tp 431
	DMTrCp 822	DMTrCp 822	DMTrCp 822	DMTrCp 822
G	M 1355	M 1331	M 1337	M 1242
	pAp 734	pCp 710	pGp 716	pTp 621
	Ap 544	Cp 520	Gp 526	Tp 431
	DMTrGp 828	DMTrGp 828	DMTrGp 828	DMTrGp 828
T	M 1260	M 1236	M 1242	M 1147
	pAp 734	pCp 710	pGp 716	pTp 621
	Ap 544	Cp 520	Gp 526	Tp 431
	DMTrTp 733	DMTrTp 733	DMTrTp 733	DMTrTp 733

Table 1. Sequence ions of protected Dideoxyribonucleotides Type:

$$d[(DMTr) X P_{OCP} Y P_{OCP} O^-]; X, Y: A^{bz}, C^{bz}, G^{ib}, T$$

We could not obtain spectra of fully protected oligodeoxyribonucleotides under any experimental conditions. Our next objective was to check out fully protected dinucleotides, where one of the protection groups is missing, namely the end phosphate, which is protected normally with tribromoethyl or cyanoethyl. This protection group must be split off during the synthesis and hence these compounds are the next logical ones to look at. We have

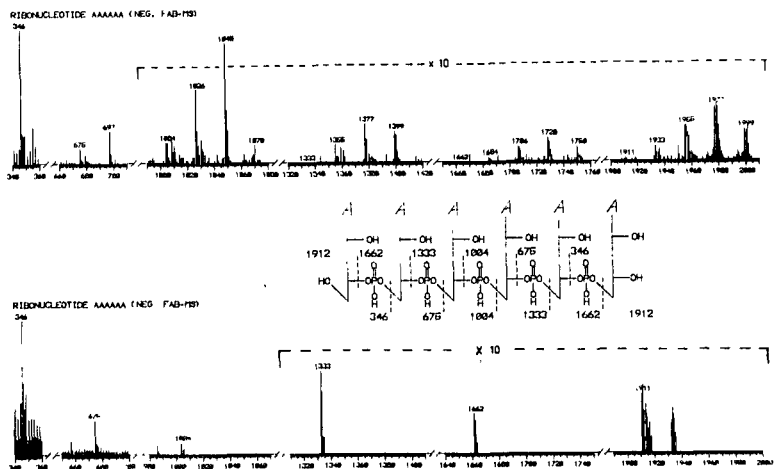


Fig. 4. Negative ion FAB mass spectrum of Ribo A₆

investigated all possible dinucleotides of a particular dimethoxytrityl protected type (Table 1), in which this protecting group was removed. In all cases we could identify and sequence these by negative ion FAB-MS (ref. 3).

Table 1 shows, that each protected dinucleotide gives a unique set of fragments corresponding to the molecular ion, diphosphate, monophosphate and dimethoxytrityl protected monophosphate ions. The isotopic pattern of the molecular and sequence ions give a check of the atomic composition of these ions. Further ions are not of interest for the sequence.

In principle we need to see only the molecular ion and the diphosphate ion, which is an intense peak, to determine the sequence.

The monophosphate and dimethoxytrityl protected monophosphate ions can be used to confirm the sequence.

A similar investigation with monomethoxytrityl protected analogues gave similar results.

The sequence behaviour of oligoribonucleotides is similar to that for oligodeoxyribonucleotides.

Figure 4. above shows the results obtained with a commercial sample of a hexamer. The spectrum is complicated by the present of sodium containing sequence ions. However, when we used the triethylammonium salt, no such complication is observed (Figure 4. below). A similar result was obtained for an octamer.

CONCLUSION

Negative ion FAB-MS is a quick and reliable method for the bidirectional sequence analysis of oligonucleotides. At present it is possible to analyse unprotected oligonucleotides up to ten base unit in length and shorter protected oligonucleotides with two and three base units.

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PROBABILITY THEORY FOR ION FRAGMENTATION IN CA/MS^{1,2}

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Mass spectrometry/mass spectrometry (MS/MS) is a new technique in mass spectrometry which is finding increasing application in the study of the structures and fragmentation mechanisms of organic ions. In this technique, collisional activation (CA) has been widely used to generate a mass spectrum of a mass-selected precursor ion. In the present work, a probability theory was formulated to describe the fragmentation yield in CA process. The theory has been applied to CA processes occurring under high collision gas pressure, where multiple collisions are prevalent.

Collision cross-section

Total collision cross-section (σ) will be divided into two parts, one contributing to ion loss (σ_L) the other to collisional activation (σ_{CA})

$$\sigma = \sigma_L + \sigma_{CA} \quad ; \quad \text{for parent ions} \quad (1)$$

$$\sigma = \sigma_L + \sigma_{CA} \quad ; \quad \text{for daughter ions} \quad (2)$$

Probability of n collisions

The number of collisions experienced by parent ions exciting the collision chamber corresponds to the number of collision gas molecules in the collision cylinder (Fig. 1), which is well approximated by Poisson distribution.

$$q_n(1) = \frac{e^{-a} \cdot a^n}{n!} \quad a = \rho \sigma l \quad (3)$$

Probability of parent and daughter ion detection in the two-step model

In the two-step mechanism for the CA process (eqn. (4)), an ion which has undergone a collision will either be lost or dissociated



Thus, probability (P_p) of detecting parent ions is given by

$$P_p = q_0(1) = e^{-a} \quad (5)$$

In the case when the parent and daughter ions have the same collision cross-section, each collision will reduce the ion current by a factor of $S (\equiv \sigma_{CA}/\sigma)$. Hence, the probability of detecting daughter ions which have undergone n collisions is given by $S^n q_n(1)$. Therefore, the probability of daughter ion detection is given by

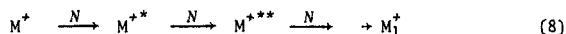
$$P_D = \sum_{n=1}^{\infty} S^n \cdot q_n(1) = e^{-(1-s)a} \cdot e^{-a} \quad (6)$$

When the collision cross-sections of daughter ions differ from that of the parent ion, eqn. (7) is obtained (details to be found in ref. 2).

$$P_D = \frac{S[e^{-f(1-s)a} - e^{-a}]}{1 - f(1-s)} \quad , \quad f \equiv \sigma_L/\sigma_L \quad (7)$$

Probability of parent and daughter ion detection in sequential excitation model

In this model (eqn. (8)), the parent ions which have not gained internal energy sufficient for fragmentation by the first collision will dissociate more efficiently by the second and successive collisions.



Therefore, the effective CA cross-section in this model at high pressure can be larger than that for the corresponding one-step excitation, resulting in daughter-ion enhancement. Defining r_n as the probability of the parent ion not gaining

sufficient internal energy for dissociation after n collisions, one obtains the probability of parent ion detection as follows

$$P_p = \sum_{n=0}^{\infty} r_n s^n f_n(1) \quad (9)$$

When the parent and daughter ions have the same cross-sections, the daughter ion detection probability is given by

$$P_D = e^{-(1-s)a} - P_p \quad (10)$$

When the daughter-ion cross-sections differ from those of the parent (ref. 2) one obtains

$$P_D = \alpha e^{-a} \sum_{n=0}^{\infty} (r_n - r_{n+1}) A_n \quad (11)$$

$$\text{with } A_{n+1} = \alpha A_n - \frac{(\alpha\beta)^{n+1}}{(n+1)!} \quad \text{and } A_0 = e^{\beta} - 1$$

$$\text{where } \alpha \equiv S/[1 - f(1-s)]$$

$$\beta \equiv [1 - f(1-s)]a$$

Analytical expressions for r_n 's were derived for some simple collisional energy-transfer probability functions ($P_C(E)$) in ref. 1. For example, for the triangular $P_C(E)$ function (Fig. 2), one obtains

$$r_n = \frac{(2r_1)^n}{(2n)!} \quad (12)$$

Comparison with the experimental data

The present results were used to fit the experimental CA data for 8.0 KeV CH_4^+ ions³ (circles in Fig. 3). Curve I in Fig. 3 was calculated using the results of the two-step model with identical cross-sections for ions. The experimental data shows the daughter ion enhancement at high collision gas pressure. In Curve II, the effect of the smaller daughter ion cross-section ($f=0.9$, estimated) was considered. This still enhances the daughter-ion intensity, but not enough to account for the experimentally observed enhancement. The results from the sequential excitation model were used to calculate Curve III.

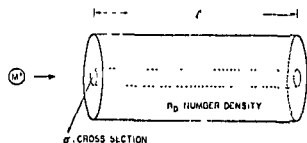


Fig.1 Collision cylinder

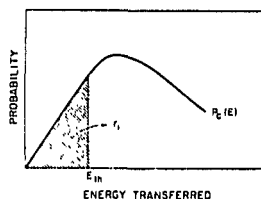


Fig.2 The triangular collisional energy-transfer probability function

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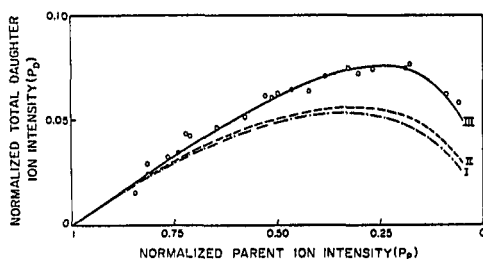


Fig.3 Experimental CA data (circles) and calculated (curves I,II,III) P_D vs P_p curves for 8.0 KeV CH_4^+ ions

ENERGY-ENTROPY TRADE-OFFS IN THE UNIMOLECULAR DECOMPOSITIONS OF SF_6^-

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The SF_6^- anion undergoes two reactions, (1) autodetachment and (2) predissociation: (1) $\text{SF}_6^- \rightarrow \text{SF}_6 + e$; (2) $\text{SF}_6^- \rightarrow \text{SF}_5^- + \text{F}$. Reaction (2) demonstrates a unimolecular metastable ion transition of statistical origin.¹ The observation of metastable ions requires the survival against autodetachment of SF_6^- having enough energy to dissociate via (2) at least for the time spent in the ion-source and up to the field free region of a magnetic sector instrument where reaction (2) takes place. RRKM-QET calculations were performed to reproduce experimental results — autodetachment and predissociation lifetimes and metastable ion intensities. Under the assumption made that reactions (1) and (2) take place competitively from the ground potential surface — the choice of threshold energy E_{01} and vibrational frequencies of SF_6^- to fit autodetachment lifetimes, has to fit experimental data for reaction (2) as well. A recent model,² whereby $E_{01} = 1.4$ eV and the structure of SF_6^- is closely similar to that of neutral SF_6 , predicts no observable metastable transition for reaction (2), provided the time spent up to the field free region is 2 μs and $E_{03} = 0.43$ eV,³ where (3) $\text{SF}_6 + e \rightarrow \text{SF}_5^- + \text{F}$.

An alternative model⁴ which treats SF_6^- as a loose $\text{SF}_5^- \cdots \text{F}$ complex can reproduce experimental results provided $E_{01} = 0.8$ eV, in agreement with a recent⁵ flowing afterglow determination of the electron affinity of SF_6 . Fig. 1 gives a sensitivity analysis for reaction (1) using the Heneghan and Benson (A) and Drzaic and Braumann (B) models. Model B, with an electron affinity of $\text{EA} = 0.8$ eV is not a unique solution. If the configuration of the reactant ion is made less loose so that $\Delta S^\ddagger$ for reaction (1) is -10 e.u. instead of -14 e.u., agreement with ion lifetimes and with metastable ion intensity is obtained for $E_{01} = 1.0$ eV, which is still within the limits of error⁵ for $\text{EA}(\text{SF}_6) = 1.0 \pm 0.2$ eV. Calculations show that autodetachment is the major reaction in the field free region.

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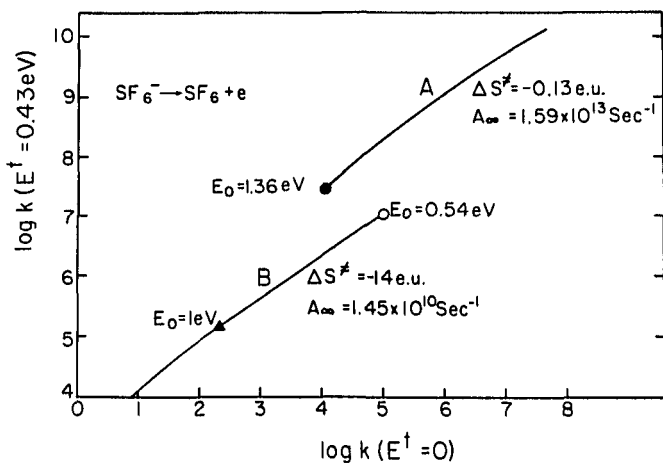


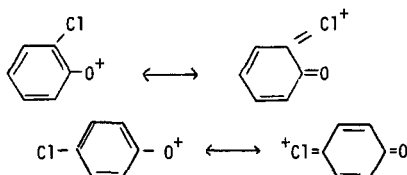
Figure 1: Sensitivity analysis for reaction (1). The value of $\log k$, at an excess energy above threshold of $E^\ddagger = 0.43 \text{ eV}$ (which corresponds to the onset of reaction (2)), is plotted as a function of $\log k$ at $E^\ddagger = 0$, the threshold for reaction (1). The two models A and B correspond to different activation entropies. The energy threshold E_0 , for reaction (1), is treated as a parameter and is varied along the curves A and B, for models A and B, respectively, between 1.36 eV and 0.54 eV.

ENERGY DEPENDENCE OF THE FRAGMENTATION OF HALOANISOLE MOLECULAR IONS

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The two major primary fragmentation reactions of anisole molecular ions involve elimination of CH_2O and loss of CH_3 ; the latter reaction is followed by elimination of CO leading to $\text{M}^+-\text{CH}_2-\text{CO}$. Cooks et al (1) have shown that elimination of CH_2O occurs by the two mechanisms shown in the Scheme for metastable ions, the four-centred reaction involving a large kinetic energy release and the five-centred reaction involving a much smaller energy release. We report on investigation of the competition between these various reactions in haloanisoles using charge exchange mass spectrometry (2) and ion kinetic energy spectroscopy.

Figure 1 shows the breakdown graphs for the chloroanisoles isomers derived from the charge exchange data. For the *o* and *p* isomers the main primary fragmentation reaction is loss of CH_3 followed by elimination of CO at high internal energies. By contrast, for the *m* isomer the main primary fragmentation reaction involves elimination of CH_2O and loss of CH_3 becomes important only at high internal energies where further fragmentation by CO elimination occurs. It is clear that the main cause of this difference is a distinct lowering of the critical energy for CH_2O loss for the *o* and *p* isomers, presumably as a result of resonance stabilization of the resulting cation, viz

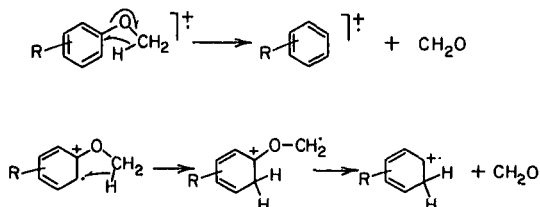


Such resonance stabilization is not possible for the *m* isomer. In addition there may be a slight lowering of the critical energy for elimination of CH_2O for the *m* isomer.

* These differences also are reflected in the metastable ions where the ratio $m^*(\text{CH}_2)/m(\text{CH}_2\text{O})$ is 8.0 (*o*), 0.024 (*m*) and 11.7 (*p*). Further, as shown in Fig. 2, metastable elimination of CH_2O from the *m* isomer involves predominately the five-membered transition state while for the *o* and *p* isomers a large contribution is made by the four membered transition state.

The monofluoroanisoles show a behaviour very similar to the monochloroanisoles. For the dichloroanisoles the results show the combined effect of *o-p* and *m* substituents. When both chlorines are *o-p* the breakdown graph shows dominant loss of CH_3 while the metastable for loss of CH_2O shows a large kinetic energy release. When both chlorines are *m* loss of CH_2O dominates the breakdown graph and the metastable shows a small kinetic energy release. The compounds containing both a *m* and an *o/p* chlorine show intermediate behaviour.

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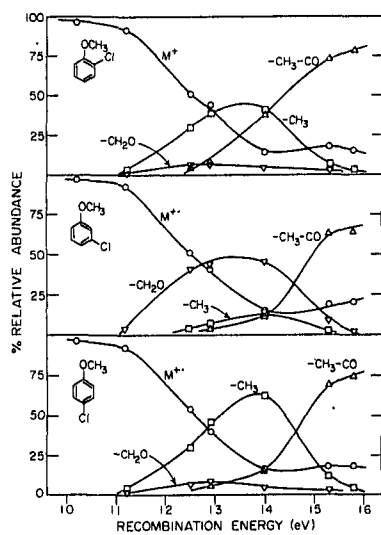


Figure 1

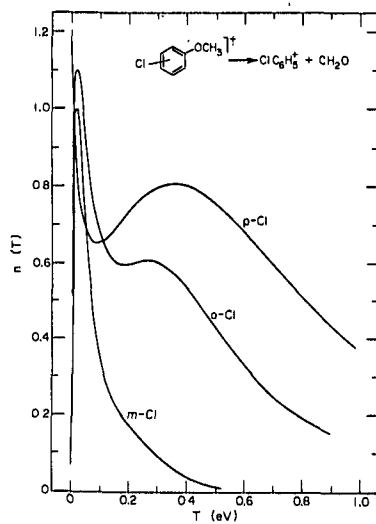


Figure 2

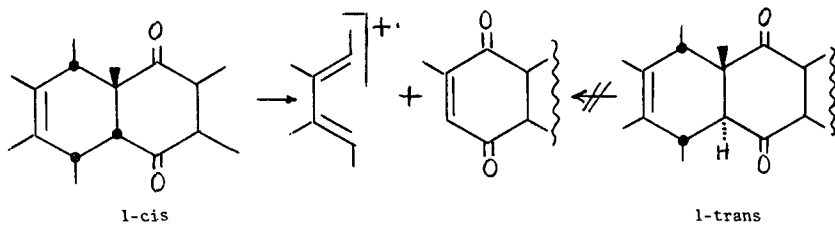
MORE ON THE MECHANISM OF THE RETRO-DIELS-ALDER FRAGMENTATION
UNDER ELECTRON IMPACT

A. MANDELBAUM and A. FRAIMAN

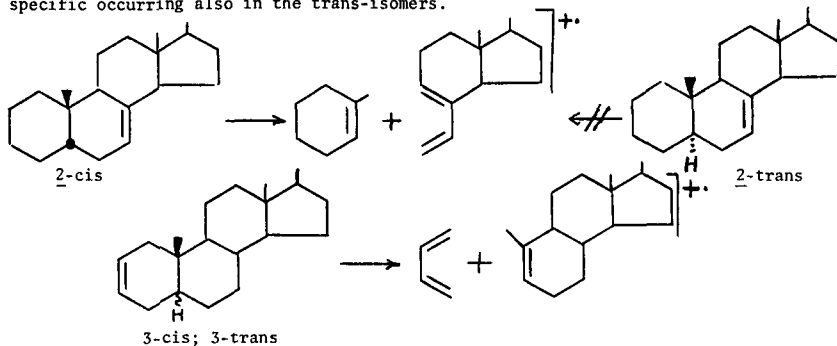
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32000 Haifa, ISRAEL

Is retro-Diels Alder (RDA) fragmentation of gas-phase radical cations under electron impact a concerted process similar to the ground state analog in the condensed phase, or are the two bonds cleaved stepwise? The most direct way to answer this question is the comparison of mass spectra of stereoisomers differing in the configuration at the ring junction which becomes a double bond after the RDA fragmentation.

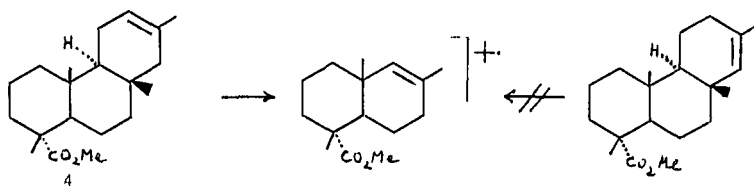
In certain systems the RDA process is highly stereospecific occurring only in the *cis*-isomers, which is an indication for the concerted mechanism. These



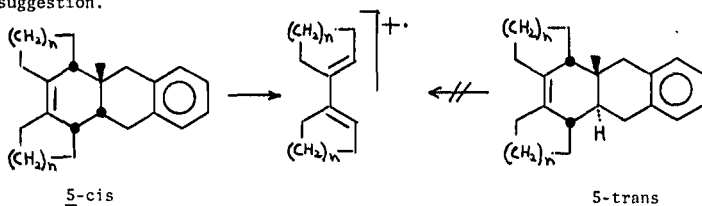
systems include various diketones 1, analogous diols, diesters and Δ^7 -steroids 2. On the other hand there are systems such as Δ^2 -steroids 3, Δ^2 -octalins and various pentacyclic triterpenes in which the RDA fragmentation is non-stereospecific occurring also in the *trans*-isomers.



A suggestion has been made that the occurrence of RDA fragmentation in *trans*-isomers may be explained by a concerted mechanism preceded by an epimerization which may take place by a series of hydrogen transfers. Such hydrogen migrations have been shown to occur in cyclohexene prior to the formation of a butadiene radical cation. The high regiospecificity of the RDA process under electron impact (also in systems which exhibit no *cis*-stereospecificity, e.g. 4) is inconsistent with this suggested isomerization by hydrogen migration.



An alternative suggested explanation is based on charge localization arguments: Systems with the charge localized in the double-bond region (e.g. 2) give rise to a stepwise non-stereospecific RDA fragmentation, while those with the charge localized at other positions formed by alternative allylic cleavages, like in 3) lead to the concerted stereospecific RDA process. The high stereospecificity found in 5 and in tricyclic analogs seems to disprove this suggestion.

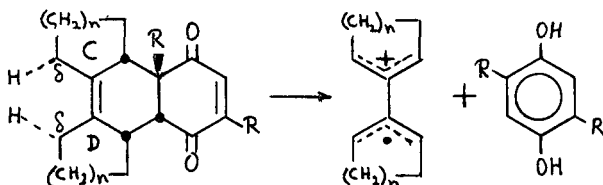


Data available from this and from previous works seem to indicate that the stereospecificity of the RDA fragmentation is affected by the degree of substitution of bonds cleaved in the course of this process. The different behavior of various systems may be explained by energetic arguments. More work will be necessary to provide evidence for this suggestion.

STEREOSPECIFIC HYDROGEN MIGRATIONS IN THE FRAGMENTATION OF ORGANIC GAS PHASE CATIONS AND IN PHOTOCHEMICAL REARRANGEMENTS IN THE SOLID STATE AND IN SOLUTION.

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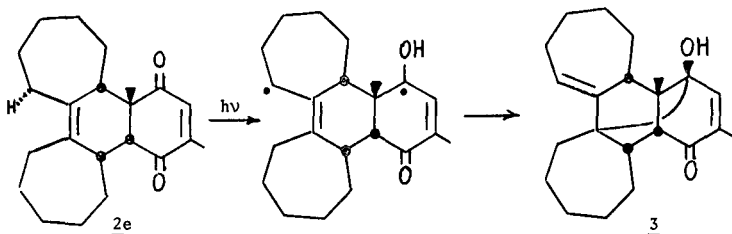
A long time ago we investigated the fragmentation behavior of diones 1 under electron impact. The most abundant ion appeared at m/z 160 corresponding to a retro-Diels Alder fragmentation accompanied by the migration of two hydrogen atoms (RDA-2H).¹ A deuterium labelling study showed that the origin of



1. $R=H$; $n,m=1,2,3$
2. $R=CH_3$

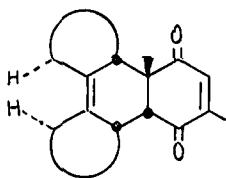
these two H-atoms was at positions δ . A similar behavior was observed for analogous diones differing in the size of rings C and D: The corresponding ions were of highest abundance when both rings were five-membered (m/z 132) or one was five- and the other six-membered (m/z 146). Lower abundance was observed for the analog with seven-membered rings C and D (m/z 188).² Similar behavior was observed in the methyl-substituted diones 2, where a high degree of stereospecificity could be established. In 2 a regular RDA fragmentation competes with the (RDA-2H) process.³

The crystalline dione 2e (seven-membered rings C and D) undergoes an interesting rearrangement on irradiation resulting in the ketoalcohol 3. It is clear that this rearrangement involves migration of a hydrogen atom from position δ to a carbonyl group. The following mechanism is suggested for the rearrangement 2e $\rightarrow$ 3. This rearrangement takes place also on irradiation of



benzene or ethyl acetate solutions of 2e. In the latter a competing $2\pi+2\pi$ cyclo-addition gives rise also to a cage cyclobutane derivative.

The obvious relationship between the electron impact behavior and the photochemical rearrangement in the solid and liquid state prompted us to undertake a detailed crystallographic study of adducts 2. This study shows that in the solid state one of the allylic δ -H atoms is within a relatively short distance (3.2 Å) from the carbonylic oxygen atom in 2e (two seven-membered rings C and D). This explains the ready migration of this H-atom resulting in the ketoalcohol 3.



	ring size		$\delta\text{H}\dots\text{O}$
	C	D	distance, Å
<u>2a</u>	5	5	3.236
<u>2b</u>	6	5	4.126
<u>2c</u>	6	6	4.820
<u>2d</u>	6	7	3.986
<u>2e</u>	7	7	3.202

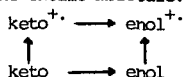
It is noteworthy that there is no simple relationship between the H...O distance in the solid state and the efficiency of the RDA-2H process in the gas-phase radical cations under electron impact. 2b, 2c and 2d give rise to abundant RDA-2H ions (m/z 146, 160 and 174 respectively) despite the large H...O distance, while 2e exhibits a relatively low abundance m/z 188 ion although the H...O is small. The photorearrangement product 3 gives rise to most abundant m/z 188 ion under electron impact.

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EFFECT OF KETO-ENOL TAUTOMERISM ON THE FRAGMENTATION OF 2-PENTANONE AS PROBED BY TANDEM MASS SPECTROMETRY; Karl V. Wood, Engine/Fuels Laboratory, Institute for Interdisciplinary Engineering Studies, Chemistry Building, Purdue University, West Lafayette, IN 47907, Scott McLuckey and R. Graham Cooks, Department of Chemistry, Purdue University, West Lafayette, IN 47907

Considerable effort has been focused on the ion chemistry of seemingly simple aliphatic ketones and the enolic isomer has figured prominently in some of these studies. In spite of the wealth of information available there appear still to be deficiencies in our understanding of these systems, for example, poor agreement has resulted from several studies aimed at determining breakdown curves, plots which show the relative fragmentation of a molecular ion as a function of internal energy. This study reports a new observation, a remarkable source temperature dependence in the fragmentation spectrum of 2-pentanone, and interprets this result in terms of an important contribution to the ion population through direct ionization of the enolic molecule.



Low collision energy mass spectrometry/mass spectrometry (MS/MS), in which relative fragment ion abundances are recorded as a function of precursor ion kinetic energy just prior to collision, enables the internal energy dependence of fragmentations of a gas phase ion to be followed. These collision energy resolved plots approximate breakdown curves although the internal energy deposited is not linearly related to collision energy.¹ A detailed study of the 2-pentanone system has shown that the MS/MS spectra of ionized 2-pentanone are highly dependent on ion source temperature. This dependence is considerably larger than can be rationalized in terms of the change in internal energy of the neutral molecules with temperature.

Three different MS/MS instruments were used in this study, a high collision energy MIKE (mass-analyzed ion kinetic energy)² spectrometer a low collision energy triple quadrupole spectrometer³ and a hybrid BQO (magnet, quadrupole, quadrupole) spectrometer.⁴ The MS/MS spectra were obtained by mass selecting the M^+ , m/z 86, ion of 2-pentanone with the first mass analyzer, subjecting this ion to an energetic collision in the collision cell (or quadrupole) and subsequently mass analyzing the resulting fragment ions.

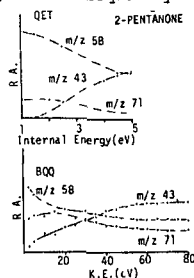


FIGURE #1

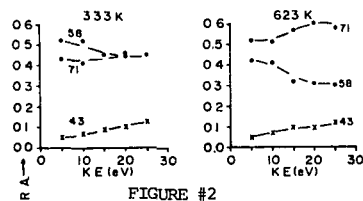


FIGURE #2

emission leaving the parent ion with insufficient energy to fragment. In this case a state-specific fragmentation could lead to the increased signal at m/z 71. However, this state must have a lifetime greater than 25 μsec (which is the approximate time delay between ionization and entrance into the collision quadrupole) and must survive a relatively low velocity collision prior to fragmentation.

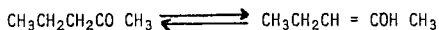
An alternate and simpler explanation for the observed temperature effects is a shift in

Figure 1 shows that the collision-energy resolved MS/MS spectra of ionized 2-pentanone agree qualitatively with the theoretical breakdown curve⁵ but the relative intensity of m/z 71 is somewhat larger in the experiment. These results were obtained at 350°K, but it was found that as the ion source temperature was increased there were dramatic changes in the relative fragment ion intensities. Figure 2 shows these fragment ion/source temperature variations as a function of collision energy using the triple quadrupole MS/MS instrument. These data were obtained with Argon as the collision gas at target gas pressure of 0.15 mtorr. As can be seen at high ion source temperatures m/z 71 is the dominant species at all collision energies studied.

This dramatic change cannot be due to the increase in thermal energy content of neutral 2-pentanone in going from 333°K to 623°K which has been calculated to be less than half an eV.⁶ Furthermore, the behavior of the m/z 43 ion does not reflect an increase in the parent ion internal energies since this ion would be expected to increase with increasing internal energy but in fact it remains constant or decreases slightly at higher ion source temperatures.

A possible interpretation of these results could result from population of an excited state of an isolated electronic state. In this case this state would have to have a lifetime sufficiently long to remain undissociated on the time scale of the experiment or to photon

the keto-enol tautomerism at higher temperatures which would result in an increased percentage of enolic ions entering the collision cell. At room temperature (289°K) the equilibrium



results in an enol concentration of $8.6 \times 10^{-5}\%$.⁷ From this the heat of formation of the enol can be determined to be approximately 5.6 kcal/mole less negative than the keto form. Assuming this difference is temperature independent over the temperature range of interest, the concentration of enol at 623°K increases to 1.2%. The enolic ion would not be expected to give an abundant fragment ion at m/z 58. However, methyl loss, by cleavage β to the double bond would give a fragment ion at m/z 71. This assumption, that the enolic ion would give an abundant m/z 71 fragment ion and little m/z 58, was tested by obtaining the MS/MS spectrum of the m/z 86 fragment ion from ionized 3-ethyl 2-pentanone. This fragment ion, resulting from ethylene elimination via the McLafferty rearrangement should have the ion structure of enolic 2-pentanone. Its spectrum can be seen in Figure 3 and the most abundant fragment ion is m/z 71.

This result suggests that if a significant fraction of the mass-selected m/z 86 ions from the experiments with 2-pentanone are in the enolic form, the fragmentation of the enolic ion in the MS/MS spectrum could explain the increased ion intensity at m/z 71 which is observed at high source temperature. Furthermore, the fraction of ionic enol which reaches the collision cell is likely to be much greater than the fraction of neutral enol in the ion source because enolic ions, in contrast to their neutral counterparts, are more stable than the keto form. In otherwords once the ions are formed there is a probability for the keto form to isomerize to the enol form. Another factor which tends to increase the relative fraction of enol reaching the collision cell is the difference in the ionization potential of the two isomeric neutral structures. The adiabatic ionization

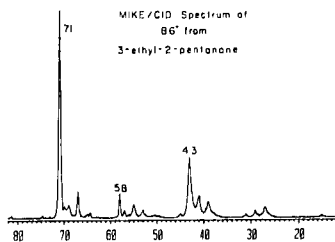


FIGURE #3

potential of 2-pentanone is 9.4 eV⁸ compared with the adiabatic ionization potential of the enol form, which is 8.2 eV, estimated from the difference in the heat of formation of neutral enol and the estimated heat of formation of the enolic ion. This difference in ionization potential results in a higher probability for ionization of the enol than the keto form, such that a larger fraction of enol ions are formed upon ionization than is present in the population of neutral tautomers.

Most mass spectrometric studies of ketones have neglected the possibility of significant contributions to the enolic form of the ion arising by direct ionization of the (low concentration of) neutral enol present. However, both direct ionization and isomerization favor production of the enolic ions. Therefore, where the rate of ionic keto → enol interconversion is rapid enough, on the time scale of the experiment, to significantly alter the distribution of ion structures, the contribution of fragmentations from the enol ion in the mass spectrum or MS/MS spectrum can be important. While this study does not disprove the existence of a long lived excited electronic state of ionized 2-pentanone keto → enol isomerization is sufficient to explain the remarkable source temperature effects observed.

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Ionised Oxy-carbenes: their Generation, Identification and Stability Relative to Conventional Ionic Counterparts.

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Following the identification^{1,2} of the (proposed) interstellar species $[\text{COH}]^+$, we have generated and identified the related carbenes $[\text{HCOH}]^+$, $[\text{C}(\text{OH})_2]^+$ and $[\text{HCO}_2]^+$. Loss of D_2 from ionised CD_3OH generates $[\text{DCOH}]^+$, free from its isomer, ionised formaldehyde (loss of H_2 from ionised CH_3OH generates $[\text{HCOH}]^+$ and $[\text{H}_2\text{CO}]^+$.³) Its CA mass spectrum allows its identification i.e. intense $[\text{CN}]^+$, weak $[\text{CHD}]^+$ signals. AE measurements using monochromatic electrons of m/z 31 in the mass spectrum of CD_3OH lead to $\Delta H_f^\circ[\text{HCOH}]^+ = 238 \text{ kcal mol}^{-1}$ vs $\Delta H_f^\circ[\text{H}_2\text{CO}]^+ = 225 \text{ kcal mol}^{-1}$, in good agreement with ab-initio calculations.⁴ The ion $[\text{DCOH}]^+$ loses both $\text{H}^\cdot$ and $\text{D}^\cdot$ in the metastable time frame with different kinetic energy releases ($T_{0.5} = 370$ and 160 meV respectively) The AE values for the metastable peaks are the same (13.9 eV) and substantially higher than that calculated for production of $[\text{HCO}]^+$, 12.8 eV . These results are explained in terms of different fragmentation products ($[\text{DCO}]^+$ and $[\text{COH}]^+$) having different ΔH_f° s. The above AE values indicate that the reverse reaction $[\text{HCO}]^+ + \text{H}^\cdot \rightarrow [\text{HCOH}]^+$ has an energy-barrier of $\sim 25 \text{ kcal mol}^{-1}$, whereas the isomerisation reaction $[\text{HCOH}]^+ \rightarrow \text{H}_2\text{CO}^+$ requires at least 34 kcal mol^{-1} . Previous work⁵ established that $[\text{HCOH}]^+$ is produced, possibly in admixture with $[\text{H}_2\text{CO}]^+$ from ionised cyclopropanol.

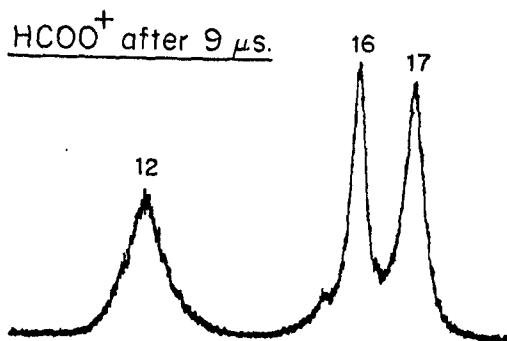
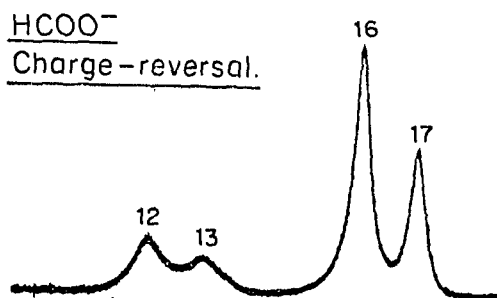
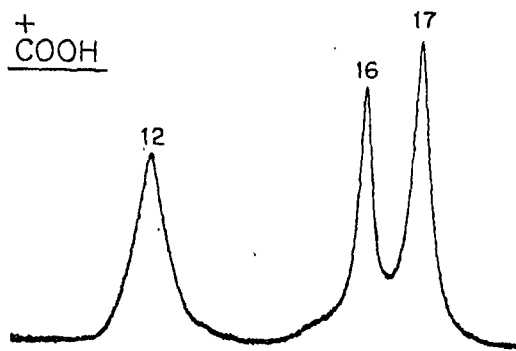
The ion $[\text{C}(\text{OH})_2]^+$, an isomer of ionised formic acid, is produced by loss of CO_2 from ionised oxalic acid and can be identified by its characteristic MI and CA spectra. AE measurements of metastable peaks lead to 1) $\Delta H_f^\circ[\text{C}(\text{OH})_2]^+ = 175 \text{ kcal mol}^{-1}$ (vs $\Delta H_f^\circ[\text{HCO}_2\text{H}]^+ = 170 \text{ kcal mol}^{-1}$); 2) barrier for the reaction $[\text{C}(\text{OH})_2]^+ \rightarrow [\text{HCO}_2\text{H}]^+$ is at least 34 kcal mol^{-1} and 3) barrier for the ion-radical reaction $\text{CO}_2\text{H}^\cdot + \text{H}^\cdot \rightarrow [\text{C}(\text{OH})_2]^+$ is ca 16 kcal mol^{-1} .

The ion $[\text{HCOO}]^+$ is produced by charge reversal of $[\text{HCOO}]^-$ (generated by negative ion chemical ionisation); see the figure for its CA mass spectrum and that for its isomer $[\text{COOH}]^+$. In this experiment reversal takes effect in the second field free region of the ZAB-2F instrument and is followed by immediate dissociation. A number of these $[\text{HCOO}]^+$ ions survive the reversal experiment and therefore have, on average, a lower internal energy than those which have decomposed. By doing the reversal experiment in the first field free region, setting the magnet current to select positive $[\text{CHO}_2]^+$ ions, the surviving ions may be analysed in the second field free region by collisional activated dissociation. The resulting CA mass spectrum is also shown in the figure. It is clear that during the time-span of 9 us extensive rearrangement into $[\text{COOH}]^+$ has taken place.

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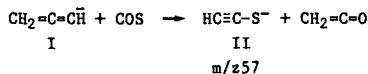
Fig: Charge reversal $\text{HCOO}^- \rightarrow \text{HCOO}^+$ and rearrangement to COOH^+ after ca $9 \mu\text{s}$.



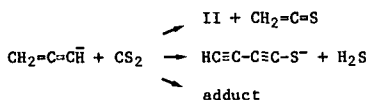
GAS PHASE REACTIONS OF CARBANIONS WITH CS₂, COS AND CO₂
 Charles H. DePuy, Veronica M. Bierbaum and Jonathan Filley
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Carbon dioxide, carbon disulfide and carbonyl sulfide all react readily with a variety of anions in a flowing afterglow (FA) apparatus.^{1,2} For example CO₂ reacts with hydroxide ion at nearly every collision by oxygen exchange and also undergoes a slow, termolecular addition reaction to form bicarbonate ion,³ while CS₂ and COS react to form HS⁻.

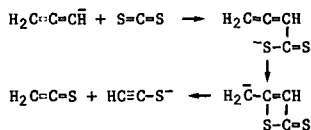
Carbanions react with CO₂ mainly by addition⁴ and, while CS₂ and COS also occasionally add, in many instances they undergo more extensive and interesting reactions. For example COS reacts with the M-1 ion from allene to form exclusively the thio ketene anion (II) and, presumably, ketene, although the neutral products are not detected. Carbon disulfide also



forms II, but two other ionic products, an adduct and an ion of m/z 81, are produced in lesser amounts. We formulate this latter ion as shown in the equation.

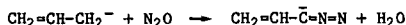


One reasonable pathway for the formation of II is as follows:

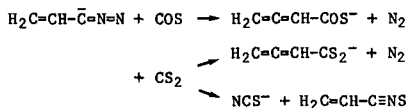


Other mechanisms can also be drawn.

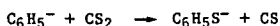
Diazoanions can readily be synthesized in a FA by reaction of a carbanion with N₂O⁴. For example the allyl anion reacts with N₂O to form the vinyl diazomethane anion.



This latter anion reacts with CO₂ to form only an adduct, but the major product of reaction with COS or CS₂ is addition followed by loss of nitrogen. Presumably the exothermicity of these latter two reactions induces fragmentation. Some NCS⁻ is also formed with CS₂.



Some anions react with COS and/or CS₂ by sulfur transfer^{2,5} COS is the more reactive of the two reagents, but sufficiently basic anions will abstract a sulfur atom from CS₂.



It is clear from these examples that COS and CS₂ are useful reagents for ion synthesis in the gas phase. The extent of molecular reorganization which their adducts undergo should also prove useful as tools for the investigation of ion structure.

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THE FAILURE TO OBSERVE THE
REARRANGEMENT BETWEEN ALKYL BENZENE
AND ISOMERIC CYCLOHEPTATRIENE CATION RADICALS IN
SOLUTION: Mark M. Green, Steven L. Mielke and
Paul Harisiades; Department of Chemistry, Polytech-
nic Institute of New York, Brooklyn, N.Y. 11201

The equilibrium between alkylbenzene cation radicals and their ring expanded cycloheptatriene isomers is well investigated in mass spectrometry. Cation radicals of both alkylbenzenes and cycloheptatrienes are also well known in solution where they can be produced by chemical oxidation and anodic electrochemistry. It is interesting that this extensive literature never mentions the rearrangement so commonly encountered in the gas phase. In the present work deuterium labelling has been utilized to conclusively demonstrate the absence of rearrangement in solution. A theoretical argument is advanced proposing that proton loss from the cation radical limits the lifetime so as to preempt the rearrangement between the six and seven membered rings.

THE GAS-PHASE REACTIONS OF TRANSITION METAL
IONS WITH NITRATES AND NITRITES: C. J. Cassidy and B. S.
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Lafayette, IN 47907.

As part of our continuing interest in the area of gas-phase transition metal ion chemistry we present the reactions of Fe^+ , Co^+ and Ni^+ with simple nitrates and nitrites. A pulsed laser has been used in conjunction with a Fourier transform mass spectrometer (FTMS) to generate the metal ions. The metal ions are then allowed to react with gas-phase organic molecules which are present in the FTMS cell. The structures of the products of these reactions have been studied by ion-molecule reactions and by collision-induced dissociation using the FTMS. In past studies involving the reactions of Fe^+ , Co^+ , and Ni^+ with simple alkanes and monofunctional alkanes, these three transition metal ions have exhibited similar reactivities; however, in this study the three metal ions have been found to react in surprisingly different manners.

A TRIBUTE TO HENRY M. ROSENSTOCK.

G.G. Meisels, University of Nebraska, Lincoln, Nebraska 68588-0312

This symposium was organized to honor Henry Rosenstock, who passed away last September 14 while participating in the NATO Advanced Study Institute in Portugal. Much as Frank Field remarked about Joe Franklin, so Henry Rosenstock also made his impact on this world and on us not only as a contributor to new knowledge, but also as a fine and empathetic human being thoroughly dedicated to science in the finest sense and without selfishness.

Henry's highest motivation as a scientist was to gain knowledge; he did not care whether he personally received credit for everything he did. He gave freely of himself, and was always willing to spend hours with his friends and colleagues discussing chemistry and mass spectrometry to help them and to learn himself. This intimate involvement with the intellectual development of his colleagues, such as my own, probably made him along with Joe Franklin one of the most powerful influences in the development of modern thinking about ion chemistry and mass spectrometry.

Henry was born in 1928 in Mannheim, Germany, the second son of a Jewish family. Hitler came to power three years later. He did not talk to me much about his very early years, but I know from personal observation that in those years teachers and others expressed their antisemitism by deliberately making those of Jewish origin feel inferior and unwanted. His family had the wisdom to emigrate to this country in 1936 when he was eight. They departed in the dead of night and left everything behind them. Surely this must have been another difficult and traumatic experience for him — the uncertainty of what he and his family would find in a strange land with a different language and no assurance of how a livelihood could be made. Indeed, his father was unemployed at times and the family had serious difficulty making ends meet. Some people are emotionally drained forever by such experiences, but Henry grew as a result of it and probably became more determined to succeed and more sensitive to others around him as a result. He made it through the tough years and through growing up in New York City, and eventually went to City College of New York where he received the B.S. in Chemistry in 1949. He ventured to Utah for his doctoral work under Professors Eyring and Warrhaftig. His dissertation is a classic, and was published the year he received his Ph.D., in 1952, at the age of 24.

He then spent four years at Oak Ridge, and two each at Operations Research, Inc., and Wm. H. Johnston Laboratories. He joined the National Bureau of Standards in 1960 and remained there until his death, serving for about 15 years as section chief, of the mass spectrometry, elementary processes, and chemical dynamics sections. One of the highlights of his life was the year 1967-68, which he spent as visiting scientist at the Centre d'Etudes Nucleaires in Saclay, France. He loved the Brittany coast, which he visited during that period, and the Megaliths at Carnac on whose purpose he had his own theory, that they were used to predict tides and as a navigational aid, a theory he hoped to develop eventually. In 1978, during the LaBaille meeting, we spent a day there together, just he and I, and he introduced me to the Megaliths and the oysters of Lochmariaquet. That day was one of the finest of my life. It was enriched by him as he showed himself intimately for what he was: a man widely interested and read, cultured, intelligent, humorous, empathetic, concerned with others, supportive, warm and friendly in spite of drifting off once in a while and whistling softly through his teeth some unrecognizable melody, shutting off the world around him for a few moments. It is obvious that he loved music; he was quite proficient on the viola and played with several community orchestras. He was also a voracious reader. He was both social and a "loner". Like most of us, at times he felt alone, isolated and unappreciated; he was always grateful and touched when someone showed appreciation.

The picture of Henry which emerges is fairly clear: a man who experienced hardship and suffering as a boy and had to learn to live with and by himself. He broke out of his environment through his innate intelligence, hard work, and sense of purpose. He reached a level of success and recognition which he had the wisdom to appreciate in his lifetime. His understanding wife Shirley, who kindly provided me with photos and other information, comments "When he surveyed his house and garden — a very modest domain — he really felt like a King." Many of us have seen him like this. Or, as someone here said, "Not bad for an immigrant boy."

I will not list his many honors and contributions. In addition to his original and many other publications on fundamental aspects of ion chemistry, he initiated and directed for 16 years the Ion Energetics Data Center at the Bureau; this compilation has made a tremendous impact on mass spectrometry and was in its initial form a critical evaluation of very high

caliber, particularly considering the state of the field at that time. It was typical of his many contributions: always strictly first class. There are no "fillers" in his nearly 80 publications (including 5 books), which began with the proceedings of the Oberlin Symposium held in 1950, after one year in graduate school, although the book did not appear until 1952. Many of his papers are classics, benchmarks, and of textbook quality. Some of them are required reading in my graduate class on mass spectrometry and for all students starting in my group. His last few papers are as typical of the quality he produced as his first. I was editor of a special journal issue to which he contributed but which appeared only after his death. His overview of benzene ionization and fragmentation, a subject in which he was interested for many years, is an outstanding example of the finest scholarship in the great humanistic tradition, carefully collecting and analyzing all available information to establish a reference point for future exploration.

This society benefitted greatly from him, and not only from his scientific talents. He began participating in the activities of ASTM Committee E-14 in 1952, chaired its subcommittee II on Fundamentals from 1962 to 1964, was vice chairman for programs from 1964 to 1966, and chairman from 1966 to 1968. He was a major moving force in the establishment of ASMS and took pride in getting ASTM to contribute \$10,000 towards its start. He was a charter member of ASMS, and on it's first board of directors. He chaired the nominating committee in 1977 and 1978. But most importantly, he was always here, and his contributions were invariably extensive in sessions and informally over lunch, at dinner, and in the hospitality suites. ASMS was his scientific home.

Henry was a leader in chemistry and particularly in mass spectrometry. He set an example for us of how to ply our trade: to work together, to solve problems, to gain insights, to understand, to appreciate. He saw the beauty of good science, and took the time to smell its flowers. He died while attending the NATO Advanced Study Institute in Portugal. He was in his element and happy, listening to talks, conversing about ion chemistry, hearing his work cited, and basking in the sun of the friendships he had established and the respect he had earned over 35 years. It is significant that a large number of papers at this meeting address concepts and models introduced and developed by him; this effort is certain to continue for many years. Let us now discuss the science to which he led us, remembering the man who has done so much for us and for mass spectrometry.

Using Ion Thermochemistry to Maximum Effect.

By J. L. Holmes, Chemistry Department, University of Ottawa,
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The enthalpy of formation, ΔH_f , of an ion is a useful characteristic which often serves as a structure identifying feature. Of equal interest are the barriers to the interconversion of isomeric ions and the minimum energy required for their fragmentation. The former can be calculated, with varying success, by ab initio methods and estimated indirectly from a study of unimolecular fragmentation characteristics. The use of appearance energies (AE) to measure fragmentation thresholds is well known.

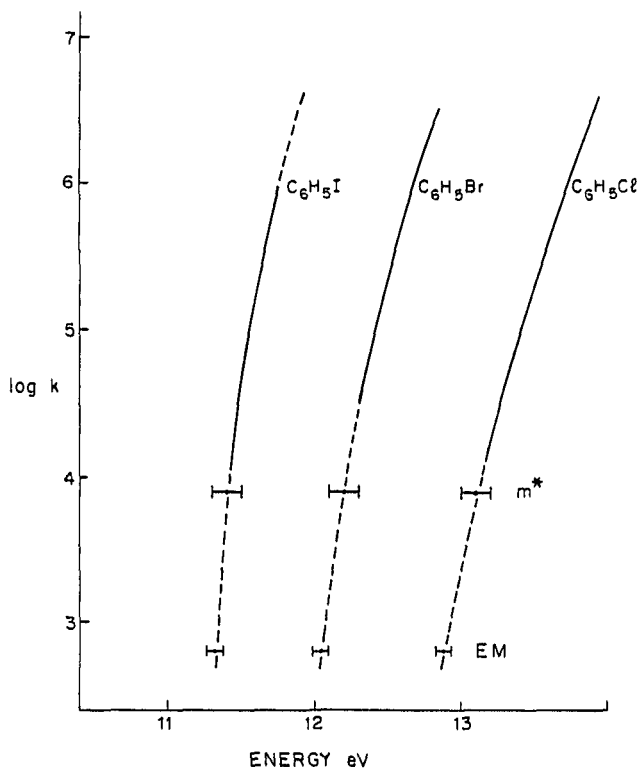
Methods for the estimation of ion enthalpies using empirical relationships and correlations based on the effects of substituents at charge bearing or non-bearing sites have been developed by ourselves^{1,2} and form the subject of a poster presentation FPA2. The substituent effect² can be used to predict ΔH_f for unknown ions in order to aid the interpretation of ion fragmentations and in particular to assign structures to daughter ions.

For example, the ion at m/z 74, $[C_3H_8O_2]^+$ derived from C_2H_4 loss from ionised 4-methoxybutan-1-ol could, were it of conventional structure, only be one among seven simple ethers or alcohols. Their neutral heats of formation lie in the range -85 to -98 kcal mol⁻¹ and their ionisation energy ≥ 9.6 eV - by analogy with similar molecules. ΔH_f for the conventional ion therefore $\geq$ ca 122 kcal mol⁻¹. ΔH_f of the above new $[C_3H_8O_2]^+$ ion, from its AE, is 112 $\pm$ 1 kcal mol⁻¹, determined on both the daughter ion and the metastable peak m/z 102 $\rightarrow$ m/z 74. This evidence alone indicates that the ion is of an unusual type and its dissociation characteristics³ lead us to propose that it is either a σ -bonded ion comprising a vinyl alcohol and a methanol moiety or a symmetrical radical-ion dipole complex.

The use of photoionisation or energy selected electron impact to determine AE values for secondary fragmentations often may lead to an ambiguous ΔH_f result because the pathway by which the secondary daughter ion has been reached is undefined. This difficulty can be satisfactorily resolved by measuring the AE of a metastable peak, a method for which has recently been described⁴. Such experiments, performed with care, lead to ΔH_f values having an uncertainty no worse than $\pm 2-3$ kcal mol⁻¹, good enough to confirm or establish an ion structure assignment and a good initial estimate for ΔH_f of a new ion.

It is worth noting that such measurements on systems accompanied by a significant kinetic shift, correlate very well with photoion-photoelectron coincidence (PIPECO) experiments and observations using energy selected electrons (EM). An example is shown in the accompanying Figure where PIPECO, m^* AE and EM results for the halobenzenes⁵⁻⁸ have been used to establish the approximate rate constant limits for the latter two types of threshold measurement.

Fig.1 Continuous lines from PIPECO experiments⁵⁻⁸. m^* peak appearance energies and those derived from measurements using energy selected electrons (EM) are shown together with error limits.



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INTERNAL ENERGY DISTRIBUTIONS IN QET

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The basic model underlying the now commonly accepted quasi-equilibrium theory of mass spectra (QET) was first presented orally in 1950 by Wallenstein, Wahrhaftig, Rosenstock and Eyring at the Oberlin Symposium on Radiobiology (1), one year after Rosenstock began his doctoral studies at the University of Utah, and appeared in print two years later (1,2). The model was developed for two purposes: to interpret the 70 eV mass spectra of organic molecules, primarily of hydrocarbons, and to contribute to an understanding of the chemical effects of high energy radiation (this research had been supported by the U.S. Atomic Energy Commission (3)).

It is of interest to note the state of mass spectrometry at that time. The first commercial instruments, made by General Electric and Westinghouse, were still widely used. The "state of the art" commercial mass spectrometer was the Dempster (180°) CEC 21-103 with a resolving power of about 200. A high temperature inlet system was capable of heating the reservoir to 150°C. Other than for a very few University laboratories, the CEC 21-103 was found primarily in Oil companies where it was used mostly for hydrocarbon analysis. Liquid inlet was achieved through micropipettes inserted through mercury seals or, if really advanced, liquid gallium above a fritted disk. Organic mass spectrometry was as yet unknown; reference mass spectra were those of API and there were perhaps 1500 of them. Thus the major data base, which Rosenstock and his coworkers tried to explain, was on hydrocarbons. The first correlation and overview concentrated on 18 octane isomers (ref. 1, table 2).

QET is based on three fundamental assumptions: 1. that ionization of gaseous molecules by 70 eV electrons leads to a distribution of vibrationally excited states of the ion; 2. that electronic excitation is degraded rapidly to vibrational energy of the ground electronic state by radiationless transitions; and 3. that daughter ions are produced by a series of competitive and consecutive first-order reactions.

THE FIRST-ORDER RATE CONSTANT

The last assumption has a priori validity because the reactions in the mass spectrometer are in a collision-free environment and are thus clearly unimolecular. The correct way of expressing this rate constant, however, was a matter of considerable debate for the first twenty years of the theory's life. Early forms of the theory used traditional RRK approaches which are in error because they are applicable only in the limit of high internal energies. There is now very little doubt that for an overwhelming majority of cases statistical fragmentation theories correctly describe dissociation when it is a result of rovibrational excitation of a single electronic state and the molecule or ion consists of six or more atoms (4-6).

THE INTERNAL ENERGY DISTRIBUTION FUNCTION

The first assumption also has a priori validity; very little effort has been expended to develop a detailed understanding of this aspect of the theory. The initial internal energy distribution function produced by electron impact is accessible by two-electron coincidence measurements (7), however, since both energies and angles must be swept such measurements are time-consuming and few data are available. The next best approach is to estimate energy deposition functions using the optical approximation and photoelectron spectra (8). The approach is based on the predominance of the interaction of the impacting electron with valence shell electrons and corresponds to treating the electron as a beam of white light with frequency distribution proportional to $1/h\nu$; the probability that such a photon of energy $h\nu$ produces an ion pair is proportional to the total ionization cross section $\sigma(\nu)$ at that energy. The resulting virtual distribution of photon energies producing ionization:

$$P(h\nu) \propto \sigma/\nu$$

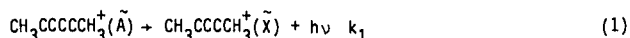
must be combined with a measure of the way the energy in excess of the ionization potential is divided between kinetic energy of the electron and internal energy of the ion, and that can of course be accomplished by examination of photoelectron spectra (PES). Since these are usually known only at one or two energies, a further approximation may be made by treating the PES as independent of photon energy and truncating them at the energy of the photon. Generally, the difference between the energy deposition function obtained by the optical

approximation and the photoelectron spectrum is a lowering of the PES intensity by a factor of two about 4 to 5 eV above ionization onset.

INTERNAL CONVERSION TO THE GROUP ELECTRON STATE

This assumption is probably the one about which we know the least; appropriately, it has received increased attention recently. Internal conversion must always compete with emission (reradiation) and dissociation. Radiative lifetimes for most states are on the order of nanoseconds or longer as dictated by the Einstein relationship (10). Degradation along the vibrational ladder occurs with rate constants on the order of 10^{12} sec^{-1} , but surface crossing may be very slow indeed (10), and the applicability of the degradation concept is therefore not clear by any means.

EMISSION. The energy deposition function, no matter how obtained, need not be equal to the internal energy distribution function. The most important factor lowering the distribution is radiation from an upper state. For example, it has been demonstrated clearly by ion fluorescence studies that the hexadiyne ions radiate from the $\tilde{A}$ state:



60% of the 2,4-hexadiyne ions in the $\tilde{A}$ state radiate [$k_1/(k_1+k_2) = 0.61$] (9); k_1 is about $4 \times 10^7 \text{ sec}^{-1}$. Thus the energy deposition function is an upper limit to the internal energy distribution function at times greater than about 10 nsec.

NONINTERCONVERTING STATES. Nonstatistical behavior has been suggested for many molecular ions; these include methylnitrite (CH_3ONO), nitromethane (CH_3NO_2), benzene ($\phi\text{-H}$), toluene ($\phi\text{-CH}_3$), nitrobenzene ($\phi\text{-NO}_2$), butyrophenone ($\phi\text{-COC}_2\text{H}_5$), anisole ($\phi\text{-O-CH}_3$), hexafluoroethane (C_2F_6), fluoroethane ($\text{C}_2\text{H}_5\text{F}$), chloroethane ($\text{C}_2\text{H}_5\text{Cl}$), dichloroethane ($\text{C}_2\text{H}_4\text{Cl}_2$) and chromium hexacarbonyl (Cr(CO)_6).² This is only a representative, not an exhaustive, list. We will discuss here just a few examples.

Hexafluoroethane. Direct dissociation from upper electronic states, sometimes called isolated states, was first suggested for hexafluoroethane (11) and later confirmed by elegant studies of energy selected ions (12). QET can almost fit the breakdown graph above 17 eV if it is assumed that CF_3^+ results from fragmentation of C_2F_5^+ , however, below that energy CF_3^+ , once it appears, should rise sharply but it does not.⁵ Moreover, kinetic energy release measurements showed that the formation of CF_3^+ was accompanied by a much smaller kinetic energy release than that of its supposed precursor C_2F_5^+ , eliminating QET as a viable interpretation even above 17 eV.

Ethylchloride. Perhaps one of the most common examples of evidence cited for noninterconverting states are decay curves of ions, usually based upon threshold photoelectron photoion coincidence (TPEPICO) time-of-flight mass spectrometry, which cannot be fitted with a single rate constant, as is required when all fragmentation processes, regardless of the identity of the product ion, arise from the same precursor. $\text{C}_2\text{H}_5\text{Cl}^+$ dissociating to HCl and ethylene ion is one of the first extensively studied systems² (13). Recently, however, Baer (14) has argued that such behavior may also reflect the existence of noninterconverting or only very slowly interconverting isomers. If ionization produces initially the ions $\tilde{A}$ and $\tilde{B}$, the following kinetics would lead also to two-component decay curves:



One must thus deal with a refinement of the second problem statement: if two "isolated" isomers are produced and react according to the above mechanism, the reaction remains on the electronic ground state surface but it is not readily distinguishable phenomenologically from noninterconverting electronic states, except as noted for methylnitrite below.

Anisole. As noted previously (15), the parent ion of this molecule disappears at 12.5 eV, and then reappears again at 13.3 eV for a narrow energy span, presumably fragmenting to C_6H_5 ($m/z = 65$) when $h\nu > 13.6$ eV. CAD experiments using a Kratos triple sector analyzer (MS50TA) indicate that the M-1 peak, which cannot be resolved from the parent ion in the TPEPICO experiment, does not fragment to $m/z = 65$ and can thus not be a complicating factor. The reappearance of the M^+ ion can be explained by noninterconverting high-lying states, reradiation to a lower level, or an isolated isomer with no ready chemical reaction exit channel accessible within a relatively narrow energy band.

Chromiumhexacarbonyl. Recent results using PEPICO by Niwa and coworkers (16) indicate that the parent ion disappears at about 13.10 eV, remains absent from the PEPICO MS until over 15.5 eV, and then again becomes an intense peak at 17.5 eV. It is difficult to see how this could be ascribed to an inaccessible isomer, and the other two explanations therefore appear more plausible. The reappearance of the parent ion coincides with the maximum energy of the 4s orbital of CO.

Nitrobenzene. As shown elsewhere in this volume (17), nitrobenzene appears to have two principal low energy fragmentation paths:



k_6 and k_7 are identical between 11.2 and 11.8 eV, at which energy the processes become too fast to measure their rate constants. At ca. 12.6 eV, however, another process leading to NO^+ sets on with a rate constant of ca. 10^6 sec^{-1} , indicating that a separate state or isomer must be formed at that energy.

Methylnitrite. We first reported several years ago that the fragmentation of the molecular ion led to m/z 30 and 31 with different rate constants (18). Subsequent investigations (19) showed that for deuteromethylnitrite the intensity of the ion at m/z 31 was reduced greatly, and that it was produced with a rate constant of $1 \times 10^6 \text{ sec}^{-1}$ over an energy range of nearly one eV. Only a surface crossing appears to be able to account reasonably for such an observation.

Other Molecules. Suggestions of deviations from QET for systems such as benzene, toluene, butadiene, benzonitrile, and benzophenone have often been explained by more refined experiments or by improved theoretical treatments (20-24). On the other hand, recent reports on alcohols (25), halocarbons (13,26) and nitromethane (19b,27) provide other examples of systems where noninterconverting electronic states isomers, may play a significant role.

ACKNOWLEDGMENTS. We appreciate the support of this investigation by the Department of Energy under contract number DE-AC02-76-ER02567.

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PHOTOELECTRON SPECTROSCOPY IN RECENT YEARS AT NBS

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Historical aspects of Henry Rosenstocks scientific career at NBS will be briefly discussed. This includes his participation in early electron impact coincidence experiments and the early development of the photoionization mass spectrometric effort with Vernon Dibeler, Jim Walker and Ken McCulloh. The development of threshold photoelectron spectroscopy and its subsequent evolution into the present variable delay threshold photoelectron-photoion coincidence effort is discussed. An application to the determination of the heat of formation of the $C_6H_5^+$ molecular ion is discussed. The importance of appropriately convoluted QET calculations in the determination of fragmentation on sets is demonstrated with the $C_6H_5^+$ example. Recent progress on dimethyl ether will be reported. New techniques of constant final energy electron energy photoelectron spectroscopy and triply differential photoelectron spectroscopy will be reviewed and their scientific importance discussed.

Work on acetylene will be used as an example to indicate the types of information that can be deduced from these techniques. The future efforts and new experiments that our colleague, Henry Rosenstock, helped develop will be detailed.

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THE QET AND IONIC DISSOCIATIONS

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The value of the statistical, or quasi-equilibrium, theory of unimolecular decay (QET(1) or RRKM(2)) is that it can be used in both a quantitative as well as qualitative manner. The ability of the QET to predict accurately the decay rates of metastable ions over a large range of energies and rates has been amply demonstrated(3-7), much of it through the work of Henry Rosenstock and his co-workers at NBS. Aside from some small molecules(8,9), often fluorinated hydrocarbons, there are no clear cut cases of ions whose dissociation rates are not consistent with the QET. In this paper we explore the value of a more qualitative use of the QET in understanding the dissociation of metastable ions.

We begin by asking the question: can we predict on the basis of ion energies whether an ion will be metastable? The answer is not entirely straight forward. If we know the energetics of both the parent ion and the energy of the transition state, and we know that the ion does not isomerize to a more stable structure prior to dissociation, we can predict its decay rate. The interesting aspect of this statement is its inverse. If we predict that an ion should dissociate rapidly, but we find that it is in fact metastable, we can learn something about its dissociation mechanism. Before we discuss some examples we need to review the statistical theory.

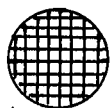
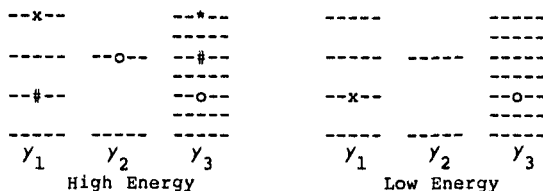
The basis of our understanding of statistical theory is through the concept of the density of states. This is best illustrated for two cases: a single harmonic oscillator, for which the number of states per unit energy interval, ie the density of states, is constant; and the case of several oscillators for which the density of states is a rapidly increasing function of the energy. The number of ways to arrange the energy in the low

A. Case of 1 Harmonic Oscillator



Density of States = $\rho(E) = \text{constant}$

B. Case of several Oscillators



← Phase space →



energy case shown above is just two. In this figure, each symbol corresponds to a particular combination of arranging the vibrational energy. If we increase the energy by a factor of 3, we have at least 7 combinations, of which only 4 are shown in the figure. It is convenient to draw this density of states as a surface, or volume which is related to the phase space. Each unit square in this surface corresponds to a particular arrangement of the vibrational energy. However the total energy is always the same.

According to the QET, the minimum dissociation rate (which means that the ion has just sufficient energy to dissociate) is given by:

$$k_{\min}(E_0) = \frac{1}{h\rho(E_0)} \quad \text{QET}$$

Classically the density of states, $\rho(E)$, is given by:

$$\rho(E, \gamma_i) \propto \frac{E^{v-1}}{\prod \gamma_i}$$

where v is the number of vibrational modes, and the γ_i 's are the vibrational frequencies. From the dependence of the density of states on E , v , and the vibrational frequencies, γ_i , it is clear that the minimum rate is a very strong function of the activation energy, E_0 , and the number of vibrational degrees of freedom, v . The dependence on the vibrational frequencies is relatively minor. The potential energy curves for two reactions, one

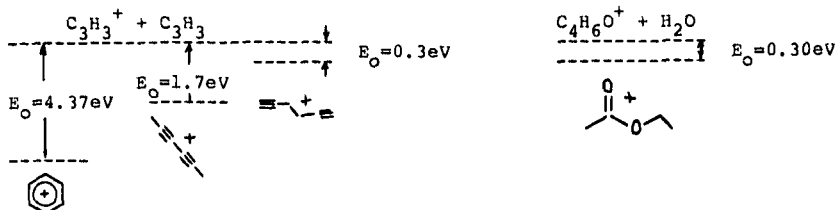


with a large, and the other a small activation energy shows how the density of states, or phase space, picture can give us a qualitative understanding of why the reaction with large E_0 should be slow. It is slow because the molecule, on the average, must explore a large region of its phase space before it finds itself in the region of the bottle neck, i.e. the transition state. One can think of it as undergoing a random walk in the phase space.

An ion is metastable if its minimum rate constant is small, of the order of 0.001 sec^{-1} . It is often stated that a reaction is slow because it is accompanied by a complicated rearrangement. This statement is very misleading. First of all, the minimum rate is independent of the transition state structure. At the minimum energy for dissociation, there is only one way of passing through the transition state, and whether this way corresponds to a peculiar arrangement of atoms or not, makes no difference. Now at energies above the minimum, the number of ways the reaction can proceed is greater for simple bond cleavages than it is for complex rearrangements, so that differences in rates up to a factor of 100 or so can be expected. Although of importance, such differences are rather minor when compared to those resulting from different values of E_0 . By far the largest effect comes from the activation energy, and no degree of complexity of a reaction mechanism can cause a reaction to become slow, if the activation energy is small. This is because the energy in the density of states is raised to the $v-1$ power.

WE CONCLUDE THEREFORE THAT THE SINGLE DOMINANT FACTOR DETERMINING WHETHER AN ION IS METASTABLE, IS THE ACTIVATION ENERGY, E_0 .

We can now look at some examples, in which these ideas are of use. Consider the dissociation energetics of isomers of benzene(10-12) and H_2O loss from ethyl acetate.(13)



Although the activation energies for the three benzene isomers are very different, the dissociation rates are identical. We conclude from this that isomerization prior to dissociation is taking place so that all isomers dissociate from the same precursor. In the case of the ethyl acetate ion, it is clear that the activation energy of 0.3 eV is too small to allow this ion to be metastable. Nevertheless, water loss from ethyl acetate is well known to proceed from a metastable parent ion. Even though, we have not yet found the isomer to which it rearranges, we can be confident that it must rearrange prior to dissociation.

A similar conclusion is drawn in the case of dissociation of dimethyl disulfide.(14) Its photoionization efficiency curve shows that the apparent activation energy is only about 1.5 eV , which is insufficient for such a small ion to be metastable. Yet it is metastable with respect to CH_2S^+ and $C_2H_5S^+$ formation. An unsuccessful search for possible isomerized structures, led to the conclusion that the ion simply undergoes a change



in the dihedral angle from 90° to 180° . By comparing the experimentally determined dissociation rates using PEPICO(15) with rates predicted by the QET, and using the parent ion energy as an adjustable parameter, it was possible to calculate the energy of the isomerized dimethyl disulfide ion. Resulting fits for the dissociation rate of both decay channels are shown as solid lines in the figure. The geometrical change in the dimethyl disulfide ion is also evident in the shape of the photoionization scan. The parent ion has an extremely slow onset which indicates that the ion and neutral structures are not the same.

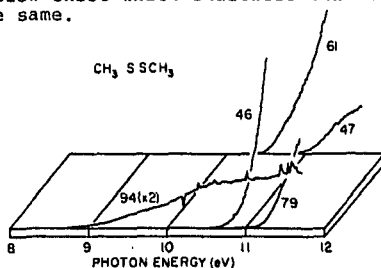
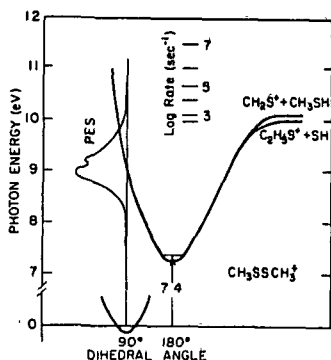
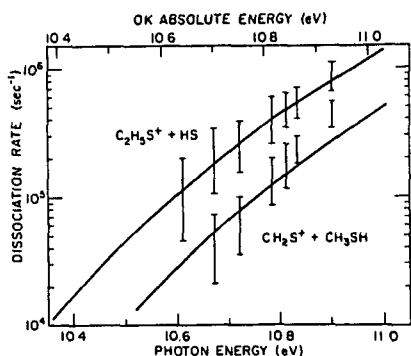


Figure 1. Photoionization efficiency curves for $CH_3SSCH_3^+$ ($m/z\ 94$), CH_3SS^+ ($m/z\ 79$), $C_2H_5S^+$ ($m/z\ 61$), CH_3S^+ ($m/z\ 47$), and CH_3S^+ ($m/z\ 46$).



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Some thoughts on Henry Rosenstock: With Henry's untimely death, many of us have not only lost a friend, drinking partner, and colleague, but also a major force in the field of ion chemistry. Through his undaunting faith that careful analysis could lead to both a qualitative and quantitative understanding of ion dynamics, Henry became our example and our leader. We will all miss him dearly.

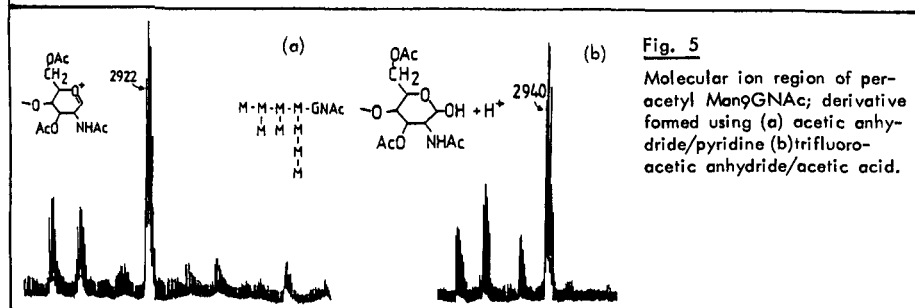
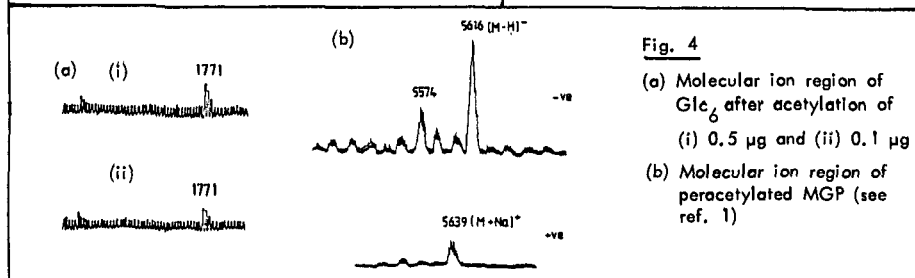
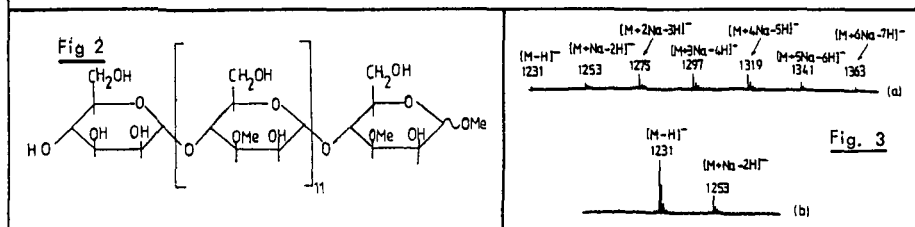
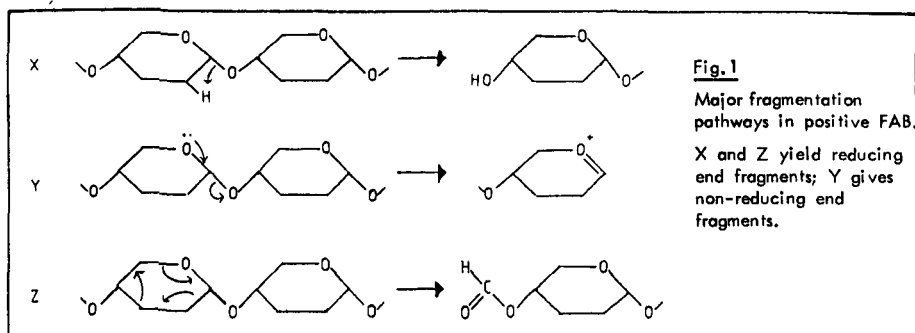
FAB-MS OF HIGH MASS OLIGOSACCHARIDES AND GLYCOCONJUGATES

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We have been applying FAB-MS to the molecular weight and sequence determination of high molecular weight carbohydrates and glycoconjugates from a variety of sources including plant cell walls, yeast glycolipids and glycoproteins, and human erythrocyte band 3 glycopeptides. Samples have been examined both as the native compounds and as their derivatives (e.g. oximes, acetyl, permethyl). Underivatized samples exhibit several fragmentation pathways; in the +ve mode, those most frequently observed are shown in Fig. 1 (we have previously reported on -ve fragment ions¹). Multiple cleavages may occur and considerable care must be exercised when interpreting spectra in order to avoid incorrect sequence assignments. For example the partially methylated mannose polymer shown in Fig. 2 yields "non-reducing end" ions $MeMan_n^+$ ($n = 1-5$) despite the fact that the non-reducing end residue is a non-methylated mannose. These ions can arise via a combination of cleavages X and Y. Cleavage X which is a dominant pathway for many fully unprotected oligosaccharides is relatively structurally uninformative, since branched molecules give the same ions as linear molecules. Cleavage Y is rarely observed in fully unprotected molecules but is often a major pathway in partially methylated polymers e.g. of the type shown in Fig. 2. We conclude, therefore, that FAB spectra of an underivatized sample will afford valuable information on the polymer's size and composition, and on the presence of functional groups such as formyl or acetyl, but is less useful for sequence assignment. M.Wt. data can normally be obtained in the 4000 dalton mass range if 5-10 μ g of non-contaminated sample is loaded. In favourable circumstances signals can be observed up to over 7000 u but good data at high mass is difficult to obtain on unprotected samples. Acylated and permethylated samples, however, frequently yield excellent spectra at high mass on less than 5 μ g of sample. Further, the quality of the data is significantly affected by the sugar composition e.g. monomethylmannose polymers give better data than mannose polymers of the same size (presumably because of the reduction in hydrogen bonding). Sensitivity is also significantly affected by the state of the matrix. This is illustrated by Fig. 3 which shows the negative FAB spectrum of a seven residue galacturonide obtained by enzymic digestion of polygalacturonic acid. Fig 3(a) shows the spectrum obtained from the sample loaded in a 5% acetic acid solution into glycerol. Fig 3(b) is obtained after 1 μ l of 0.1 M HCl had been additionally added to the target without further sample being applied. Note the potential gain in sensitivity when the experiment is performed at pH 1.0 since the molecular ion current is concentrated into only two ions rather than a multitude of sodium salts. Other parameters which may affect molecular ion intensity are salts (careful dosing is frequently advantageous), the presence of denaturing agents (e.g. DMSO), and the volatility of the matrix (e.g. replacing glycerol with monothioglycerol). Manipulation of the matrix may, however, alter the data obtained. For example, the methylmannose polymer shown in Fig. 2 exhibits mainly cleavages X and Y from the protonated molecular ion. In contrast, addition of a small amount of NaCl to the matrix results in a sodiated molecular ion which yields sequence ions arising via fragmentation pathway Z.

In order to generate unambiguous sequence information from FAB spectra we have examined a variety of derivatives including acetylated, permethylated and reducing end modified samples. Very high sensitivity for molecular ion detection can be achieved with many of these samples. For example we showed earlier² that ammonium dosing of permethylated sugars can be used to obtain molecular weight data on submicrogramme quantities up to over 6000 dalton. The acetyl derivative is particularly useful because it can be simply prepared and affords very good data (see Fig. 4(a)) even at high mass (see Fig 4(b)). We are using a modification of the procedure of Boume et al³ (trifluoroacetic anhydride in acetic acid) to effect rapid, clean acetylation. The method is valuable for submicrogramme quantities since purification is not required and we are using it routinely in our band 3 glycopeptide work. Polymers with very labile linkages are, however, susceptible to cleavage, and should be derivatised with acetic anhydride/pyridine. We have observed that the molecular ion species may vary according to the acetylation procedure used. This is illustrated in Fig. 5. Acetyl derivatives yield similar sequence data to permethyl derivatives. A-type sequence ions predominate (cleavage Y) allowing sequence assignment from the non-reducing end and location of branch points. For polymers containing 1,6-linkages e.g. high-mannose glycoproteins, FAB molecular weight determination of the acetylated products of acetylation can be used to ascertain branch lengths, phosphate substitution etc..



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AN IMPROVED METHOD OF TARGET PREPARATION BY ELECTROSPRAY. R. Beavis, W. Ens, and K. G. Standing, Physics Dept., University of Manitoba; J. B. Westmore, Chemistry Dept., University of Manitoba, Winnipeg, Canada R3T 2N2; P. W. Schiller, Clinical Research Institute of Montreal, Montreal, Canada H2W 1R7.

The electrospray technique is one of the few SIMS/PDMS compatible sample preparation techniques for the study of large, involatile organic molecules(1). A dissolved sample flows slowly out of a needle tip subjected to a high electric field. This field draws the liquid out into a point, emitting streamers of liquid, which then break up into very fine droplets(2,3,4). The droplets then impact on the target, drying rapidly so that no visible droplets of liquid form on the target backing material. Typical 'mounds' of sample produced on the target by these droplets are .1 to 1 microns in diameter. The main problem with this technique is that using standard methods (1), the reproducibility of the spray, ie. its direction and dispersion, are subject to wide variation, making target preparation a somewhat hit-or-miss proposition.

In order to increase the reproducibility of our target preparation and to use as little material as possible, we decided to develop a focussing technique for our electrospray. The diameter of a normal electrosprayed target was 1 to 2 centimeters. Our SIMS primary beam was only 1 millimeter in diameter, however, so that the majority of the sample on the target was never used in taking a mass spectrum and was therefore wasted.

The first stage in the development of a focussing technique was the introduction of a field plane above the electrospray needle. This plane was kept at the same potential as the needle. This served to straighten the fringing electric field of the needle by the superposition of a parallel plate capacitor field onto the field of the needle. The use of a field plane decreased the spot diameter by a factor of 2 to 4.

The second stage was the introduction of an electrostatic lens. It was found that a simple metal ring was an effective lens for this purpose. Coupled with the field plane, this lens yielded a reduction in sample spot size to 1 mm, comparable to the size of our primary ion beam. This reduction in total sample area led to a reduction in sample usage by at least two orders of magnitude. The final arrangement is shown in Figure 1.

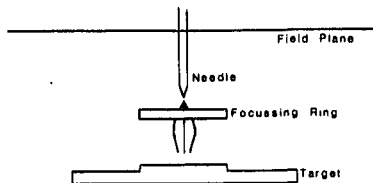


Figure 1 Focussed electrospray setup.

Another problem with the standard electrospray method was that it did not work if the water concentration in the solvent was much greater than 20% V/V(5). It was also poor for solvents of relatively low volatility, such as isopropanol. In both cases, visible droplets formed on the target, which lead to no or poor molecular ion yields.

After some experimentation, we determined that the use of a heat lamp gave dramatically better water handling capacity for our spray. Solvents containing up to 80% water could easily be sprayed. Presumably, the heat lamp increased the temperature of the target and needle, the latter prewarming the solvent leading to enhanced evaporation during the droplet's flight, minimizing the amount of solvent introduced to the target surface.

In any spray deposition process, as the level of surface coverage increases, so does the probability that the next droplet to impact on the surface will at least partially cover a previously deposited droplet. This effect rapidly becomes important as surface coverage increases producing deviations from a linear relationship between surface coverage and sample dose at only 20% surface coverage. Using both calculated and Monte Carlo simulated models of the spray process we were able to account for the increase in yield, shown in Figure 2, strictly on the basis of surface coverage. This same analysis was used to explain a four fold decrease in background peaks, ie. those due to the target backing material.

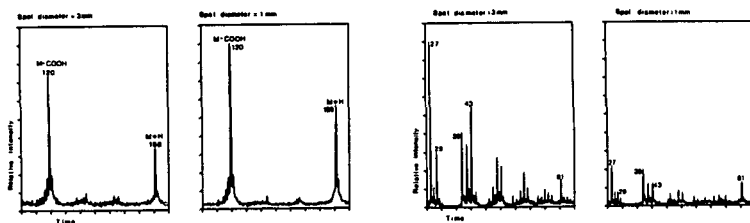


Figure 2 Yield and background changes with spot size for .5 µg phenylalanine.

A major difficulty with of the electrospray procedure was that, occasionally, a seemingly properly sprayed sample did not yield good results. One explanation of this could be that the flow rate of the sample out of the needle is important. This was found to be very much the case. The results are shown in Figure 3. They showed the dramatic increase in SIMS molecular ion yield in going from 5 µl/min to .9 µl/min. The reason for this drastic change in yield is still not understood.

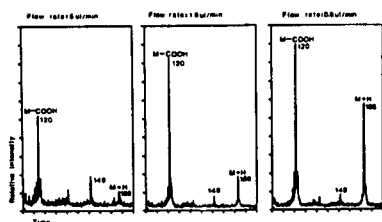


Figure 3 Yield variation with flow rate (.5 µg Phenylalanine)

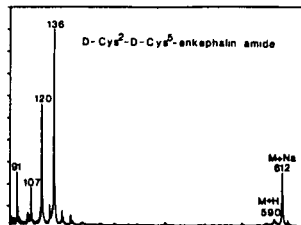


Figure 4 Mass spectrum of (2-D-Cys,5-D-Cys) enkephalin

As the determination of the structure of synthetic polypeptides is an important application of SIMS, we have undertaken to examine several novel cyclic enkephalin analogs (6). A spectrum of one of these potent analogs is shown in Figure 4. This spectrum showed that the analog was not a cyclic dimer, something not determinable by purely chemical means.

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PROTEIN TIME-OF-FLIGHT MASS SPECTROSCOPY BY FAST HEAVY ION INDUCED DESORPTION

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A Californium-252 fission fragment time-of-flight mass spectrometer has been constructed. The principle of the spectrometer is the same as that originally proposed by Macfarlane and coworkers (1). Here we report on a study of proteins with this spectrometer. The experiment involved molecules in the mass range 1000-14000 amu. The samples were prepared with the electrospray (2) technique using trifluoroacetic acid as solvent. The acceleration voltage used was 17 kV. Time-of-flight spectra of positive ions were collected. The spectra took 1-5 hours each to accumulate. All the molecules studied gave good spectra i.e. a quasi-molecular ion peak could be identified and a molecular weight estimated. In Table I the results of the molecular weight determinations are summarized. The secondary ions are post-accelerated with a few kV at the stop detector. For the largest molecules the metastable component in the peaks therefore give a centroid which is shifted towards smaller flight times and for these cases the molecular weights extracted will be underestimated (see Table I). The general features of mass spectra from the large molecules are the importance of metastable ion decay contributions and production of multiply charged ions. Besides doubly charged ions also triply charged ions were observed for the largest molecules. In Fig. 1 the positive ion spectrum of porcine iso-phospholipase A₂ is shown. This molecule is a 124 amino acid residue protein of mass 13980 amu. The spectrum illustrates the effects of metastable ion decay, multiply charged ions and polymer ions.

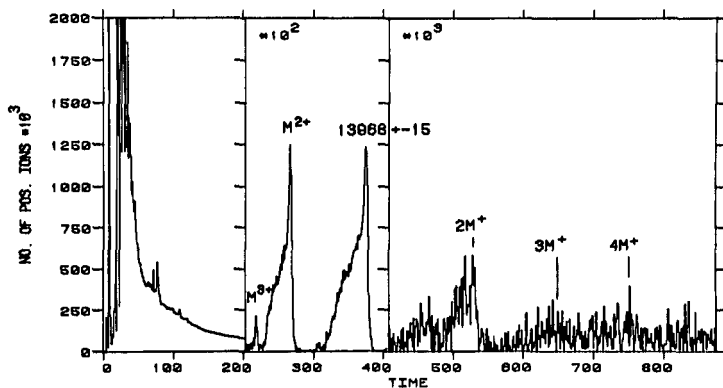


Fig. 1. Positive ion spectrum of porcine iso-phospholipase A₂ (MW 13980).

Table I. Summary of molecule weight determinations

Molecule	Calculated mass (CM)	Measured mass (MM)	$\Delta(\text{CM}+\text{l-MM})$	Relative precision o/oo
LH-RH	1182.3	1184.0 $\pm$ 0.3	-0.7 $\pm$ 0.3	0.6
Glucagon	3482.8	3483.0 $\pm$ 0.5	+0.8 $\pm$ 0.5	0.2
Bovine insulin	5733.5	5734.3 $\pm$ 2.0	+0.2 $\pm$ 2.0	0.03
Siamensis neurotoxin	7821	7818.7 $\pm$ 4.0	+3.3 $\pm$ 4.0	0.4
Porcine proinsulin	9082	9090 $\pm$ 10	-7 $\pm$ 10	0.8
Cytochrome C	12384	12335 $\pm$ 30	+50 $\pm$ 30	4.0
Tiger Snake II-1	13309	13285 $\pm$ 20	+17 $\pm$ 20	1.2
Bovine ribonuclease	13682	13529 $\pm$ 50	+162 $\pm$ 50	12
Porcine phospholipase A ₂	13980	13968 $\pm$ 15	+13 $\pm$ 15	0.9

This work was supported by the Swedish National Science Research Council and the National Swedish Board for Technical Development.

References:

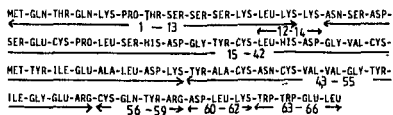
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PEPTIDE AND PROTEIN SEQUENCING BY MASS SPECTROMETRY

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Our early work on the use of FAB-MS in the study of peptide fragmentation mechanisms, and of mixtures of peptides from protein digests (Morris et. al. 1981) has laid the foundations for the solution of a number of important problems in protein chemistry. This work falls into two categories, studies on small neuroactive peptides of unknown structure, and studies on larger proteins either of unknown structure or structure anticipated from nucleotide sequence data. In this latter category our original work on protein digest mixtures (above) has proved extremely useful in the analysis of more complex digest mixtures of complete proteins. Quasimolecular ion signals are normally obtained for the majority of components of the tryptic digest under one set of running conditions (Xe, glycerol, HCl). Varying conditions will then normally lead to the appearance of the remaining peptide(s) (negative ions, thioglycerol etc.). In the area of Recombinant DNA Protein Products, we have applied our mapping procedure - which we have christened FAB MAPPING - with great success. Here the molecular biologist must define that the protein has the expected sequence and that the cell line used to grow it has not "rebelled" producing mutations, insertions, deletions etc. Normal classical microsequencing (Hunkapillar-Hood type) whilst powerful in terms of sensitivity is unable to accomplish sequence assignment of such a protein in one experiment since 50 or 100 steps of Edman degradation will be the limit of such techniques. Our FAB MAPPING procedure allows the complete sequence to be mapped in terms of tryptic or other fragments. Assignments by mass are very easily confirmed by further single or multiple steps of, for example, CARBOXYPEPTIDASE B, LEUCINE AMINO PEPTIDASE, EDMAN CYCLES, ACETYLATION etc. The MS signals observed should all conform to the anticipated sequence, and if not, or if additional signals are seen, this provides an excellent method for spotting modifications. The commercial application of the methods we originated has now been successfully launched by M-SCAN Ltd., and a number of pressing problems in the Biotechnology Industry have already been solved by our FAB-MAPPING technique. Examples of this type of study are detailed in this lecture with reference to the screening Urogastrone produced by recombinant techniques (Fig. 1).

Fig. 1.
Sequence of Urogastrene
(First determined by
ICI Ltd.)



One of our main research interests is in the field of Neurochemistry beginning with the structure elucidation of the Enkephalins in 1975. In this study the mass spectra were obtained and interpreted to give the then surprising result (not anticipated from classical data) that Enkephalin was a mixture of two pentapeptides and not an 8-10 residue molecule with a modified Tryptophan unit. This structure was achieved using 50-100 nanomoles of natural product. In contrast we now demonstrate that equally difficult structural interpretations of neuropeptides can be determined at the 1-5 nanomole level, using a combination of FAB-MS and judicious choice of protein chemical manipulation. To illustrate this, details of the structure elucidation of a new cardioactive excitatory peptide isolated from the giant neurones of *Aplysia* are shown. Here the combination of FAB-MS with 1:1 acetylation of amino groups and tryptic digest FAB mapping was crucial to the interpretation of the spectrum shown below (Figure 2). We have noted that our structure :

H-MET-ASN-TYR-LEU-ALA-PHE-PRO-ARG-MET-NH₂

is homologous with another neuropeptide which is also cardioexcitatory although less active, and we postulate the important unit for this activity is PHE-X-ARG-Y-NH₂-C-terminus.

Another area of our studies where FAB-MS has been critically important is in hormonal calcium regulation in man. Cloning and subsequent analysis of recombinant DNA resulted in a partial nucleotide sequence of human calcitonin precursor mRNA (Craig et.al. 1982). A synthetic peptide corresponding to the C-terminal portion of the nucleotide sequence was found to be highly biologically active in lowering plasma calcium. We have now determined the structure of the natural product isolated from adrenal tumors as the non-postranslation-

ally modified polypeptide ASP-MET-SER-SER-ASP-LEU-GLU-ARG-ASP-HIS-ARG-PRO-HIS-VAL-SER-MET-PRO-GLN-ASN-ALA-ASN. The FAB molecular ion region of this natural tumor-isolated product is shown in Fig. 3.

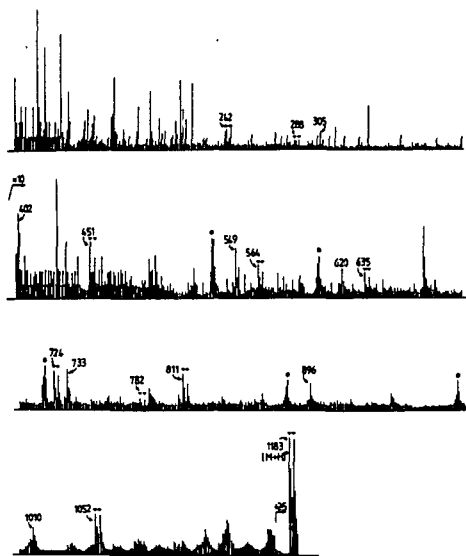


Fig. 2. Fast Atom Bombardment Mass Spectrum of Cardioactive Neuropeptide

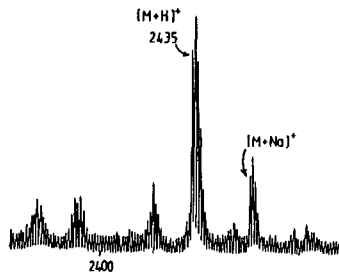


Fig. 3. Fast Atom Bombardment Mass Spectrum of Natural PDN-21 Katsacalcin

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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF ANGIOTENSIN II
ANALOGUES CONTAINING L-MALIC ACID

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Synthetic analogues of biologically important peptides have been used extensively to enhance biological activity or to provide clues to how the structure of a peptide is related to its biological function. However, once synthesized, structural analysis of these analogues may no longer be accomplished using classical amino acid analysis and protein sequencing via Edman degradation. Mass spectrometry offers an attractive alternative for analysis of peptide analogues since it is compatible with the presence of terminal blocking groups and unnatural amino acid components. With the development of fast atom bombardment (FAB), routine mass spectral analysis can be done on underivatized peptides.

Efficient utilization of FAB for analysis of synthetic peptide analogues requires rapid assignment of major ions consistent with what is known about the characteristics of FAB peptide spectra. Generally speaking, the following characteristics apply:

1. Significant ions are nearly always even electron species.
2. The strongest ion in the high mass region of the spectrum is MH^+ .
3. Sequence information is found in one or more fragment ion series.
4. Prominence of an ion series is determined by the location of easily ionized functional groups.

The analysis of peptide spectra is greatly assisted through the adoption of a systematic method of naming the fragment ion series. In positive ion spectra, ions whose m/z values correspond directly to portions of the molecule are named as outlined in Fig. 1. Additionally, ion series exist whose m/z values are two mass units higher. Those we have chosen to call the prime series. Thus $B' = B + 2$, etc. Of the twelve possible series ten have been found to exist in published spectra. No evidence for series A' and C has been found. Six series (A, B, C', X, X' , and Y') are sufficient to assign the major fragment ions in most positive ion FAB spectra.

The structural similarity of L-malic acid to L-aspartic acid suggests that peptides incorporating an isosteric malic acid residue may possess interesting biological activities. Two analogues of Angiotensin II:

- a) β -malyl-Arg-Val-Tyr-Ile-His-Pro-Phe-OH
- b) β -malyl-Arg-Val-Tyr-Ile-His-Pro-Leu-OH

were synthesized¹ and their FAB spectra analyzed according to the principles outlined above. Confirmation of the molecular weight for both compounds is provided by the strong MH^+ ion in each spectrum.

Since an arginine residue is present near the amino terminus, it is expected that N-terminal fragmentation series would be more prevalent than C-terminal fragmentation series. Indeed, of the four series which have more than two members (see table 1), three are N-terminal series with series A being dominant. The combined information from all the series is sufficient to confirm the amino acid sequence. Additionally, the spectra of the malic acid derivatives closely resemble a published spectrum of Angiotensin II² (AII). A listing of the fragment series members for AII and the malic acid analogues is given in Table 1.

¹p. Cordopatis, J. Matsoukas, A. Michel, and D. Theodoropoulos; *Experientia*, in press.

²W.A. König, M. Aydin, U. Schulze, U. Rapp, M. Höhn, R. Pesch, and V.N. Kalikhevitch, *Int. J. Mass Spectrom and Ion Phys.*, **46**, 403, 1983.

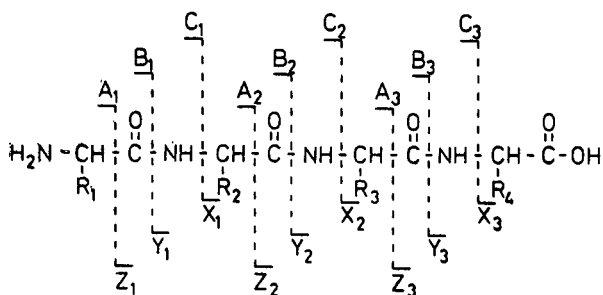


Figure 1

Table 1

TABLE OF FRAGMENTS IN MASS SPECTRA OF ANGIOTENSIN II AND ANALOGUES

PEPTIDE	SERIES MEMBERS PRESENT			
	A	B	C'	Y'
Ang	3, 4, 5, 6	3, 4, 5, 6	3, 4, 5, 7	1, 3, 4, 5
[6-MAL ¹] Ang	2, 3, 4, 5, 6, 7	2	2, 3, 5,	1, 5, 6
[6-MAL ¹ -LEU ⁸] Ang	2, 3, 4, 5, 6, 7	2, 4, 6, 7	2, 3, 4, 5, 7	1, 2, 4, 5, 6

ANALYSIS OF MIXTURES OF HUMAN FIBRINOGEN GLYCOPEPTIDES BY FAB-MASS SPECTROMETRY; D. Heller and C. Fenselau; Mid-Atlantic Mass Spectrometry Facility, Department of Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD; R. Townsend and Y. C. Lee, Department of Biology, Johns Hopkins University, Baltimore, MD 21218.

Fibrinogen is a plasma glycoprotein which circulates as the precursor of fibrin, the structural component of the blood clot. The structure of the oligosaccharide chains has been previously determined (1) based on carbohydrate composition, ^1H -NMR spectroscopy, sequential exoglycosidase digestion and GCMS of derived partially methylated alditol acetates. It was found that the carbohydrate consists of biantennary chains with either zero, one, or two sialic acid residues at the opposite end from the site of attachment to protein. The carbohydrate is linked to the protein via an amide bond to Asparagine. Additional heterogeneity exists in isolated glycopeptides due to incomplete enzyme degradation. Since the sialic acids in complex glycopeptides in other systems have been found to act as recognition agents, it is important to gain information about the presence of species with zero, one, or two sialic acid residues.

Positive FAB-MS yields molecular weight information about the components of these mixtures. Conditions used for FAB analysis consist of placing about 10 micrograms of sample dissolved in about 1 microliter of thioglycerol on the probe tip and irradiating with xenon. Raw data scans were collected under DS-55 protocols for the lifetime of sample ions, and were then averaged (2), centroided, and mass-assigned (Figure 1).

Three sequentially degraded derivatives of the original mixture (Figure 2), which had been prepared for NMR, were studied by FAB-MS, and the relative amino acid composition calculated from the relative intensities of protonated and cationized molecular ions. Intensities from each isotope peak were summed after the subtraction of a value for the average background ion level, if present, for about 10 amu around the peaks. The data is compared to hydrolysis data, as well as values obtained from the starting mixture (Table 1). Close agreement is obtained; noting that glycine values determined by amino acid analysis are frequently high due to its widespread presence in trace contaminants. Also, average sialic acid content calculated from the spectrum of the mixture is compared to carbohydrate analysis data.

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2. J. A. Yergey, unpublished work.

FIGURE 1.

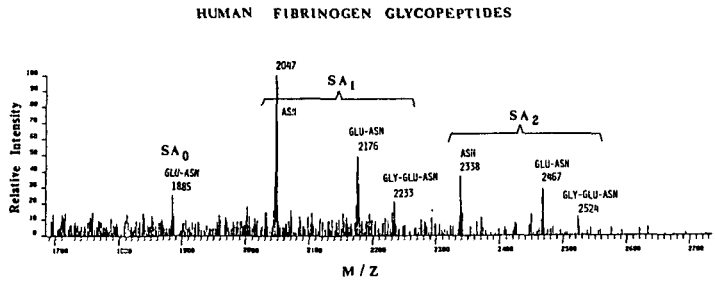


FIGURE 2.

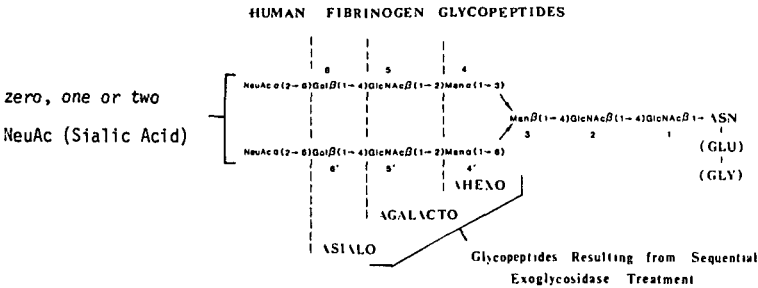


TABLE 1.

AMINO ACID AND CARBOHYDRATE COMPOSITION
(NORMALIZED TO ASN CONTENT)

	FAB-MS			HYDROLYSIS	
	ASIALO	AGALACTO	AHEXO	HFG	HFG
ASN	1.00	1.00	1.00	1.00	1.0
GLU	0.45	0.39	0.38	0.44	0.4
GLY	0.06	0.05	0.05	0.09	0.2
GLCNAC				4*	4.0
MAN				3*	3.0
GAL				2*	2.3
NANA				1.5	1.8

*ALL COMPONENTS MUST HAVE THIS STRUCTURE

MEASUREMENT WITH OPTIMAL MOLECULAR SPECIFICITY
OF ENDOGENOUS PEPTIDES IN NERVE TISSUE
BY MEANS OF FAB-CAD-B/E-B'/E'-SIM METHODOLOGY

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The objective of this research program is twofold: to develop the capability of measuring subnanogram amounts of endogenous neuropeptides g^{-1} of wet weight tissue and significantly, to attach unambiguous molecular specificity to that measurement. The aims of this research program are manifold: calibrate an immunoassay antibody; describe molecular processes involved in nociception¹, extend the mass range of endogenous peptides to be measured², synthesis of individual stable isotope-labeled peptide internal standards³, measure⁴⁻¹⁰ endogenous neuropeptides in several peripheral and central nervous system tissues to compare those peptide amounts in unstimulated control tissue versus electrostimulated tissue, and utilize a radioimmunoassay to "screen" antibody-responsive RP-HPLC peaks.

A Finnigan MAT 731 mass spectrometer outfitted with a fast atom bombardment (FAB) source (Ion Tech) and operating in the collision activated dissociation (CAD) mode¹¹⁻¹³ is utilized. Individual ion currents are analyzed by two alternating linked field scans, B/E for the peptide of interest and B'/E' for the ¹⁸O-labeled peptide four mass units higher. An Apple II microcomputer¹⁴ collects the two ion currents from sequential scans, integrates those currents, and calculates the ratio of the two integrated ion currents. Tissue extracts are purified by utilizing a combination of commercially available C18 Sep-Pak (Waters) cartridge and reverse phase C18 high performance liquid chromatography utilizing a volatile triethylamine formate buffer^{15,16}. The organic modifier is acetonitrile and peptide UV absorption is monitored at 200 nm. HPLC fractions are collected and subjected to FAB (Xe) to produce (M+H)⁺ ions which in turn are subjected to CAD with Xe. The C-terminal tripeptide fragment (GFL or GFM) is selected for selected ion monitoring. This analytic measurement mode provides the optimum specificity available. Furthermore, several peptides can be measured in one extraction experiment.

Table I collects preliminary analytical data obtained for FAB-CAD-B/E-B'/E'-SIM measurement of the two endogenous pentapeptides methionine enkephalin (ME = YGGFM) and leucine enkephalin (LE = YGGFL) in tooth pulp tissue for unstimulated controls versus electrostimulated tissue.

TABLE I
MEASUREMENT OF NEUROPEPTIDES IN TOOTH PULP TISSUE

	(ng of enkephalin g^{-1} wet weight tissue)	
	Unstimulated	Stimulated
ME	355 (n=2)	240 (n=2)
LE	20 (n=3)	8 (n=2)

This data indicates a decrease of 20% of the ME and 40% of the LE following electrostimulation of canine tooth pulp. While these peptide decreases also may be due to an increased metabolic breakdown of those enkephalin pentapeptides, it is difficult to assess the extent of preenkkephalin breakdown in the metabolic cascade precursor + pentapeptide + metabolite(s).

Table II collects data from FAB-CAD-B/E-B'/E'-SIM measurement of endogenous enkephalin pentapeptides in the canine anterior and posterior pituitary.

TABLE II
MEASUREMENT OF NEUROPEPTIDES IN PITUITARY TISSUE

	(ng pentapeptide g ⁻¹ wet weight tissue)	
	<u>Anterior Pituitary</u>	<u>Posterior Anterior</u>
LE	70	2
ME	2,950	3,760

These data demonstrate that there is clearly more methionine enkephalin in both lobes, more methionine enkephalin in the posterior pituitary versus the anterior pituitary, and the reverse distribution for leucine enkephalin. This type of analytic data, obtained with high molecular specificity in the FAB-CAD-B/E-B'/E'-SIM mode indicates the possibility that differential peptide processing mechanisms may be involved in the canine anterior and posterior sections of the pituitary reflecting different metabolic profiles and post-translational events. The opioid peptide precursors may be processed in several ways because different metabolism pathways are available in different tissue and different peptide metabolic products may be found in those different tissues. A variety of post-translational modifications (tyrosyl O-sulfation, phosphorylation, oxidation of the methionine enkephalin thioether to the sulfoxide, amidation, and acetylation) may alter the peptide hydrophobicity and shift the HPLC enkephalin peak.

It is important to understand and accurately delineate the metabolic profile of opioid neuropeptides represented by these two enkephalin pentapeptides because a current hypothesis indicates that these neuropeptides may decrease neuronal firing via neuromodulatory pathways.

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STRUCTURE DETERMINATION BY FAB MASS SPECTROMETRY

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A. Tuinman,^b P.L. Wessels,^b C.C. Viljoen,^b and H. Kruger.^b

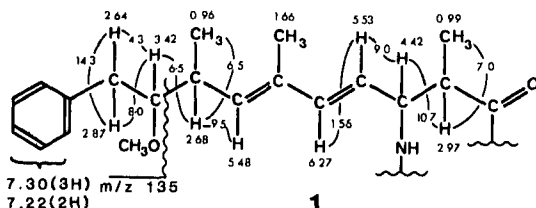
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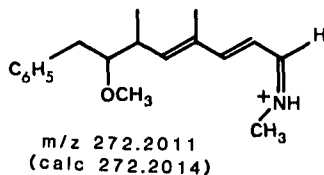
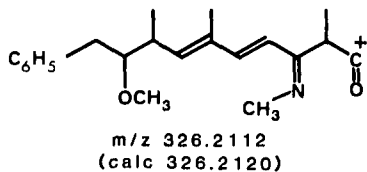
The blue-green algae *Microcystis aeruginosa* produces a peptide toxin which is a potent animal poison.¹ This paper illustrates structure determination by FAB MS through structural studies on the toxin variant BE-4.²⁻⁴

The reported³ amino acid composition of BE-4 is (molar ratios in parentheses): erythro- β -methyl-D-Asp(1); D-Glu(1); D-Ala(1); L-Ala(1); L-Leu(1); and N-methyldehydroalanine (N-MeDha) (1). The peptide has a blocked N-terminus (no acetylation with Ac₂O/H₂O), and is resistant to enzymic digestion. The molecular weight is determined as 909 Daltons by fast atom bombardment (FAB) mass spectrometry, but neither this spectrum, nor the EI spectrum of permethylated BE-4, showed evidence of the sequence ions normally observed in the spectra of linear peptides^{5,6}. On the basis of the above evidence, we conclude that BE-4 is likely to be a cyclic peptide. If the peptide is monocyclic, then the component(s) to be added to the already identified units has a mass of 313 Daltons. The identification of this component as a novel β -amino acid is first presented.

In addition to the presence of the above-mentioned amino acids, the 400 MHz ¹H nmr spectrum of BE-4 in D₂O showed carbon-bound protons corresponding to the β -amino acid residue (1). The connectivity was established by spin decoupling experiments (J values indicated in (1)) and negative NOEs [(5.53) + 1.66(-12%), (6.27) + 5.48(-15%), and (5.48) + 6.27(-15%)], the NOEs being determined in (CD₃)₂SO solution at 303K.



The nature of the functional groups in (1) was established by EI mass spectrometry. An abundant fragment ion at m/z 135 in the mass spectrum of BE-4 (or permethylated BE-4) is in accord with a methoxy substituent β to the phenyl group. This conclusion is consistent with the chemical shift of the adjacent proton (3.42 ppm), and the observation of a methyl resonance at 3.35 ppm (D₂O solution). Fragment ions in EI mass spectra of permethylated BE-4 before, and after, pulse hydrolysis (6N HCl, 5 min, 100°C, followed by acetylation) correspond to structures (2) and (3), respectively. These assignments are in accord with high resolution mass measurements, and the shifts to m/z 329 and 275 when CD₃I replaces CH₃I in the permethylation step.

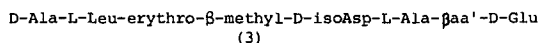


Reduction of the N-MeDha residue in BE-4 with NaBH₄ (or NaBD₄) gives a product with a molecular weight increased by two (or three) Daltons, as established by FAB-MS. The reduced toxin was subjected to pulse hydrolysis (6N HCl, 5 min., 100°C) and two major products, BE-4I and BE-4II, were isolated by reversed phase HPLC; their molecular weights (FAB-MS) were 812

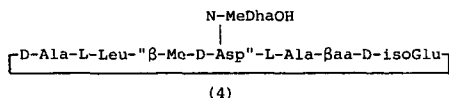
and 794 Daltons, and both showed loss of a C-terminal "Glu" residue⁶ (where "Glu" represents either Glu or β -MeAsp, since these amino acids have the same molecular weights). These molecular weights were unchanged when reduction of BE-4 was carried out with NaBD₄. Thus, the residue which has been lost is reduced N-MeDha. FAB-MS analysis of the esterified (MeOH/HCl) or acetylated (Ac₂O/H₂O) products revealed that both have a free amino group; while BE-4I has three, and BE-4II has two, carboxylic acid groups. These results suggest that BE-4I is a linear peptide produced from hydrolysis (+18 Daltons) of monocyclic BE-4, with losses of the reduced N-MeDha unit (-85 Daltons) and methanol (-32 Daltons). The last loss is readily accommodated from (1). Product BE-4II is concluded to differ from BE-4I by dehydration involving one carboxyl group.

BE-4I was acetylated (Ac₂O/d₆-Ac₂O, 1:1 in H₂O) and then deuterio-permethylation. The EI mass spectrum of the product shows pairs of acylium sequence ions at *m/z* 131/134, 261/264, 424/427, 512/515, 810/813, and 973/976. These, in conjunction with the above FAB data, define the sequence: Ac-Ala-Leu-"Glu"-Ala- β aa'-"Glu" where β aa' is the β -amino acid (1) minus methanol. A similar experiment on BE-4II gave the partial sequence Ac-Ala-Leu-"Glu"-Ala...

Analysis of the N-trifluoroacetyl isopropyl esters of the amino acids derived from acid hydrolysis of BE-4II, by a GC-MS system fitted with a chiral capillary column, showed D-Ala: L-Ala in the ratio 1:1.5. After one Edman degradation cycle on BE-4II, the FAB mass spectrum of the remaining peptide⁷ showed that N-terminal Ala was cleanly removed. Analysis of the remaining amino acid components of the peptide as above showed L-Ala as before, but no D-Ala was detected. Thus D-Ala is the N-terminal residue and L-Ala the fourth residue from the N-terminus. The second Edman cycle on BE-4I or BE-4II removed L-Leu, but the third Edman cycle failed as evidenced by no change in the molecular weight as determined by FAB-MS. Dansylation of this tetrapeptide, followed by hydrolysis, gave DNS- β -MeAsp, establishing β -MeAsp as residue 3. Since it is not removed by the Edman procedure, it must be β -linked to the next residue. Thus, the structure of BE-4I (except the stereochemistry of β aa') is (3), where Glu is now restricted to Glu or isoGlu in the parent peptide.



To determine the positions of free carboxyl groups in BE-4, its ¹H nmr spectrum was recorded as a function of 'pH' (NaOH added to a solution in D₂O). Only the resonance of the C α -proton of the Glu residue (4.14 ppm) shifted significantly upfield (to 3.86 ppm) with increasing 'pH'. We conclude that the α -carboxyl of Glu is free, whereas the γ -carboxyl, and both the α - and β -carboxyls of β -methylAsp (or β -methylisoAsp) are blocked. Since the molecular weight of the toxin requires two free carboxyl groups in the structure, the nmr data can be satisfied if the second carboxyl group is attached to the α -carbon of N-MeDha (lacking an α -CH). This conclusion is additionally supported by the observation that NaBH₄-reduced BE-4 forms a dimethyl ester (0.02M HCl/MeOH, followed by FAB-MS), whereas BE-4 itself forms a monomethyl ester; evidently under these conditions, the conjugate carboxyl group is not esterified. Thus (4), where " β -Me-D-Asp" is either erythro- β -methyl-D-Asp or erythro- β -methyl-D-isoAsp, satisfies all structural information we have for BE-4.



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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF NUCLEOTIDES

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Di- and tri-nucleotides from both the ribo and deoxyribo series have been examined by both positive and negative ion fast atom bombardment mass spectrometry (FABMS). The negative ion mode is clearly preferable for this class of compounds for reasons which are apparent from Fig. 1 and 2.

Figure 1 shows positive and negative FABMS of an identical sample of the dinucleotide d(TpA) and illustrates the quite general observation that ions due to Na substitution are much more abundant in positive ion FABMS.

Figures 2a and 2b show the negative ion FABMS of the trinucleotide d(TpTpC). All the major ions observed (excluding those from the glycerol matrix) carry valuable sequence information and the order of the constituent nucleosides is easily read.

It is important, however, to distinguish between cleavages at the 5' sugar carbon from those at the 3' carbon. Grotjahn et al.(1) have found in their study of several octa- and one deca-deoxyribonucleotide that ions due to cleavage at the 3' carbon are more abundant than those at the 5' carbon, however, among the 11 trinucleotides studied here three (ApUpU, ApApU and ApGpU) show a reversal of this order. In the spectra of the dinucleotides a fragment ion due to cleavage of the sugar ring with the 3' carbon-phosphate link is invariably observed and, as shown in Fig. 3a and 3b, isomers like ApC and CpA can be readily distinguished. Unfortunately, corresponding sugar ring cleavage ions are not observed in trinucleotide spectra.

This study was carried out on an AEI MS9 mass spectrometer which has been modified for negative ion detection and spectra are routinely obtained with ample sensitivity at scan rates of 10 sec/decade into a DS55 data system. Because the mass range is limited to about 1300 daltons at 6 kV, it has not been possible to extend the study to large nucleotides. Fig. 4 shows a penta- and a hexa-nucleotide positive ion spectrum recorded on our MS50 which has yet to be modified for negative ion detection. The Na substitution problem is very evident.

(This work has been submitted for publication in Biomedical Mass Spectrometry).

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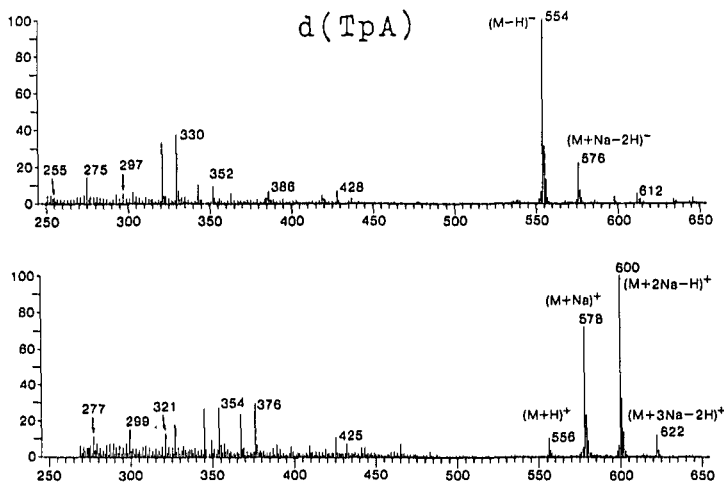


Fig. 1

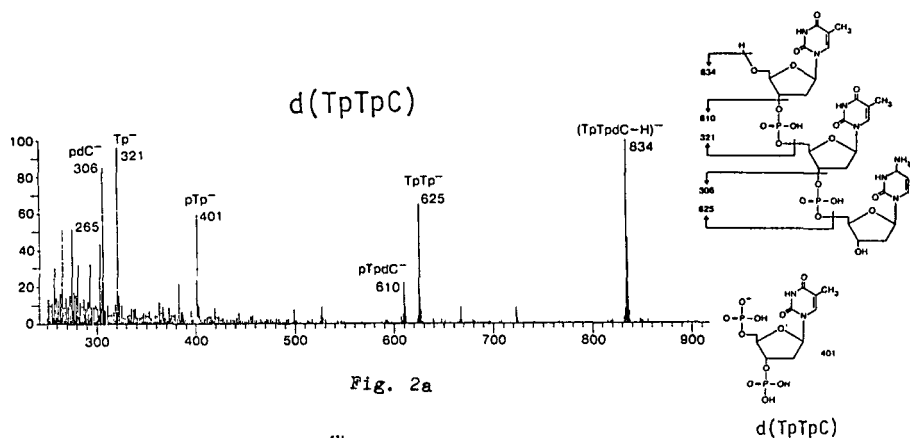


Fig. 2a

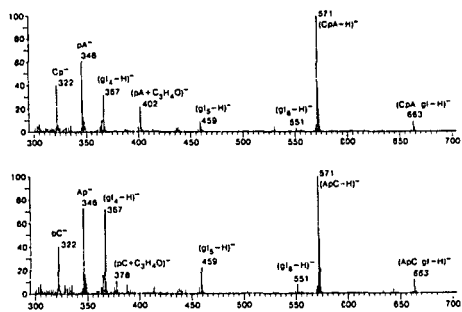


Fig. 2b

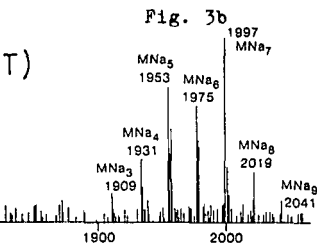
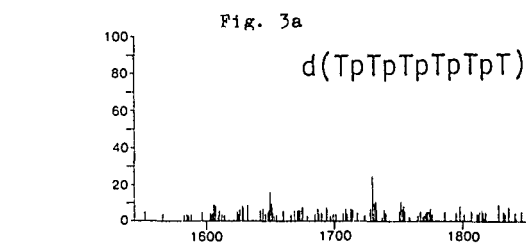


Fig. 4

DETERMINATION OF ACTIVE HYDROGEN CONTENT BY FAST ATOM BOMBARDMENT
MASS SPECTROMETRY FOLLOWING HYDROGEN-DEUTERIUM EXCHANGE

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Fast atom bombardment mass spectrometry following hydrogen deuterium exchange in glycerol-(OD)₃ and D₂O has been studied as a means of establishing active hydrogen content in molecules of unknown structure. A preliminary account of this work has been published (1).

The procedure for exchange simply involves making a solution of the dry sample (1-10 µg) in a glycerol-(OD)₃/D₂O mixture and immediately transferring the sample to the FAB probe tip for analysis. The number of active hydrogens is established by the mass shift between the spectrum of unlabeled material, and that following exchange. In many cases this can be unambiguously determined by visual inspection of the spectra (Fig. 1). Ion m/z 517 (Fig. 1)

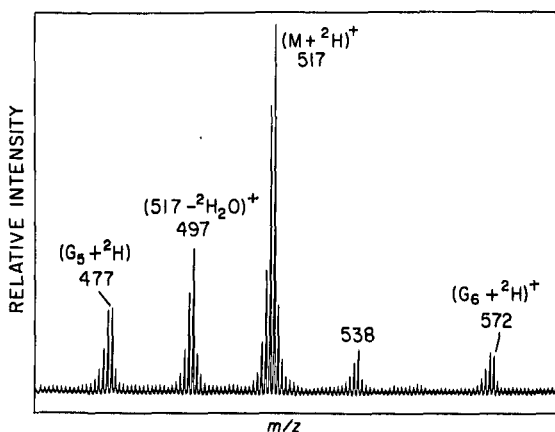


Fig. 1 Fast atom bombardment mass spectrum of melezitose in [hydroxy-²H₃]glycerol (G) and H₂O. The m/z 517 ion contains 12 deuterium atoms.

corresponds to melezitose, with all 11 active hydrogens exchanged with deuteriums, attached to a deuterium, in analogy with protonated molecule MH^+ (unexchanged) usually produce in FAB. The extent of exchange in our experiments ranged from 90-93% of the maximum possible. Because the [glycerol-(OD)₃]_nD⁺ cluster ions (m/z 477 and 572 in Fig. 1 for $n = 5$ and 6, respectively) represent the deuterium content of the sample at the time of ionization, they can be used to measure the extent of exchange.

For larger molecules with number of exchanges greater than 15 (examples in ref. 1), the isotopic pattern may appear visually confusing. To gain clarification in such cases, two computational methods have been developed: (a) comparison of the observed isotope cluster with the one calculated from a known or estimated elemental composition and using the percent exchange measured from glycerol ions and (b) by correcting the isotope pattern for natural heavy isotope contributions, which can either be determined from the elemental composition or from the mass spectrum of the unlabeled compound. The remaining ions, after normalization, give a cluster which is due to the exchanged deuteriums only. This cluster can then be used to count the number of deuteriums and the extent of exchange. An example of these methods is shown in Fig. 2.

Isotope exchange similar to that of molecular species is also observed in the adduct and fragment ions (e.g., $M \cdot Na^+$, m/z 538 and MD^+-D_2O , m/z 497 in Fig. 1). The knowledge of the exact hydrogen content of the fragment ions along with that of molecular ion of an unknown molecule provides an important constrain on the overall structure of the molecule. This is

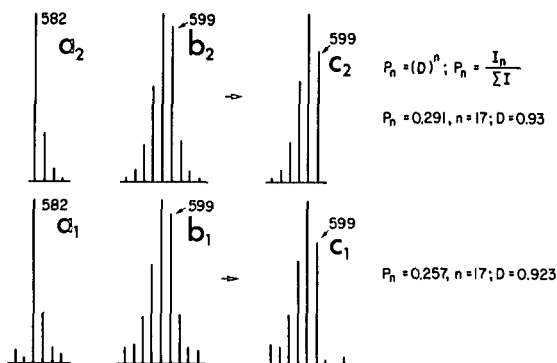


Fig. 2 Ion clusters a_1 and b_1 are observed in the FAB mass spectrum of unexchanged and exchanged streptomycin (MW = 582), respectively. a_2 and b_2 are calculated for unexchanged and exchanged (number of deuteriums, $n=17$ and % exchange, $D = 93\%$) streptomycin. c_1 and c_2 are derived from b_1 and b_2 respectively by correcting for natural heavy isotope contributions. P_n is the probability of ion n with intensity I_n .

illustrated in the exchange of bleomycins (Fig. 3). Analyzed as a mixture, Blenoxane (Bristol Laboratories), all active hydrogens were exchanged in the molecular ions at 93% level. Two structurally very important fragment ions a and b (Fig. 3) were observed with exchange of all active hydrogens. Ions a and b lend support to the structure of the part of the molecule which was historically the most difficult to be assigned (2).

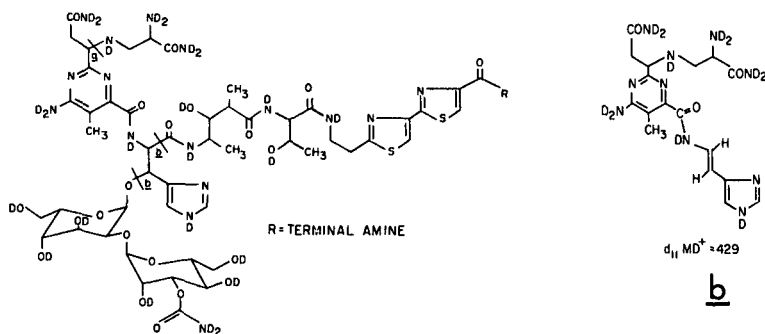


Fig. 3 Exchanged bleomycins (BLM). Molecular ions observed, $d_{25}(\text{demethyl-BLM-A}_2)D^+ = 1426$, $d_{25}(\text{BLM-A}_2)D^+ = 1439$ and $d_{29}(\text{BLM-B}_2)D^+ = 1455$. Notation $d_n(M)D^+$ indicates n active hydrogens exchanged in the molecule, attached to a deuterium.

Further fragmentation studies of nucleotides, carbohydrate and peptides will be published elsewhere.

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GC/MS DETERMINATION OF CHOLESTEROL USING ^{18}O INTERNAL STANDARD

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An ^{18}O -cholesterol isotope dilution procedure was devised to measure the amount of cholesterol in samples. The method studied included acylation (usually acetyl, propionyl, or trifluoroacetyl) of the cholesterol and GC/MS isotope ratio measurements using the OH^- negative chemical ionization technique (1). With this technique the carboxyl anion is the dominant fragment ion in the spectrum (2) and is used for quantitation. Its formation entails the transfer of the cholesterol oxygen atom. If one uses ^{18}O -labeled cholesterol as an internal standard for quantitation, the low contribution of the ^{13}C isotope in the carboxyl anion (generally of 2 or 3 carbon atoms) permits accurate determination of the $^{18}\text{O}/^{16}\text{O}$ ratio even for relatively low degrees of enrichment.

Of the three cholesteryl esters studied, the propionate ester appeared to be the most useful. The OH^- CI method produces a background ion at m/z 61, which interferes with the determination of the ^{18}O -acetate ion. The utilization of the trifluoroacetate ester was hampered by the high baseline level at m/z 113 (trifluoroacetate anion) generated by the imperfectly removable excess of the acylating agent trifluoroacetic anhydride (TFAA). Only recently, we could overcome this difficulty using N-methyl-bis-(trifluoroacet-)amid (= MBTFA) instead of TFAA.

No background interference was noted with propionate ions and the relative intensity of this ion is $\approx 50\%$ of the total ionization.

A 1 % SE 54 (5' x 1/8") glass column was used in a temperature programmed operation, and selected ion monitoring was used for MS data acquisition. A series run with calibration standard 0.0 - 50 % $^{18}\text{O}/^{16}\text{O}$ -cholesteryl propionate resulted in a linear graph.

The precision of the method is very good: at 0.0 % enrichment the coefficient of variation (CV = rel.std. deviation) was 0.34 % (N = 4) using a model mixture of "neutral sterols" (coprostanol, cholesterol, campesterol, stigmasterol, β -sitosterol).

Repetitive runs of a cholesterol sample of biological origin which was the "neutral sterol fraction" isolated from feces (3) showed also a very good agreement yielding a CV value of 0.82 %. The enrichment in ^{18}O -cholesterol was in this sample 0.76 %.

This method, which is probably the first OH^- /NCI method for isotope ratio measurement seems to be very flexible. The synthesis (4) which was used to obtain the internal standard ^{18}O -cholesterol is simple and relatively inexpensive, and easily adaptable for other sterols; in fact, for many other kinds of alcohols. All of these compounds can then be assayed using the same $^{18}\text{O}/^{16}\text{O}$ isotope ratio measurement method as described above.

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CI MASS SPECTROMETRY OF FATTY ACID ESTERS OF 3-CHLORO-1,2-PROPANEDIOL AND THEIR IDENTIFICATION IN CONTAMINATED SPANISH OLIVE OIL; J. A. G. Roach, W. C. Brumley, A. Joshi, P. Yurawecz, A. M. Gardner and J. A. Sphon; Bureau of Foods, Food and Drug Administration, Washington, D. C. 20204

In May 1981, a mysterious new illness began to claim victims in the area around Madrid, Spain. The cause of the outbreak was traced to cooking oil sold in working class neighborhoods by door to door vendors.(1) The Spanish government removed the oil from the market by offering a free exchange of virgin olive oil for suspect oil. The U. S. Food and Drug Administration has examined 16 suspect oil samples as part of the effort to determine the cause of this illness. In general, only trace levels of those chemicals or elements which are ordinarily determined by FDA procedures were found. High levels of fatty acid anilides were detected in some of the oils.(1,2) Most of the toxicological investigation of the oil has focused on these anilide contaminants. However, many of the samples contained high levels of chlorinated material. The bulk of the chlorinated material in the oils was found to be fatty acid esters of glycerol chlorohydrins.

Electron ionization spectra of fatty acid esters of glycerol chlorohydrins have previously been described.(3,4) Ambiguity in the EI data regarding molecular weights and the particular fatty acids involved in chloropropanediol diesters led to the use of positive ion-negative ion chemical ionization with hydroxide reagent ion to characterize these compounds.

The positive ion CI (methane or methane/nitrous oxide) mass spectra afford (M+H)+, (M-H)+, (M+H-HCl)+, (M+H-RCOOH)+, (RCO)+ and (RCO-H2)+ ions. The use of methane/nitrous oxide to generate hydroxide reagent ion unambiguously confirms the fatty acid content of these esters in the negative ion CI spectra by yielding abundant (RCO)- ions for each fatty acid ester component. (M-H)- species were also observed in the negative ion spectra. The positive ion and negative ion CI spectra of dichloropropyl oleate in figure 1 were obtained by capillary GC/MS analysis of synthesized material. The diesters of chloropropanediol were usually analyzed by solid probe. This presented a much more complex mixture to the instrument for analysis. The complementary nature of the positive and negative ion data was best utilized in the analysis of these complex mixtures *via* pulsed positive ion-negative ion operation of the mass spectrometer. The spectra in figure 2 were obtained from the diester fraction of a Spanish oil sample. The 279 dalton base peak in the negative ion spectrum is consistent with a linoleic acid anion. There are abundant positive ions at 263 and 355 daltons corresponding to (RCO)+ and (M+H-RCOOH)+ species arising from a dilinoleate ester of chloropropanediol. The molecular weight assignment is supported by (M+H)+ at 635 daltons, (M-H)+ and (M-H)- at 633 daltons. There is evidence for a dioleate ester in the form of a 281 dalton oleic acid anion, 637 dalton (M-H)- and (M-H)+ species, a 639 dalton (M+H)+ and appropriate (RCO)+ and (M+H-RCOOH)+ species at 265 and 357 daltons. Similar arguments may also be made for the presence of lesser amounts of palmitate and stearate in the diesters in this fraction.

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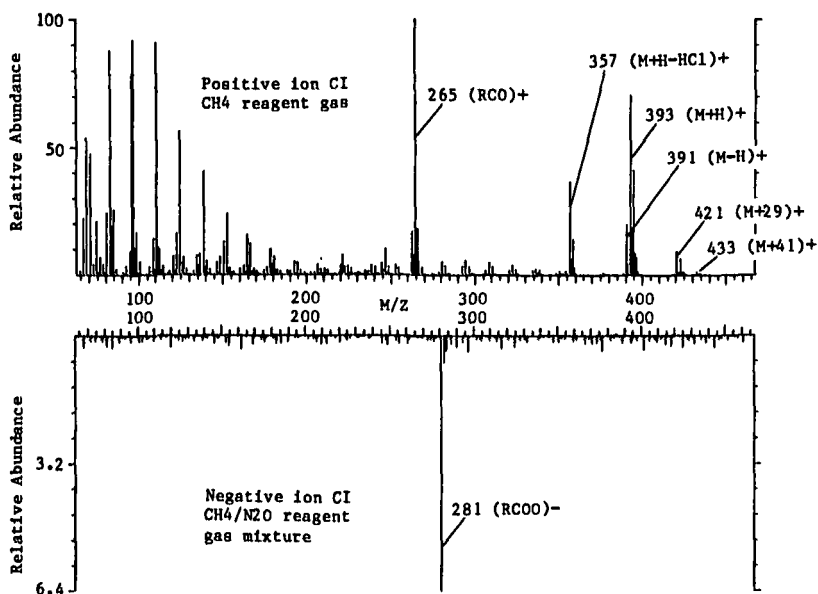
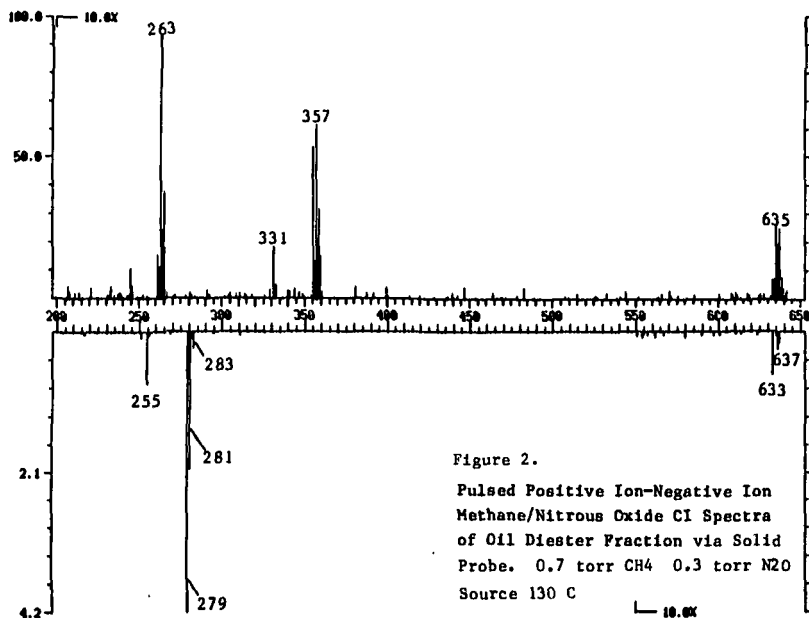


Figure 1. Positive ion and negative ion CI spectra of dichloropropyl oleate



ANALYSIS OF COMPLEX MIXTURES OF DEUTERIUM-LABELED BLOOD LIPIDS USING CAPILLARY CHROMATOGRAPHY AND CHEMICAL IONIZATION MASS SPECTROMETRY

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INTRODUCTION

An analytical method using capillary gas chromatography and isobutane chemical ionization mass spectrometry with multiple ion detection has been developed to analyze human blood lipids from subjects fed mixtures of triglycerides containing labeled fatty acids.

EXPERIMENTAL

The blood fractions separated on TLC plates were converted to methyl esters and submitted for mass spectrometry. One microliter of an isooctane solution containing one microgram of ester was injected into a 30 meter, wide bore (.313 mm) fused silica column, with a thick film (1 μ m) DB1 bonded phase. The exit end of the column was run to within 3/8" of the electron beam of the source of a Finnigan 4000 mass spectrometer. The isobutane reagent gas pressure was varied with .4 to .06 Torr with the maximum sensitivity occurring at .1 Torr.

Methyl esters of the fatty acids fed are shown below. The monoene mixture plus the methyl ester of naturally occurring oleic acid made up a four-component mixture that cannot be separated by GC and that has only 2 mass numbers between each compound. Considerable measurement care and computer programming effort must be expended to correct for the second isotope peak, which interferes with the next heavier compound.

MONOENE	Methyl <u>trans</u> -10-Octadecenoate-14,15-d2
MIXTURE	Methyl <u>cis</u> -10-Octadecenoate-14,14,15,15-d4
	Methyl <u>cis</u> -9-Octadecenoate-14,14,15,15,17,18-d6
DIENE	Methyl <u>cis</u> -9-Hexadecenoate-9,10-d2
PLUS	Methyl <u>cis</u> -12, <u>trans</u> -15-Octadecadienoate-9,10-d2
MIXTURE	Methyl <u>cis</u> -9, <u>cis</u> -12-Octadecadienoate-15,15,16,16-d4
	Methyl <u>cis</u> -9-Octadecenoate-14,14,15,15,17,18-d6
	Methyl Octadecanoate-9,10,13,13,14,14,-d6

The table below shows what appears to be hydrogenation occurring during the chemical ionization of the unsaturated fatty esters. Two atoms of hydrogen are added to the pseudo molecular ion to produce M+3 mass peaks more than 5 times the theoretical intensity.

HYDROGENATION DURING ISOBUTANE CHEMICAL IONIZATION

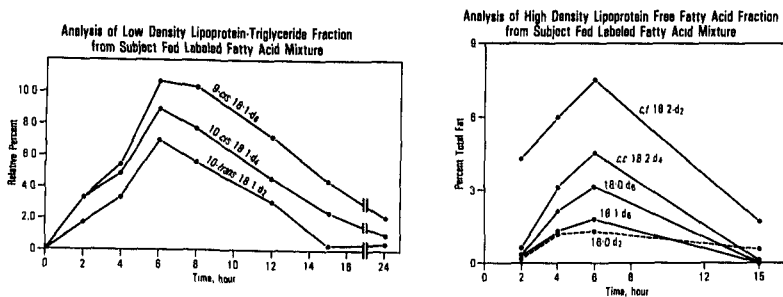
ISOBUTANE PRESSURE	METHYL LINOLEATE 2 DOUBLE BONDS			METHYL OLEATE 1 DOUBLE BOND			METHYL STEARATE SATURATED		
	M+1	M+2	M+3	M+1	M+2	M+3	M+1	M+2	M+3
.11	100	20.7	4.50	100	20.8	3.70	100	20.9	2.3
.20	100	21.6	9.22	100	21.3	7.38	100	21.2	2.2
.40	100	23.3	13.2	100	22.2	11.0	100	21.9	3.4
Theory	100	21.1	2.5	100	21.2	2.5	100	21.2	2.5

A Finnigan INCOS multiple ion detection (MID) program was set up to scan the odd mass numbers 269 to 279 to measure the C16 region, mass numbers 283 to 293 to measure the C17 region where the 17:0 was used as an internal standard, mass numbers 295 to 305 to measure the C18 esters and mass numbers 319 to 327 to measure the C20 esters. In general the GC provided complete separation of the methyl esters, with the exception of the labeled compounds. The d6 labeled esters eluted about a half-peak width ahead of the unlabeled esters, and the *trans* isomer eluted about a third of a peak width behind the unlabeled ester. Thus the naturally occurring 9-*cis*-18:1, the 10-*trans*-18:1-d2, the 10-*cis*-18:1-d4, and the internal control 9-*cis*-18:1-d6 all interfere with each other. A computer program is necessary to find the corrected intensities for these mixtures.

A major part of the project was a computer program that identifies and determines the area of each mass peak, corrects for mass interferences of the labeled components, and also provides a base for searching for very small mass peaks that might occur if the labeled fatty acids are lengthened or shortened during metabolism.

RESULTS

The two plots below are examples of the results of the analysis and computation. The left plot is the low-density lipoprotein triglyceride blood fraction of a subject fed the monoene mixture. The monoene mixture contains equal weights of esters, and it is obvious from the plot that the subject's system is discriminating against the delta-10 fatty acids as compared to the delta-9 control. The right plot is the high-density lipoprotein free fatty acid blood fraction from a subject fed the diene plus mixture. In the diene plus mixture the subject was fed equal weights of the dienes c,t-18:2-d2 and c,c-18:2-d4, and it can be seen that his system has discriminated in favor of the c,t fatty acid, which is formed during hydrogenation, as compared to the labeled analogue of the naturally occurring c,c fatty acid.



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The Quality of Positive Ion Spectra from Alternating
EI and CI Scans with a Capillary Gas
Chromatographic Inlet System

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The development of high resolution fused silica capillary columns and their widespread application to environmental sample analyses have led to the revelation of numerous resolved components in many samples. Electron impact (EI) ionization gas chromatography/mass spectrometry (GC/MS) and the ancillary retention index information often permit the identification of many, but usually not all sample components. Additional components may be identified with additional information such as that supplied by chemical ionization (CI), but this requires a second GC/MS experiment which is usually not considered during routine environmental monitoring. The second experiment requires as much time as the first, but usually does not provide as much information.

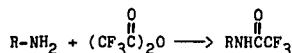
It has been suggested that application of a fast switching system based on solenoid valves should permit alternate pressurization and evacuation of the ion source. This should permit alternate acquisition of CI and EI spectra during an otherwise routine GC/MS experiment. This work was performed to determine the quality of the CI and EI spectra obtained during capillary column GC/MS with alternate acquisition of CI and EI spectra and repetitive one second scans. This type of data acquisition, if acceptable quality spectra are obtained, should be ideal for routine screening of environmental samples. A mixture of compounds of environmental interest was prepared and analyzed by alternating CI/EI (ACE) GC/MS. Results are presented in terms of the quality of the EI and CI spectra as judged by established criteria and literature spectra.

DISTINCTION OF ISOMERS OF POLYCYCLIC AROMATIC AMINES
BY THEIR REACTIVITY UNDER SEVERAL IONIZING CONDITIONS

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In the trace analysis of organic pollutants it is frequently necessary to be able to distinguish between structurally similar isomers. The polycyclic aromatic amines (PAA) found in samples of coal-derived liquids is such a class of compounds where certain isomers have been found to exhibit much greater mutagenetic activity than others. We report here the signals produced for aminoanthracenes and aminophenanthrenes under various ionizing conditions. Emphasis will be given to the results obtained with the simplest of the detectors used here, the electron capture detector (ECD) with oxygen sensitization. The degree of isomer distinction achieved by the ECD method is then compared with that obtained by APIMS, EIMS, and positive and negative ion CIMS.

In order to increase their propensity for forming negative ions and also to facilitate their separation by capillary column chromatography, each PAA was first reacted with trifluoroacetic anhydride as shown,



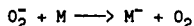
and was then introduced to one of the various ionization sources via gas chromatography.

Responses to the PAA derivatives measured by ECD are shown in Table I. The values listed are the ratios of responses observed with and without oxygen added to the detector make-up gas. The magnitude of ECD oxygen sensitization of the ECD has been thought to be proportional to the ratio of reaction rates of O_2^- and the electron with the sample molecule at one atmospheric pressure.¹ It is seen that the magnitude of oxygen sensitization is quite varied ranging from 1.0 to 107 and is very dependent on the subtle structural variations within this set of eight isomers.

The negative ion APIMS spectrum of each PAA derivative was extremely simple; only the M^- ion was observed in all cases, with and without oxygen added to the nitrogen carrier gas. Obviously, no structural information is thereby provided by the APIMS spectra, alone. However, the intensity of the M^- ion was generally increased by oxygen's presence in a manner which is structure dependent. The relative magnitude of this APIMS enhancement is also listed in Table I. Inspection of the two columns indicates that the relative order of oxygen sensitization to the set of isomers in the ECD and the APIMS is the same. (For the two methods the absolute magnitudes of individual enhancement measurements are not expected to be the same.) Thus, it appears that with both of these atmospheric pressure ionization detectors the relevant reactions are:



and



and the only measurable parameter which is sensitive to structural differences is the relative rates of these two reactions. This parameter is more easily and reproducibly determined by the ECD rather than the APIMS.

¹D. A. Miller and E. P. Grimsrud, *Anal. Chem.*, **51**, 1979 (851).

Table I. Oxygen Sensitization of the Responses of the ECD and APIMS to Trifluoroacetic Derivatives of Polycyclic Aromatic Amines

PAA	ECD ^a	APIMS ^b
1-anthracene	3.1	2.1
2-anthracene	1.0	1.0
9-anthracene	8.1	4.0
1-phenanthrene	19	9.1
2-phenanthrene	72	20
3-phenanthrene	107	29
4-phenanthrene	34	13
9-phenanthrene	43	16

^aECD response with 2.0 ppth O₂ in the nitrogen bulk gas at 250°C relative to response without added oxygen.

^bObserved intensity of M⁻ ion with 2.0 ppth O₂ in the nitrogen bulk gas at 250°C relative to that observed without added O₂.

The EI and CI spectra of isomeric set of PAA derivatives can be briefly summarized as follows: With EI some degree of differentiation was provided by small differences in the intensity of fragment ions, so that 3 of the 8 isomers displayed clearly unique spectra. With positive ion CI using both isobutane and methane, again unique spectra was observed for 3 of the 8 isomers. The results obtained by negative ion CI in methane and in isobutane at first appeared to be much more promising than that obtained by positive ion EI or CI. Upon closer inspection of all negative ion CI spectra, however, it was noticed that the relative intensities of the negative fragment ions were dependent on the concentration of the analyte. Apparently, reactions in addition to simple electron attachment are responsible for the total negative ion CI spectra observed.

In summary, negative ion CI shows considerable promise for the differentiation of isomeric PAA's but appears to be complicated by presently unknown ionization processes. Positive ion EI and CI spectra provided only partial differentiation of the isomers. The ECD with oxygen sensitization was observed to provide a unique and reproducible response ratio to each isomer and in view of its simplicity appears to be a particularly promising tool for this type of measurement.

THE CHEMISTRY OF REACTIONS OCCURRING IN A MICROWAVE-POWERED GC/MS INTERFACE. Fred P. Abramson and S.P. Markey, Department of Pharmacology, The George Washington University Medical Center, Washington, DC 20037 and The Laboratory of Clinical Science, NIMH, NIH, Bethesda, MD 20205.

We report on the chemistry of reactions occurring in a medium pressure (ca. 1 Torr) microwave-powered reaction chamber which serves as an interface between a capillary gas chromatographic column and a mass spectrometer. The discharge causes dissociation of the bonds in the eluting compound and a reagent gas scavenges the atoms which are formed. As a result, each element or nuclide originally present in a molecule is converted into small polyatomic species characteristic of both the element and the reagent gas. Thus, rather than observing the mass spectrum of the eluting compound, we observe the several spectra of the reaction products simultaneously. This work characterizes the reactions which occur in this apparatus, and addresses the question of whether reaction to completion or chemical equilibrium is characteristic of this technique.

When oxygen is the reagent gas, the carbon initially contained in an eluting compound is converted to CO_2 and CO and the hydrogen is converted into water. Labeled carbon or hydrogen can be detected through these reactions. If a molecule originally contained sulfur, SO_2 and SO will be present in the simple spectra which now represent the original compound. Similarly, any nitrogen in the analyte will appear primarily as N_2 with smaller amounts present as NO and NO_2 . Halogens appear both as X_2 and HX.

When hydrogen is the reagent gas, carbon is detected as a mixture of hydrocarbons: methane, acetylene, and ethylene. Oxygen is converted to both H_2O and CO, and halogens appear as HX. Although molecular nitrogen is relatively unreactive in the hydrogen-scavenged discharge, organic nitrogen is converted efficiently to HCN. Sulfur appears as CS_2 with smaller amounts of H_2S . This provides a practical advantage for the hydrogen discharge system since CS_2 can be used as a solvent without affecting the normal channels which detect carbon.

While the appearance of C_2H_2 or CO in a hydrogen-scavenged discharge seems inappropriate to the highly reductive environment of the reaction scheme, we believe that it represents an apparent chemical equilibrium as has been observed in other sorts of discharge reaction devices. For example, hydrogen and CO will be in equilibrium with the water and methane which they produce. Although the position of the equilibrium can be affected by the concentration of the reacting species, all 4 compounds will be present due to a back reaction. To test this idea, a solution of d-6-acetone in another solvent not containing any hydrogen was injected over a tenfold range of sample sizes. The production of D_2 and HD was compared with the production of deuterated methanes. As the sample size increased, so did the contribution of deuterium to the total hydrogen signal. As a consequence, the presence of CH_3D and CH_2D_2 grew, consistent with the increased availability of deuterium as a reactant gas subsequent to the initial decomposition of the injected acetone.

The chemistry of the discharge can be used to optimize an analysis for a particular compound or a particular instrument. For a nominal mass spectrometer, the most effective scheme for detecting ^{14}C would be as $^{14}\text{CO}_2$ at m/z 46. Only a small background due to residual nitrogen producing NO_2 at m/z 46 would interfere. However, if the compound in question contained a significant amount of nitrogen compared to its ^{14}C , a false signal not representing $^{14}\text{CO}_2$ might be observed. Because ^{14}N and ^{14}C both have a nearly identical exact mass, high resolution would not remove this difficulty. An alternative would be to use hydrogen as a reagent gas and to detect $^{14}\text{CH}_4$ at m/z 18 where it can be separated from any water which is present using a resolving power of 1000.

This work has demonstrated the range of chemical reactions which occur in both hydrogen and oxygen discharges. The simple species which are formed are useful in determining which atoms were initially present in a molecule, and could be used to selectively detect these species in complex mixtures.

The choice of reactant gas permits optimization of a particular analysis to suit both the compound in question and the capabilities of the mass spectrometer used.

Finally, we believe that the reaction system approximates an equilibrium so that complete reactions may not always be observed, multiple pathways are possible, and that side reactions between products or instrument background are possible.

DIFFERENCES IN THE CHEMICAL IONIZATION AND ATMOSPHERIC PRESSURE IONIZATION MASS SPECTRA OF SOME ISOMERS OF CHLORO, METHYL AND NITROANTHRACENE

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The intentional addition of oxygen to an electron capture detector has been previously shown to significantly enhance the ECD response to certain polycyclic aromatic hydrocarbons (1-3). The measured response enhancement values were found to be dependent upon subtle structural variations (1,2), and, in fact, have been used to quantitate mixtures of coeluting substitution PAH isomers (3,4). The response of the oxygen-doped ECD is thought to be proportional to the rate of an ion-molecule reaction between the negative ion, O_2^- and the sample molecule (1,2,5,6). To further understand the ion chemistry responsible for the oxygen-sensitized response of an ECD, we have obtained the chemical ionization and atmospheric pressure ionization mass spectra of several isomers of chloro, methyl and nitroanthracene in the presence and absence of intentionally added oxygen.

The positive ion CI spectra of 2-methyl and 9-methylanthracene are identical ($M+H$)⁺ and M^+ are the major ions observed. The negative ion CI spectra of these isomers are also identical with M^- being the predominant negative ion. The positive ion API spectra of the two methylanthracene isomers are identical, ($M+H$)⁺ is the major ion observed. In the APIMS, M^+ with a trace of ($M+O$)⁺ is observed for 2-methylanthracene whereas ($M-CH_3+O_2$)⁺ and ($M+O_2$)⁺, relative intensities 5:1, respectively, are observed for 9-methylanthracene. With oxygen doped nitrogen carrier gas, only ($M-CH_3+O_2$)⁺ is observed for 9-methylanthracene whereas ($M+O_2$)⁺ and ($M+O$)⁺, relative intensities 4:1, are observed for 2-methylanthracene.

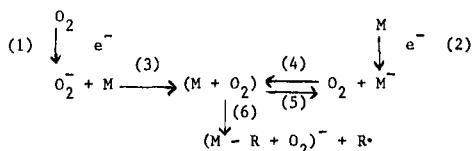
The negative ion CI spectra of 1-chloro, 2-chloro and 9-chloroanthracene are identical; M^- is the predominant negative ion. The positive ion CI spectra of 2-chloro and 9-chloroanthracene are identical yet differ distinctly from that of the 2-chloro isomer. Whereas ($M+H$)⁺ m/e 213, is the predominant ion in the CI spectra of all three isomers, the 2-chloro isomer is uniquely characterized by an intense ion at m/e 177 presumed to be ($M-Cl$)⁺. This spectral difference aids in the qualitative identification of the chromatographically unresolvable 1-chloro and 2-chloroanthracene isomers. The positive ion API spectra of the three chloroanthracene isomers are identical; ($M+H$)⁺ is the major ion. In the APIMS, M^- and ($M+O_2$)⁻, relative intensities 4:1, are the major ions observed for 1-chloro and 2-chloroanthracene whereas ($M+O_2-Cl$)⁻ and ($M+O_2$)⁻, relative intensities 3:1, are observed for 9-chloroanthracene. With oxygen-doped nitrogen carrier gas, the predominant negative ions in the APIMS are exclusively ($M+O_2-Cl$)⁻ for 9-chloroanthracene and ($M+O_2$)⁻ for 1-chloro and 2-chloroanthracene. With oxygen-nitrogen or oxygen-isobutane reagent gas mixtures, the negative ion CI spectra of the three isomers are identical, M^- is predominant, and there is no indication of oxygen attachment to the chloroanthracene molecules. Interestingly, several PAHs such as phenanthrene, pyrene, triphenylene, tetracene, and benzo(e)pyrene showed considerable oxygen incorporation under CI conditions as evidenced by an intense ($M+O-H$)⁻ ion when an isobutane-oxygen reagent gas mixture is used. Hunt and coworkers (7,8) have previously observed the formation of phenolic anions of various PAHs when using oxygen and oxygen-hydrogen reagent gases. ($M+O-H$)⁻ ions have also been identified in the API negative ion mass spectra of several PAHs (1).

The negative ion CI spectra of 2-nitrophenanthrene, 2-nitroanthracene and 9-nitroanthracene are identical, M^- is the predominant ion with a small amount of ($M-O$)⁻ m/e 207 also observed. ($M+H$)⁺, ($M+H-NO$)⁺ and ($M+H-O$)⁺ are the major ions observed in the CI positive ion spectra of the three nitroanthracene isomers. ($M+H$)⁺ is the predominant ion in the API positive ion spectra of the three isomers. In the APIMS, for 2-nitrophenanthrene and 2-nitroanthracene M^- is the only ion observed whereas M^- and ($M-NO_2+O_2$)⁻ or ($M-N$)⁻, relative intensities 4:1, are observed for 9-nitroanthracene. With oxygen-doped nitrogen carrier gas, only M^- is observed for 2-nitrophenanthrene and 2-nitroanthracene, whereas M^- and ($M-NO_2+O_2$)⁻, relative intensities 4:1, are

observed for 9-nitroanthracene. With oxygen-nitrogen and oxygen-isobutane reagent gases there is also no indication of oxygen attachment to the nitroanthracene isomers under CI conditions.

From our data three main conclusions can be drawn. First, atmospheric pressure ionization mass spectrometry is capable of distinguishing structural differences, amongst PAH isomers, that cannot be resolved by other forms of mass spectrometry. Secondly, oxygen attachment to the various isomers of anthracene is occurring selectively at positions 9 and 10 on the anthracene molecule. Thirdly, the ion chemistry responsible for the oxygen-induced ECD response enhancement of the chloroanthracene and methylanthracene isomers is different than that of the nitroanthracenes.

Though the exact ion-molecule chemistry occurring for PAHs under API/ECD conditions is unknown, our results lead us to postulate the following reaction sequence similar in nature to that of Horning and coworkers (9,10).



For the isomers of chloroanthracene and methylanthracene, an intermediate $(\text{M} + \text{O}_2)^-$ is formed either by reactions 1 and 3 or 2 and 4. This intermediate may remain intact, as is the case for 2-methylanthracene and 1-chloro and 2-chloroanthracene, or dissociate, reaction 6, to form $(\text{M} - \text{R} + \text{O}_2)^-$ ions such as those observed for 9-chloro and 9-methylanthracene. A charge transfer sequence (reactions 1, 3, and 5) between O_2^- and M leading to M^- is also possible. The ECD oxygen-induced response enhancements observed for the nitroanthracene isomers and other nitro PAHs probably occur as a result of this charge exchange reaction. A charge transfer reaction between O_2^- and M is also postulated to be responsible for the ECD response enhancement observed for derivatized PAH amines (3).

The amount of π resonance energy lost by various PAH molecules when one of their carbon atoms is removed from the total resonance system is easily calculated from simple Huckel theory. This value might be expected to provide an estimate of the relative ease with which nucleophilic attachment by O_2^- on some site in the PAH molecule might occur (1). For anthracene there are essentially only three distinct sites of possible oxygen attachment. The atom localization energies of these sites are: position 1, 2.238; position 2, 2.428 and position 9 or 10, 2.018. Since oxygen attachment will most likely occur at the position which corresponds to a minimum loss of π resonance energy, positions 9 and 10 should be most susceptible to nucleophilic attachment by O_2^- . Our data indicates that oxygen attachment is indeed occurring at positions 9 and 10 for the chloranthracene and methylanthracene isomers.

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DIFFERENTIATION OF ISOMERIC HETEROATOM-SUBSTITUTED AROMATICS
USING CHEMICAL IONIZATION

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Chemical ionization techniques employing gas phase hydrogen-deuterium exchange have been used to differentiate isomeric aromatic compounds substituted with oxygen-, sulfur-, or nitrogen-containing functional groups with respect to the substitution at the heteroatom. Using a protonated CI reagent, the compound is first ionized via proton transfer. Using a deuterated CI reagent, not only is the compound ionized by the transfer of a deuterium ion, but any active hydrogens on the heteroatom exchange with the deuterium atoms of the reagent as well. When the protonated and deuterated spectra of the compound are compared, the differences in mass observed yield information on the type of substitution at the heteroatom.

Ammonia and deuterated ammonia (ND_3) have previously been used to differentiate simple primary, secondary and tertiary amines (1), which exhibit changes of three, two and one mass units, respectively. This technique has been recently extended to the differentiation of isomeric primary, secondary, and tertiary aromatic amines, including heterocyclic amines and compounds containing more than one nitrogen (2). However, unknown compounds which yield changes in mass of one or two mass units cannot be unequivocally assigned as being either N-alkyl substituted or heterocyclic amines based on the CI data alone.

Methanol and deuterated methanol (CH_3OD or CD_3OD) have been used to differentiate isomeric oxygen-containing aromatics, including hydroxyaromatics (PhOH), aromatic ethers (PhOR), and aromatic substituted alcohols (PhROH), which yield differences of two, one and zero mass units, respectively. The observed change of zero mass units for aromatic substituted alcohols arises from the loss of H_2O (or D_2O) from the protonated (or deuterated) species prior to detection. In a similar manner methanol may also be used as a CI reagent to differentiate the analogous sulfur-substituted aromatics, including thiophenols, aromatic thioethers and aromatic substituted mercaptans.

Using GC/MS, these CI techniques have been successfully employed to differentiate isomeric compounds in complex mixtures (3). Additionally, in complex mixtures containing primarily hydroxyaromatics, trace amounts of isomeric amines have been differentiated

without interference from the hydroxyaromatics using ammonia as the CI reagent. This selective ionization of the amines arises from the higher proton affinity of the ammonia reagent relative to oxygen-containing aromatics.

A full account of this work is being prepared for publication in the near future. This research is sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

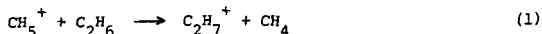
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RADICAL COMBINATION IN A CHEMICAL IONIZATION SOURCE

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Radicals are major products of the interaction of electrons with CH_4 .¹ Recently, products of radical/molecule reactions were reported under chemical ionization (CI) conditions.^{2,3} Large concentrations of H^+ , CH_3^+ , and C_2H_5^+ were observed in the CH_4 CI plasma.² We have observed the formation of C_2H_6 and higher alkanes by radical combination under CH_4 CI conditions.⁴

Ethane is detected under CH_4 CI conditions as C_2H_7^+ from the exothermic proton transfer reaction:



Small abundances of C_2H_7^+ reported in previous high pressure mass spectrometric studies of CH_4 were attributed primarily to trace impurities of C_2H_6 in the CH_4 . Significant increases in the abundance of C_2H_7^+ are observed under slightly modified conditions which can be attributed to C_2H_6 formed by radical combination reactions.⁴ Figure 1(a) shows the effect of

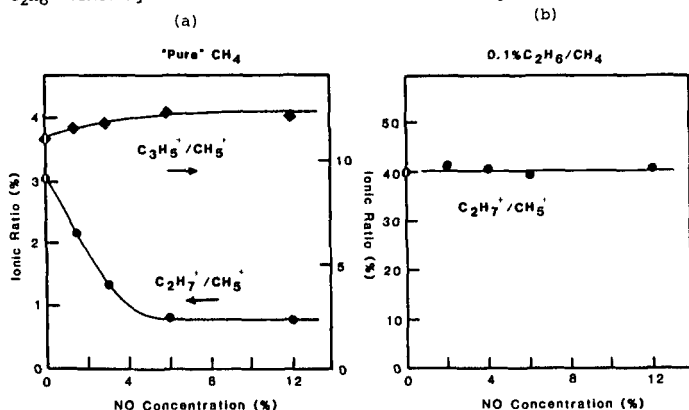


Figure 1. Effect of Radical Scavenger (NO).

adding the radical scavenger NO to "pure" CH_4 . The ratio of ionic abundances, $\text{C}_3\text{H}_5^+/\text{CH}_5^+$, is essentially independent of the NO concentration because neither ion reacts rapidly with NO. The ionic ratio, $\text{C}_2\text{H}_7^+/\text{CH}_5^+$, decreases markedly with the addition of a small amount of NO. In Fig. 1(b), a small amount of C_2H_6 has been added to the CH_4 so that the preponderance of the C_2H_7^+ results from the reaction of CH_5^+ with the added C_2H_6 . The $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ ratio in Fig. 1(b) is independent of the added NO concentration establishing that C_2H_7^+ does not react rapidly with NO. The large decrease in the $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ ratio with the addition of NO, in Fig. 1(a), therefore, results from a decrease in the concentration of radical precursors. The small abundance of C_2H_7^+ at large NO concentrations in Fig. 1(a) can be attributed primarily to trace C_2H_6 impurities in the CH_4 .

The abundance of C_2H_7^+ depends on the nature of the source chamber surface, the ionizing electron current, and the source temperature. Table 1 shows the relative abundances of C_2H_7^+ observed using ion sources with different reaction chamber surfaces. It is clear that the surface plays an important role in the formation of the C_2H_7^+ .

Figure 2(a) shows the effect of varying the ionizing electron current. Since both C_3H_5^+ and CH_5^+ involve one electron in their formation, the ratio of their abundances, $\text{C}_3\text{H}_5^+/\text{CH}_5^+$, is essentially independent of the ionizing current. The $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ ratio increases linearly with increasing ionizing current. In Fig. 2(b), a small amount of C_2H_6 has, again, been added to the CH_4 so that essentially all of the C_2H_7^+ results from reactions with the added C_2H_6 . The $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ ratio in Fig. 2(b) is independent of the ionizing current indicating that there are no complications from possible differences in ion/electron neutralization rates for C_2H_7^+ and CH_5^+ under these conditions.

The concentration of radicals in the CI plasma can be shown to be approximately proportional to the electron concentration in the ion source.

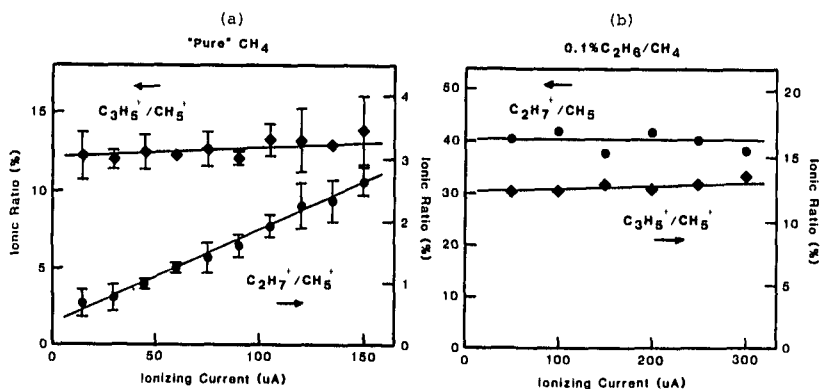


Figure 2. Effect of Ionizing Electron Current.

$$[\text{Radical}] \propto [e] \quad (2)$$

Similarly, analysis of the detection step, reaction (1), shows $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ to be proportional to the concentration of C_2H_6 .

$$\text{C}_2\text{H}_7^+/\text{CH}_5^+ \propto [\text{C}_2\text{H}_6] \quad (3)$$

The $\text{C}_2\text{H}_7^+/\text{CH}_5^+$ is proportional to the ionizing current or $[e]$ in Fig. 2(a).

$$\text{C}_2\text{H}_7^+/\text{CH}_5^+ \propto [e] \quad (4)$$

Substituting equations (2) and (3) into (4) gives the result:

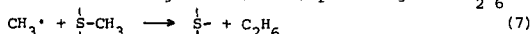
$$[\text{C}_2\text{H}_6] \propto [\text{Radical}] \quad (5)$$

The C_2H_6 is produced by a process which is kinetically first-order in radical concentration.

These experimental observations can be reconciled by a combination mechanism involving reversible adsorption of a radical on the source chamber wall,



followed by reaction of the bound radical with a gaseous radical producing free C_2H_6 .⁵



Preliminary evidence has been obtained for other radical combination reactions under CH_4 CI conditions. Hydrogen produced by radical combination cannot be observed under these conditions because no exothermic ion/molecule reaction is available to detect the H_2 . Propane and butane can be detected by reactions with CH_5^+ producing C_3H_7^+ and C_4H_9^+ , respectively. The $\text{C}_3\text{H}_7^+/\text{CH}_5^+$ and $\text{C}_4\text{H}_9^+/\text{CH}_5^+$ ratios, Table 2, increase with increasing ionizing current and decrease with the addition of a small amount of NO . These observations establish that radical combination reactions forming propane and butane also occur under CH_4 CI conditions.

Table 1. Effect of Source Chamber Surface

Source Surface	Rel. C_2H_7^+ Yield*
Au/Al	0.32±0.02
Stainless Steel	1.00
Al(Al_2O_3)	6.6±0.9

Table 2. Other Radical Combination Products

Ionic Ratio	"pure" CH_4		Added NO
	50 μA	300 μA	300 μA
$\text{C}_3\text{H}_7^+/\text{CH}_5^+$	5.4%	6.6%	5.3%
$\text{C}_4\text{H}_9^+/\text{CH}_5^+$	3.0%	5.8%	3.7%

* Relative to stainless steel = 1.00.

Corrected to constant ionic reaction time.

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ROF 10 | A KINETIC THERMOMETER FOR MEASURING THE
TEMPERATURE OF ION-MOLECULE REACTION CELLS; M.J. Henchman,
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It is in no way simple to measure an effective temperature for reaction cells containing gaseous ions and neutral gas at low number density. Such difficulties are responsible in part for continuing uncertainty in ionic thermochemistry. For example, the difference in proton affinity between ammonia and water was quoted as 32.0 kcal/mol in 1978; today a prudent estimate would be 37 ± 2 kcal/mol.

A chemical reaction can provide an effective thermometer if either the rate constant or the product distribution shows a marked dependence on temperature. A reaction meeting the latter requirement is $\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3$, with deuterium labelling of one reactant. The yield of CHD_4^+ from $\text{CH}_4^+ + \text{CD}_4$ is representative. It would be 7% if statistical; experimentally it is 10% at 80K and 25% at 300K; and an ICR study in the literature reports 63%.

ROF 11 | HYDROGEN-DEUTERIUM EXCHANGE USING FOURIER
TRANSFORM MASS SPECTROMETRY
Sahba Ghaderi and Gerald E. Parisod; Nicolet Analytical
Instruments, Madison Wisconsin 53711-0508

Hydrogen-deuterium exchange reactions, involving deuterated reagent ions such as D_3O^+ , ND_4^+ , CH_3OD_2^+ , etc., have been reported under both conventional CI conditions and ion cyclotron resonance conditions. These reactions appear to be useful for counting the number of exchangeable protons and, therefore, bear similarity to the corresponding proton magnetic resonance technique except that much smaller quantities of sample can be used. The information obtained may be helpful for assigning ionic structures on the basis of exchange behavior, characterizing isomeric molecules and assigning acidity of hydrogen atoms in cations and anions.

The method is readily applied by using Fourier transform mass spectrometry (FT/MS). Long ion trapping, low pressure chemical ionization and ion ejection capability of the FT/MS make it an ideal tool for deuterium exchange studies.

In this paper, results of some model reactions will be presented along with a discussion on the selection of the exchange reagent and the value of the technique in distinguishing isomeric compounds.

ELECTRON CAPTURE NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY: APPLICATION TO THE ANALYSIS OF FUSARIUM TRICOTHECENES.*

Introduction:

Trichothecenes, produced by fusarium fungi, are polar and toxic. They have been implicated in the intoxication of animals and men for decades. Consumption of fungi infested food or exposure to even trace amounts of these trichothecenes could produce lethal effects. Hence, it is of utmost importance to develop specific and sensitive methods of detection and accurate quantification of these toxins at ultra-trace levels in complex sample matrices.

The developed method is comprised of the conversion of the polar trichothecenes (as shown below (in figure 1) along with the internal standard, into their volatile heptafluorobutyl (HFB) esters and subsequent analysis by the Electron Capture Negative Ion Chemical Ionization (NICI) technique for increased sensitivity. Deoxyverrucarol (DOVE), obtained by the selective reduction of the 4 α -hydroxyl group of verrucarol, has been found to be an internal standard well suited for the quantification of these fusarium trichothecenes by the GC/MS - NICI technique.

Experimental:

Residues, obtained by the evaporation of solutions containing various amounts (5-500 ng) of all the above mentioned trichothecenes and deoxyverrucarol (250 ng) were treated with 10% acetonitrile/toluene (500 μ l) and heptafluorobutyl imidazole (500 μ l). The reaction mixture was heated at 100°C for 15 minutes, cooled, and treated with 5% NaHCO₃ solution (2 X 500 μ l) and distilled water (2 X 500 μ l). The organic layer containing the derivatives was stored at -5°C until analysis. The organic layer (1 μ l) and toluene (1 μ l-solvent wash) were injected splitless into a 30 m 0.25 mm ID fused silica SE-30 capillary column and analyzed by NICI at a source temp of 100°C, using methane as the reagent gas. A Hewlett-Packard 5985-B GC/MS/DS was used throughout the investigation.

Results and Discussion:

Optimum conditions for the derivatization, separation (GC), and detection (MS-NICI) were established. All seven HFB esters were well resolved, as indicated in figure 2, and their mass spectra were obtained. With the exception of T-2 (HFB), where (M-82)⁺ was the base peak, the most abundant ion was either M⁺, or (M-HF)⁺. In each case, the most abundant ions were used for the quantification of the analytes by SIM experiments.

Several solutions containing varied amounts of HFB esters of T-2, HT-2, DON, verrucarol, 8 α - and 8 β -hydroxyverrucarol and fixed amount of DOVE (HFB) were analyzed by the SIM mode and their corresponding peak areas (ion counts) were measured. Each solution was analyzed four times and the average of the best three values were used. In each case, when the amount of derivatives (trichothecenes) and their relative responses with respect to the DOVE (HFB). This is indicated in figures 3 - 5.

An aliquot (1 μ l) of solution containing the analytes (500 pg/ μ l) and the internal standard (500 pg/ μ l) was analyzed at various intervals after the time of derivatization. The measured relative responses, over a period of 14 days, varied less than 20%. A detailed, systematic study on the stability of HFB esters is underway.

Commercial bee pollen was spiked with T-2, HT-2, Verrucarol, DON, 8 α - and 8 β -hydroxyverrucarol in ppb level. A known amount (0.54 mg) of the spiked pollen was extracted with methanol (2 X 5 ml). The filtrate was evaporated and the residue was treated with DOVE and evaporated again. The HFB esters were prepared and analyzed by NICI. The results are indicated in Table 1.

SUMMARY

1. Deoxyverrucarol (DOVE), closely related in structure to simple trichothecenes, has been found to be an excellent internal standard for the detection and quantification of fusarium trichothecenes.
2. Trace amounts of HT-2, T-2, DON, Verrucarol, 8 α - and 8 β -hydroxyverrucarol (5-500 ng) have been effectively converted into their HFB derivatives.
3. The relative responses of the HFB esters of these six trichothecenes vary linearly with their concentration, over a range of 200 - 1000 pg.
4. The derivatives are reasonably stable over a period of 14 days, if stored at -5°C.
5. The percent recoveries of verrucarol, 8 β -hydroxyverrucarol, T-2, and HT-2 from spiked bee pollen are in the range of 17-44%.

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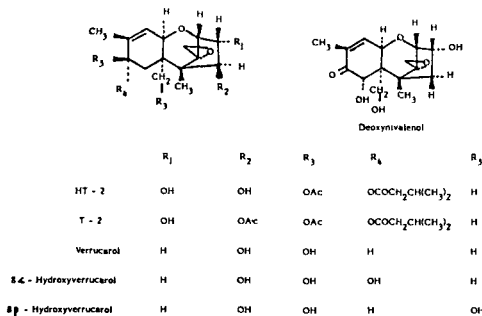


FIGURE 1.

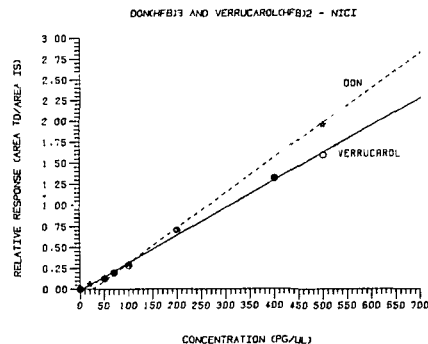
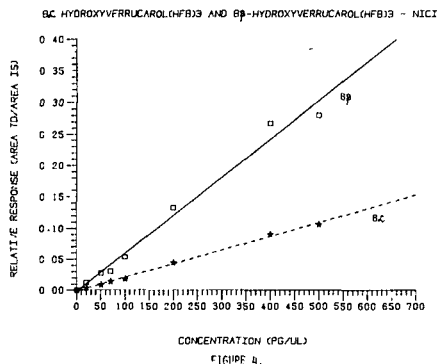
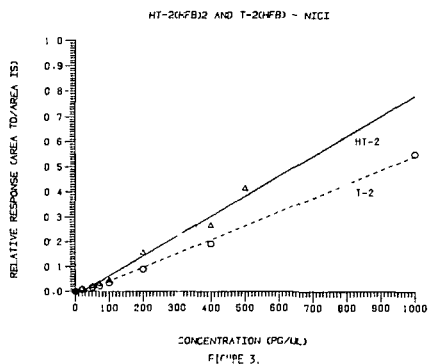
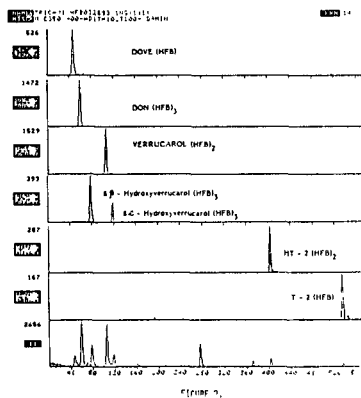


TABLE 1.

Amount of Compound Reagent (ng)	Compound	Spiked Amount (ng)	Concentration of Analyte in Sample Matrix (ng)	Amount of Analyte Recovered (ng)	Recovery
0.5621	DON	240 ng	66.3	3.78	5.66
0.5621	Verrucarol	240 ng	66.3	41.98	23.79
0.5621	8,9 - Hydroxyverrucarol	240 ng	66.3	10.42	17.25
0.5621	8,6 - Hydroxyverrucarol	240 ng	66.3	2.817	0.61
0.5621	HT - 2	240 ng	66.3	22.94	34.13
0.5621	T - 2	240 ng	66.3	195.70	61.83

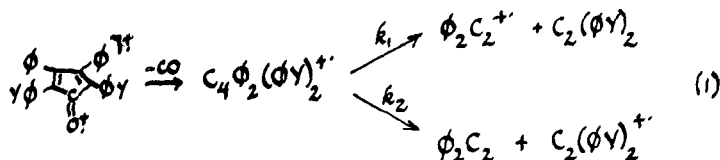
A Collision Product Ion Intensity Correlation in the Absence of Precursor Thermalization

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The first correlation of collision product ion intensities when the precursor has been generated by electron ionization, and when the bonds broken are full covalent bonds, has been obtained. The competitive products in equation 1 have appearance potentials correlated by Hammett sigma constants, and we now find that the ratio of ion intensities also correlates reasonably well. In the past such correlations have been observed for hydrogen-bound dimers generated under chemical ionization conditions, and an explanation was advanced making use of the very reasonable concept of an effective temperature (1).



An alternative explanation may be offered. The great similarity of the reactions suggests that in general the rate expression from RRKM theory (equation 2) has many, virtually identical terms: $L^\ddagger$, $Q_1^\ddagger/Q_1$, h , $N^*(E^*)$.

$$k(E^*) = L^\ddagger \frac{Q_1^\ddagger}{Q_1} \frac{1}{h N^*(E^*)} \sum_{E_v^* = 0}^{E^*} P(E_v^*) \quad (2)$$

If rotational freezing out is similar, rotational terms may also be removed from the $P(E^*)$ functions being summed. The ratio of rate constants then reduces to a ratio of summed vibrational states, equation 3.

$$\frac{k_1}{k_2} = \frac{\sum_{E_v^* = 0}^{E^*} P_1(E_v^*)}{\sum_{E_v^* = 0}^{E^*} P_2(E_v^*)} \quad (3)$$

A simple function (equation 4) has long been used to approximate the sum of states successfully even at low energies.

$$\sum P(E_v^*) = \frac{(E_v + \alpha E_E)^5}{5! \prod \hbar \omega_i} \quad (4)$$

Since the denominator is the same for the two competitive reactions we may rewrite the ratio as equation 5.

$$\frac{k_1}{k_2} = \left(\frac{E_{V1} + aE_z}{E_{V2} + aE_z} \right)^5 \quad (5)$$

Defining E as $E^* - aE_z$ and ΔE as the difference between the forward energy barriers, we arrive at equation 6.

$$\log \frac{k_1}{k_2} = \frac{5}{2.3} \ln \left(1 + \frac{\Delta E}{E - E_2} \right) \quad (6)$$

Given typical values of E^* (4 eV), aE_z (2 eV), and ΔE (0.3 eV), saving only the first term of the series expansion is justified (equation 7).

$$\log \frac{k_1}{k_2} = \frac{5}{2.3} \frac{\Delta E}{E - E_2} \quad (7)$$

So for each energy equation 8 very nearly holds, and the correlation holds.

$$\log \frac{k_1}{k_2} = (\text{const}) \Delta E = (\text{const}') \sigma \quad (8)$$

Some of the assumptions about rotational terms would not be justified in general and this lack of generality raises interesting questions about how limited the extensions of this discovery may be.

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Title: Collision Activated Decompositions of Positive and Negative Ions of Esters

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Much work has recently been performed in this laboratory toward the goal of devising comprehensive schemes of analysis for classes of organic compounds in complex mixtures by use of tandem mass spectrometry. Thus, studies of carbonyl compounds and phenols⁽¹⁾, polycyclic aromatic compounds⁽²⁾, organosulfur compounds⁽³⁾ and chloro compounds have been carried out.

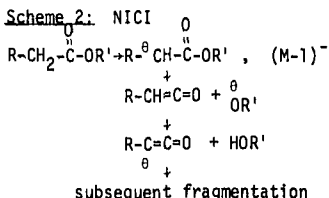
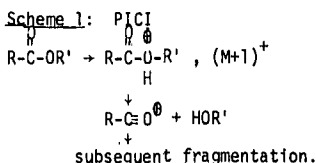
Under CI conditions, many of these compounds produce abundant amounts of ions characteristic of molecular weight. These can frequently be induced to fragment by CAD in known ways, often yielding a given neutral. Some compounds which do not display this behavior can be derivatized, in order to produce such a characteristic ion.

Thus, many compounds and several classes of compounds can be analyzed for by CI CAD MS.

In an attempt to extend the number of classes of compounds which can be analyzed for, carboxylic acid esters were studied. These compounds possess a functionality coupling two portions of the molecule, each of which may vary in structure independently. Also, reactions producing a suitable derivative are not easy to envision. Transesterification is expected to violate criteria upon which simple analysis are based. High yield is not anticipated in a reasonably short time. Removal of excess reagent is not necessarily facile.

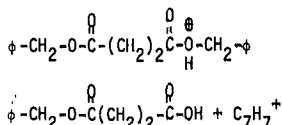
Even, so, the CI CAD MS method offers some promise for the analysis of esters.

The most frequently observed CAD mechanisms involved loss of the alcohol moiety. This yields ions of predictable structure, $R-C=O^{+1}$ and $R-C=C=O$.



Some exceptions to these schemes were found. Of the 49 compounds studied, the $(M+1)^+$ ion of dibenzyl succinate was the only one which decomposed by a mechanism different from Scheme 1:

Scheme 3: dibenzyl succinate, $PICI$



Here, $C_7H_7^{+}$ and the neutral acid are formed instead of the alcohol and $R-C=O^{+}$.

Esters which do not possess within the acid chain a $-CH_2-$ group α to the carbonyl cannot form the neutral ketene. Thus, more exceptions to Scheme 2 were found, including the phthalates, m- and p-phenylene diacetate, diethylphenyl malonate, and methyl and ethyl cinnamate.

While loss of a particular neutral or formation of a given ion cannot be expected for this whole class of esters, some types of esters do show analytical possibilities.

Acetates always show the $m/z 43^+$ ion and most also form $m/z 41^-$. Ethyl esters often display loss of 28 amu in the PICI. Methyl esters show loss of 32 amu. Phthalates may be distinguished from other similar compounds by the formation of $m/z 147^-$ and $m/z 148^-$.

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RPA 3. ISOTOPE EFFECTS IN COLLISION-INDUCED DISSOCIATIONS OF SIMPLE IONS.

T. A. Lehman, Bethel College, No. Newton, KS 67117; J. R. Hass, NIEHS, Research Triangle Park, NC; F. W. Crow and K. B. Tomer, Midwest Center for Mass Spectrometry; and L. G. Pedersen, Chemistry Department, University of North Carolina at Chapel Hill.

Competitive collision-induced reactions $(AB\bar{B}^*)^+ \rightarrow AB^+ + B$ and $AB^+ + B^*$, where B is the lighter and B* the heavier isotope, have been studied. Experiments have been done on the ZAB-2F at NIEHS and on the triple analyzer (TA) at the Midwest Center for Mass Spectrometry. Results of the same experiments on the two instruments are generally in good agreement, though a few results are discordant.

Several instrumental variables have been tested for possible influence on the product isotope ratios, corrected for an assumed linear dependence of detector response on ion mass. We report that ratio as

$$\frac{\frac{I(AB^*)^+}{m_{AB^*}}}{\frac{I(AB)^+}{m_{AB}}}$$

and find that the variation of the z slit width and the accelerating voltage produces a small fluctuation in the ratio that rules out any functional dependence. Variation of the collision gas pressure does affect the ratio; the few cases for which we have signal-averaged results in the absence of gas show quite different ratios. This requires further study.

An analysis of the energy transferred from translation to vibration has been developed by Landau and Teller (1) and expanded by Rapp (2). It assumes a linear collision between a monatomic and a diatomic particle. We have applied it to both diatomic and triatomic ions. It is assumed that product ion intensities depend linearly on the amount of energy transferred to vibration. The table presents some results.

Ion subject to CID	Product ion ratio as defined above		
	predicted	observed	
		TA	ZAB
diatomic:			
$^{16}\text{O}^{18}\text{O}^+$	1.265	1.06	1.18
$^{32}\text{S}^{34}\text{S}^+$	1.129	1.05	
triatomic:			
CHD^+	2.15	2.4	3.3
$^{16}\text{O}-^{18}\text{O}^+$	1.21		1.12
$^{35}\text{Cl}-^{37}\text{Cl}^+$	1.10	~1.06	~1.06
$^{32}\text{S}-^{34}\text{S}^+$	1.12	1.00	~1.09

Given the uncertainties in some of the data, the theory provides a good indication of the magnitude of the product isotope ratios.

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1. L. Landau and E. Teller, Z. Sowjetunion 1936, 11, 18.
2. D. Rapp, J. Chem. Phys. 1960, 32, 735.

A Systematic Study of the Low Energy Collision-Induced Dissociation Spectra of
Protonated Methyl n-Alkanates

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The collision-induced dissociation (CID) spectra of a series of protonated methyl n-alkanates $\text{CH}_3(\text{CH}_2)_n\text{COOCH}_3$ ($n=1-9$) were studied at collision energies in the range 8-58 eV using a Sciex TGA 6000 triple quadrupole. Argon was used as the collision gas. A linear relationship was found between the reciprocal of the required target thickness and the chain length of the esters (Figure 1). The reciprocal of this target thickness is equivalent to the cross-section for loss of parent ions.

The influence of hydrocarbon chain length and collision energy on the fragmentation process was examined. It was found that characteristic fragment ions such as those due to the loss of methanol dominated in the CID spectra of the short chain esters but became less important with increasing chain length (Figure 2). The CID spectra obtained at lower collision energies seem to be more useful since these spectra usually contain characteristic ions in relatively high abundances. At high collision energy, non-characteristic ions resulting from fragmentation of the hydrocarbon part of the esters are the major products. An example is given in Figure 3. Note that the parent ions are not shown in the spectra.

The present results indicate that the relative importance of a fragmentation process may change within a class of compounds. It should be noted that a spectrum obtained from a neutral loss of methanol may not adequately represent the entire series of methyl esters since there is a high probability of underestimating higher esters when using the linked-scan technique.

FIGURE 1

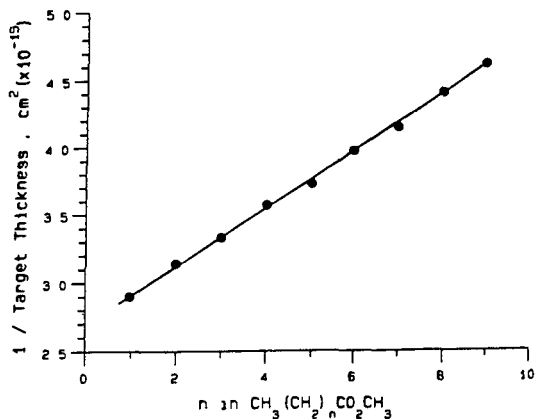


FIGURE 2

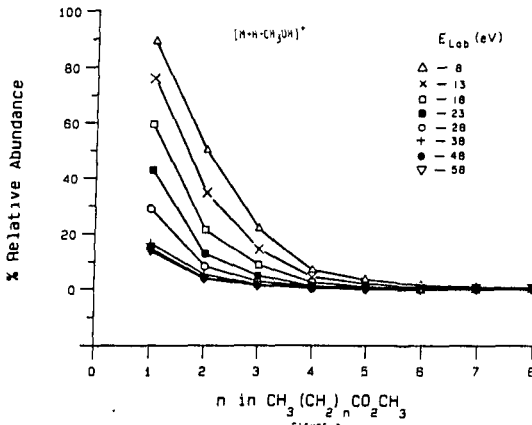
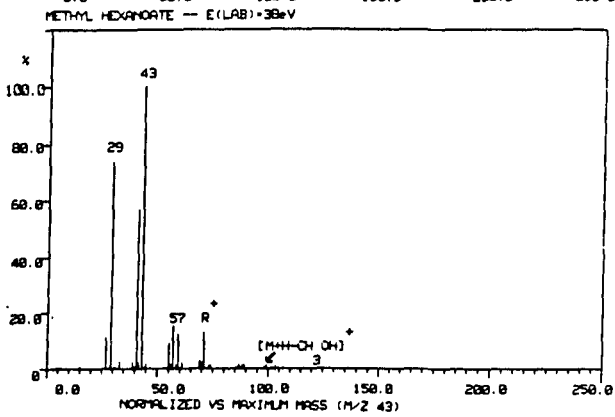
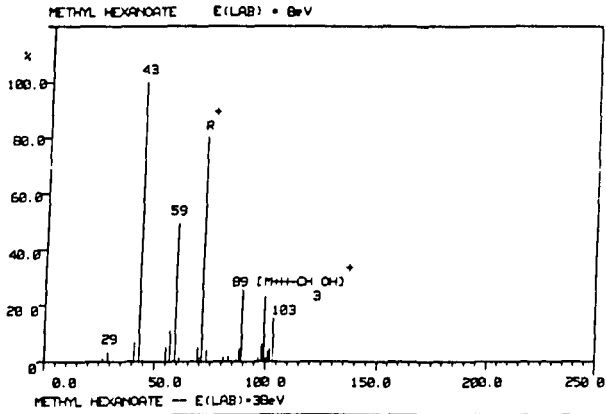


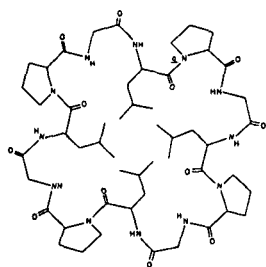
FIGURE 3a



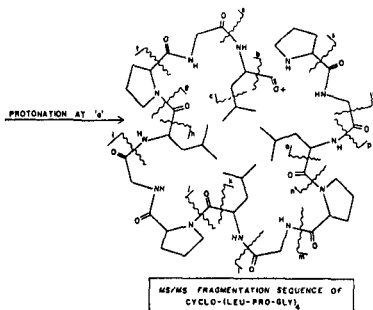
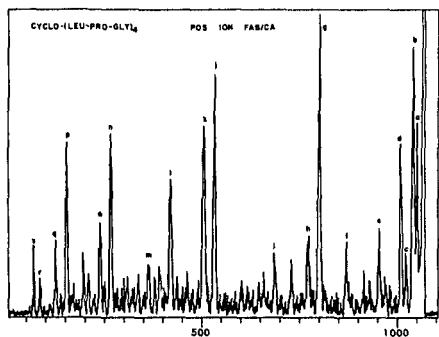
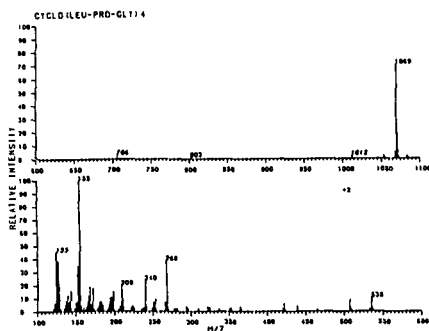
FAB MS/MS OF CYCLIC PEPTIDES, K.B. Tomer, F.W. Crow and D.L. Lippstreu, Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, NE 68588

The mass spectral analysis of underivatized cyclic peptides has been quite difficult. These compounds are often very involatile and rearrangements often occur during their electron impact induced fragmentation.

It was hypothesized that FAB would overcome the volatility problem. Often the M+H ions observed in FAB spectra do not exhibit significant fragmentation and there is often background due to the mobilizing matrix. MS/MS of the M+H ions should yield the necessary fragmentation data free from interferences and the high energy collisionally induced decomposition spectra emphasize simple cleavage processes. Thus the combination of FAB and MS/MS appeared to offer a potentially useful tool for the analysis of cyclic peptides.



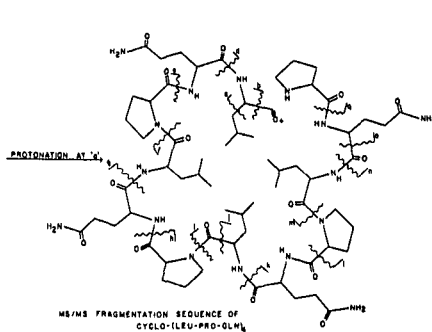
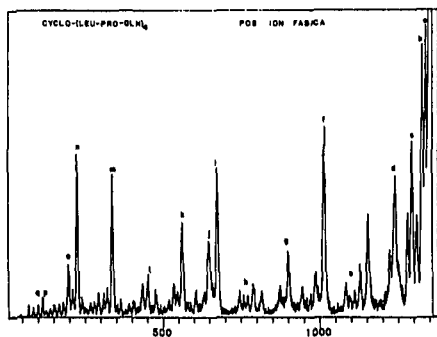
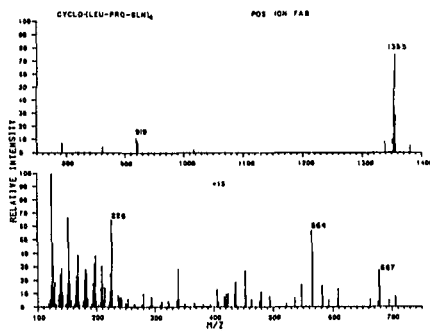
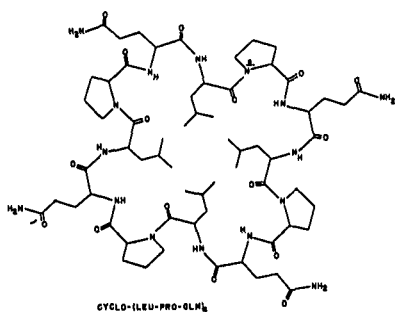
CYCLO-ILEU-PRO-GLY₁₄



To test these hypotheses, a series of cyclic peptides¹ were investigated by FAB and FAB MS/MS. The representative cyclic peptides, cyclo(Leu-Pro-Gln)₄ and cyclo(Leu-Pro-Gly)₄, their spectra and fragmentation patterns are illustrated here. In all cases the major ions of the spectrum can be explained on the basis of initial protonation on nitrogen followed by ring opening between the protonated nitrogen and the adjacent carbonyl to form a linear acylium ion. This ion then fragments by successive losses of amino acid residues. Thus the combination of FAB and FAB MS/MS is shown to be a powerful tool for the analysis of cyclic peptides.

¹The cyclic peptides were obtained from Professor Kenneth Kopple of the Illinois Institute of Technology.

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COLLISIONALLY ACTIVATED DECOMPOSITION SPECTRA OF DIPEPTIDES FOR USE IN PEPTIDE SEQUENCING; Joseph M. Marasco and Donald F. Hunt; Dept. of Chemistry, Univ. of Virginia, Charlottesville, VA 22901.

One of the many recent advances in protein sequencing methodology includes the use of dipeptidyl peptidase enzymes.¹ After digestion of the peptide sample, the resulting dipeptide products are derivitized and analyzed by GC/MS. Overlapping dipeptides are obtained when another sample of the peptide is digested after modification at the amino- or carboxyl-terminus, depending on which dipeptidyl peptidase was used. With the advent of fast atom bombardment mass spectrometric sequencing, techniques such as the above lend valuable supporting evidence to proposed amino acid sequence information, and offer the advantage of sensitivity.

In support of this methodology, we have investigated the negative ion collisionally activated decomposition (CAD) spectra of a large number of N-acyl-0-pentafluoro benzylated dipeptides. By obtaining sequence information from the CAD spectra, the need for chromatographic separations is eliminated. Dipeptides were derivitized in melting point capillary tubes or on a glass solids probe tip by treating the dried sample with a solution of d_0/d_6 (1:1) acetic anhydride (1000 fold molar excess) in aqueous methanol for three minutes at room temperature. After removal of excess reagent in *vacuo*, the acyl peptide is treated with an acetonitrile solution of diisopropyl ethyl amine and pentafluoro benzyl bromide (50 fold molar excess of each) for five minutes at room temperature followed by drying in *vacuo*. Care must be taken to avoid methyl ester formation in the acylation process, which will prevent pentafluoro benzyl ester formation and hence greatly decrease sensitivity under electron capture conditions.

Behavior of the N-acyl-0-pentafluoro benzylated dipeptides is predictable in the ion source under electron capture conditions, with loss of 181 amu (corresponding to loss of pentafluoro benzyl radical forming the carboxyl anion) for mono carboxylic dipeptides being the only ion observed. For di- and tri- pentafluoro benzyl esters, loss of 379 amu and 577 amu are the only ions observed corresponding to loss of $(C_6F_5CH_2 \cdot + C_6F_5CH_2OH)$ and $(C_6F_5CH_2 \cdot + 2 C_6F_5CH_2OH)$, respectively. Exceptions occur when either serine or threonine are present in the molecule, in which case the additional loss of 18 amu (H_2O) is observed. A wealth of sequence information is derived from the collisionally activated decomposition of the parent carboxylate anions. The major and diagnostically most useful fragmentation pathway leads to cleavage at the peptide bond, forming the anion of the C-terminal amino acid. Other major pathways include: loss of 44 amu (CO_2); loss of 86 amu (CO_2 plus ketene); formation of the acylated N-terminal amino carboxamide anion. Fragmentation dependent upon the nature of the side chains include: loss of 59 amu (acetamide), occurring in dipeptides in which the N-terminal side chain can cyclize or contains an acidic proton β to the carbonyl, as seen, for example, in asp-gly and his-leu; loss of 106 amu from tyrosine-containing dipeptides (loss of methylene quinone); loss of 30 amu and 44 amu from serine and threonine containing dipeptides respectively, (corresponding to loss of formaldehyde and acetaldehyde); and loss of 48 amu (CH_3SH) from methionine residues. Mechanistic studies suggest that several of these eliminations may occur *via* a four-membered transition state.

The above study simplifies the dipeptidyl peptidase approach to peptide sequencing, since the analysis of the dipeptides produced results in unambiguous structure assignment for dipeptides of the same molecular weight. The general fragmentation patterns noted above will free the investigator of chromatographic cataloging necessary when using GC/MS, while also providing picomole sensitivity.

¹H. C. Krutzsch and J. J. Pisano, *Methods Enzymol.*, **47**, 391-404 (1977).

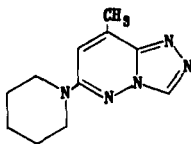
MS/MS: TRACE STRUCTURE DETERMINATION FROM COMPLEX CHEMICAL MATRICES -
APPLICATIONS TO DRUG METABOLISM

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This paper shows the applicability of tandem mass spectrometry (MS/MS) for the structural elucidation of drug metabolites directly from complex biological matrices such as urine. Yost (1) and others have suggested that by using the special operating modes of a triple stage quadrupole mass spectrometer, metabolites can be identified and their structure determined directly from plasma or urine samples.

Urine samples from a study designed to define the pharmacokinetic behavior and metabolism of a 400 mg oral dose of MDL-257 in man were examined. Data from this study



9-Methyl-6-(1-piperidinyl)(1,2,4)triazolo[4,3-b]pyridazine

MDL-257

indicated that more than 80% of the ^{14}C dose was rapidly absorbed, that the pre-systemic first pass metabolism of MDL-257 was extensive, and that from 55 to 73% of the ^{14}C dose was recovered in the urine within four hours of administration. Samples were analyzed by use of direct insertion probe on a Finnigan TSQ-45 MS/MS operated in the electron impact ionization mode.

Since MDL-257 and many of its metabolites contain common structural daughter ions, parent spectra of these daughter ions were collected. These resulting spectra contained all the parent ions including the molecular ions of possible metabolites. Additional urine samples were then run in the daughter ion mode by selecting the observed parent (molecular) ions in sequence with the first quadrupole, passing these ions through the collision quadrupole chamber, and then scanning the third quadrupole to obtain complete daughter spectra of each parent. These resulting spectra were interpreted for structural information.

In all cases the quality of the daughter ion spectra obtained from urine samples were excellent and agreed with the daughter ion spectra of pure standards.

We were able to confirm one major metabolite and identify two previously unsuspected metabolites of MDL-257.

In conclusion this study confirms the utility of MS/MS to do rapid metabolic profiling and identification directly from complex samples such as urine with minimal time for sample preparation and analysis.

References

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Positive and Negative FAB and FAB MS/MS of Natural and Modified Nucleosides, F.W.Crow, K.B.Tomer, M.L.Gross, Midwest Ctr. for Mass Spectrometry, Univ. of Nebraska, Lincoln, NE 68588; J.A.McCloskey, Dept. of Pharmaceutical Sci., Univ. of Utah, Salt Lake City, UT 84112 and D.E.Bergstrom, Dept. of Chemistry, Univ. of N. Dakota, Grand Forks, ND 58202

Nucleosides and nucleotides are members of a class of polar and thermally labile compounds which cannot be analyzed by conventional mass spectrometric methods without derivatization or modification. This research employs positive and negative ion Fast Atom Bombardment (FAB) and FAB MS/MS techniques to probe the structures of nucleosides and nucleotides. This work will be extended to mixtures of either group of compounds.

Approximately 1 ug of sample was analyzed. All data was taken using a Kratos MS-50-Triple Analyzer. All of the compounds studied produced intense MH^+ and $(M-H)^-$ ions in the positive and negative ion modes respectively. A major peak in both the positive and negative ion FAB spectra corresponds to the base portion of the molecule. Positive ion FAB produces a protonated molecule cation of the neutral base, while negative ion FAB produces the deprotonated $(M-H)^-$ ion of the neutral base. Weaker ions corresponding to the sugar portion and fragments from the sugar tend to be lost in the background generated by the FAB matrix. These same fragments are present in the CID spectrum without suffering from interferences seen in the magnet scans.

SUBSTITUTION EFFECTS: Base vs. Sugar Substitution

The base in guanosine does not fragment to any great extent. This is a result of its ring structure and unsaturation. Therefore, substituting an N-methyl for an N-hydrogen simply alters the weight of the protonated base peak in the CID spectrum. Since the sugar portion of the molecule fragments more, O-substitution yields ions which not only identify that the methyl group resides on the sugar moiety, but also indicates its position.

Substitution on the Base Portion

The positive ion FAB/CID spectra of 5- and 6-bromotubercidin are virtually identical. The only difference is the existence of an ion at $m/z=265$, the loss of HBr from MH^+ . This ion is observed for the 5- isomer and is not present in the spectrum of the 6- isomer. This implies that the hydrogen which is lost as part of the HBr is located on the amine function. When the bromine is not located near the amine one does not observe HBr loss taking place.

C-N vs. C-C Bonding

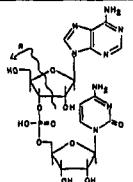
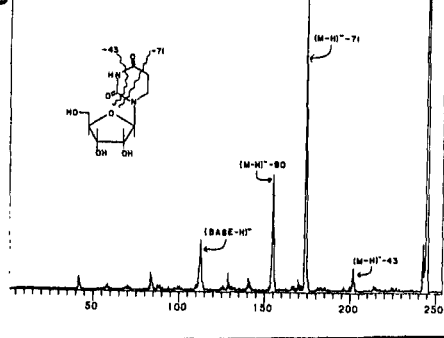
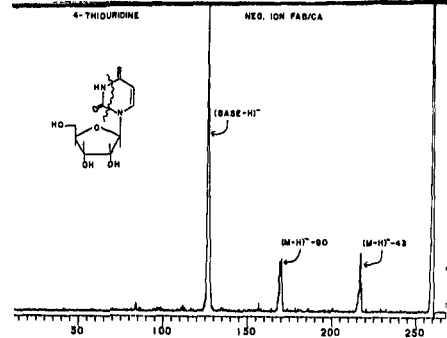
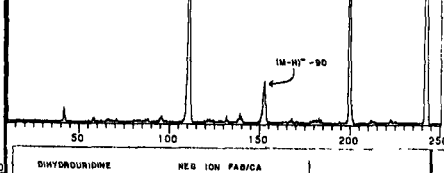
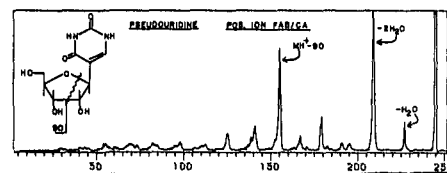
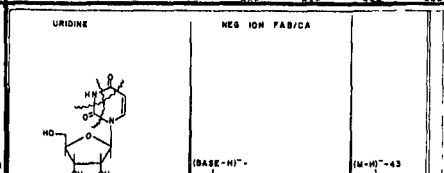
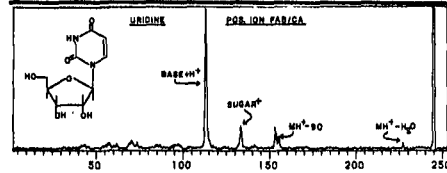
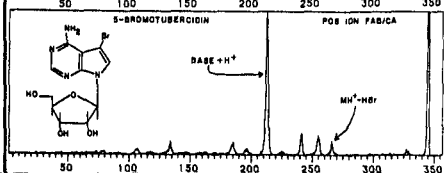
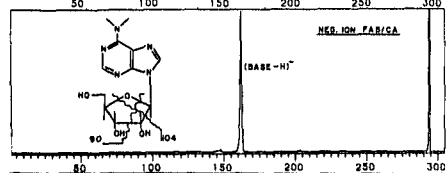
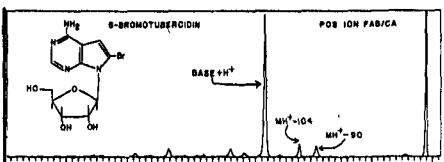
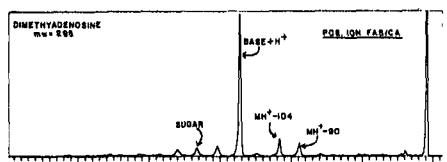
In uridine the base and sugar are bonded together by a C-N bond. This is an easily broken bond and the CID spectrum is dominated by the $Base+H^+$ ion. Other smaller ions result from fragmentation of the sugar ring. When the C-N bond is replaced by a C-C bond as in pseudouridine the CID spectrum is dominated by fragments from the sugar portion of the molecule. Loss of water molecules and the characteristic MH^+-90 ion now are the base peaks. Thus a nucleoside and a pseudonucleoside are easily distinguished.

Fragmentation of the Base

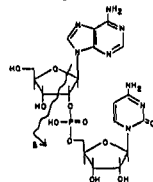
Some nucleosides contain bases which are capable of fragmenting. This has been seen in the negative ion CID spectrum of uridine which loses 43 amu from two possible fragmentation routes. The spectrum of 4-thiouridine shows clearly which route must be the dominant one since no loss of $HN-SO$ is observed. Hydrogenation of the double bond in the base of uridine opens up additional fragmentation pathways not available in the more unsaturated uridine.

Dinucleotides

The dinucleotides adenylyl 3',5'-cytidine and adenylyl 2',5'-cytidine serve as good examples of where MS/MS can be a valuable structural probe for complex non-volatile molecules. Both of these compounds produce intense but nearly identical positive and negative ion FAB and FAB/CID spectra. Only in the negative ion FAB/CID mode is any difference observed. Specific sugar ring cleavage takes place in order to retain negative charge on the phosphate moiety. These fragments alone allow one to distinguish these two molecules.



ADENYLYL 5'-CYTIDINE



ADENYLYL 5'-CYTIDINE

THE ANALYTICAL UTILITY OF METAL ION ATTACHMENT IN FAST ATOM BOMBARDMENT MASS SPECTROMETRY. J. Allison, B. Musselman, G. Dolnikowski, and J. Watson, Departments of Biochemistry and Chemistry, Michigan State University, E. Lansing, MI 48824

The usefulness of adding inorganic salts (KCl, NaCl) to solutions of analyte in glycerol has been widely documented in FAB experiments. These inorganic salts generally dissociate to generate cations and anions which combine with the analyte to form adduct species which can provide valuable information about the molecular ion of the sample and its functional groups.

The use of reactive inorganic complexes has been investigated. Silver perchlorate (a powerful oxidant) and silver nitrate have been added to the analyte in glycerol to generate silver adduct ions of the organic molecule.(Fig.1) The silver isotopes of 107 and 109 with relative abundances of 52% and 48%, respectively, generate spectra similar to those of a molecule containing a single bromine atom. Increased sensitivity has also been noted for some peptides and long chain fatty acids. This may result in the lowering of detection limits, provided that experimental parameters are optimized.

The main benefit of silver adduct ion formation lies in its simplifying the process of examining the FAB spectra of unknown compounds. Normally, when low levels of analyte are present, the varied contributions of glycerol or other matrix components can overwhelm the signal from the analyte.(Fig.2) The addition of the silver ion species results in the generation of an isotope cluster in the mass spectra (Fig.3) which is not normally found in the FAB spectra. Thus, instead of looking for single peaks in an ever-changing background one can examine the spectra for this adduct species, subtract the mass of the cation in the adduct, and subsequently obtain the mass of the appropriate molecular ion.

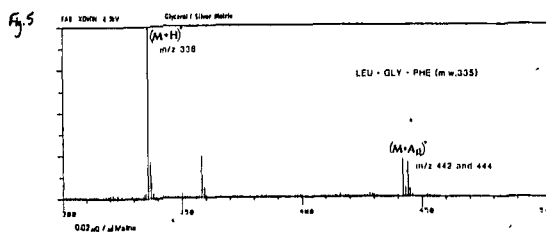
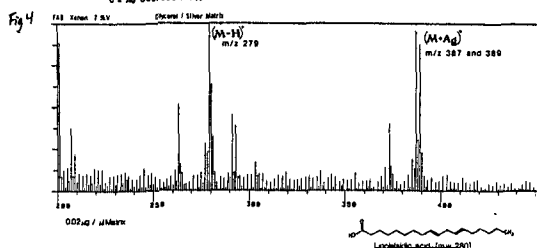
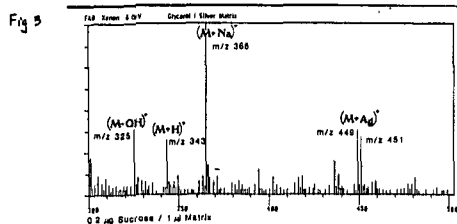
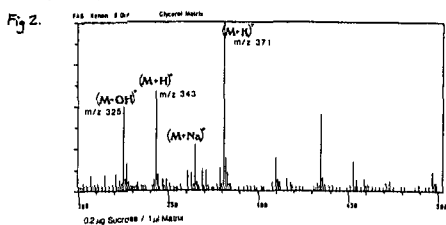
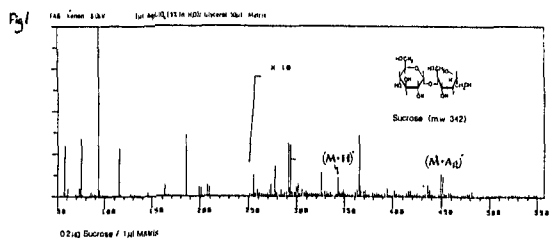
The success of this method for Ag⁺ adduct formation from FAB of Ag salts parallels results from analysis of organics on Ag surfaces by SIMS.(1).

Experimental

A matrix of 1 μ l 5% AgClO₄ in water added to 50 μ l glycerol has been found to be most useful in these experiments. Use of higher concentrations of the AgClO₄ seems to lead to an overproduction of elemental silver (m/z 107 and 109) and may chemically modify the glycerol as well as the analyte, if it is a reactive species. Lower concentrations of the salt result in low abundances of adducts. Analysis of the samples should be completed as soon as possible after addition of the (potentially reactive) salt because the usefulness of such solutions frequently decreases with time.

Representative groups of compounds have been analyzed using this methodology including fatty acids(Fig.4), peptides(Fig.5) and polysaccharides(Fig.3). All of these compounds have yielded (M + Ag)⁺ species.

1. Grade H. and Cooks R.G.; "Secondary Ion Mass Spectrometry. Cationization of Organic Molecules With Metals." J.A.C.S., 100; p. 5915; (1978).



Environmental Applications of
Triple Quadrupole Mass Spectrometry:
Aroclors in Industrial Oils,
Dioxin Screening and Hazardous
Waste Analysis

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A recent review of MS/MS applications concluded that "there are insufficient data available to establish exactly where the quadrupole MS/MS instrument's performance fits into the analytical picture."¹ We present three examples of MS/MS applications of environmental importance and we briefly discuss the significance of the Triple Quadrupole Mass Spectrometer (TQMS) from a spectroscopic and programmatic perspective.

For the applications reported here, a moving belt LC/MS interface was employed to rapidly and directly introduce microliter quantities of sample into the ion source without chromatographic separations. Using, in most cases, stable isotopic variants of analytes of interest and this introduction approach, linear calibration curves and good to excellent sensitivity are demonstrated for a variety of molecules of environmental interest. Sample throughput rates using this introduction mode in limited objective analytical strategies of approximately 50 - 150 determinations/hour are shown.

Aroclors in Industrial Oils

Aroclor mixtures introduced into the ion source without chromatography in full scan mode under positive ion electron impact and positive/negative methane chemical ionization were found to yield readily differentiable "mixture" mass spectra, for Aroclors 1221, 1232, 1242, 1248, 1254 and 1260. However, direct analysis of low level Aroclors in transformer oils requires the scanning options provided by a TQMS. In negative CI, and parent ion scanning, we could directly analyze and distinguish the higher Aroclors 1242, 1248, 1254 and 1260 at regulatory levels of interest with effectively no sample work-up (other than addition of labelled internal standards and dilution). Using this approach, complex Aroclor mixtures produce simplified mixture mass spectra, not complex chromatograms. These data suggest that such approaches are adequate for regulatory decisions.

"Dioxin" Screening

Based on our laboratory's recent involvement in a "dioxin" monitoring program, we have reported that a TQMS based screening procedure for 2,3,7,8-tetrachlorodibenzo-p-dioxin could be employed to significantly reduce the costs and decrease the response time of programs of this nature.² Low picogram sensitivity for pure 2,3,7,8-TCDD solutions is demonstrated under oxygen Townsend discharge negative ion conditions. Detection and confirmation of pg/ μ l quantities of 2,3,7,8-TCDD in the presence of ng/ μ l amounts of Aroclor 1260 by daughter ion scanning is also shown. The combination of sensitivity (negative ion mode), specificity (daughter ion scan) and high sample throughput rate via the moving belt LC/MS interface appears to provide a viable alternative to established analytical strategies.

Hazardous Waste Extract Analysis

Results from hazardous waste extracts prepared by the National Enforcement Investigation Center at Denver, Colorado, and analyzed at our laboratory by TQMS are presented. Positive and negative ion, full scan, methane CI data for "clean" and "complex" extracts spiked with selected priority pollutants is presented. In

the former case, pseudo-molecular ions (positive or negative) for all spiked compounds were readily observed. Positive ion daughter analysis of adjacent molecular ions (M/Z 139 and 140) for isophorone and 2-nitrophenol, clearly confirm the spiked analytes in the sample without chromatography. Similar analysis of the complex extract demonstrated that many of the spiked compounds were obscured in the positive ion full scan spectra; however, negative ion full scan and daughter ion techniques qualitatively confirm the presence of nitroaromatics, chlorinated phenols and large polynuclear aromatic compounds.

Full scan Q1MS positive and negative ion CI data is presented for a priority pollutant standard containing most of the extractable priority pollutants at 25 ng/ μ l/analyte to demonstrate the "best" case potential for such screening analysis, i.e., no matrix. The fact that most priority pollutants can be qualitatively confirmed down to 1 ng/ μ l/analyte and the fact that the sample volume can be significantly increased (in this introduction mode), provides the user with great flexibility in pollutant analysis of actual hazardous waste extracts.

Using a crude, but rapid, introduction technique, isotopic variants of analytes of interest, and the scanning options of a TQMS, the ability to analyze samples in limited objective analytical problems at high throughput rates is demonstrated. While instrument and sample related limitations exist, our TQMS data and our experience with large environmental monitoring programs,^{3,4} lead us to conclude that TQMS based analytical methods (for screening or confirmation) are practical. We expect that the routine application of TQMS to large environmental monitoring or testing programs will be driven both by technical and programmatic considerations.

¹Burlingame, A.L.; Dell, A.; Russell, D.H.; Anal. Chem., Vol. 54, No. 5, April 1982, 371 pp.

²Sauter, A.D.; Betowski, L.D.; Ballard, J.M.; "Rapid Analytical Methods Development, the Triple Quadrupole Mass Spectrometry Potential," presented at the Sixth Annual Symposium on Priority Pollutant Analysis, March 1983, Norfolk, VA.

³Shackleford, W.M.; Cline, D.M.; Burchfield, L.; Fass, L.; Kurth, G.; and Sauter, A.D., Computer Survey of GC/MS Data Acquired in the U.S. EPA Screening Analysis: Systems and Results, Advances in the Identification and Analysis of Organic Pollutants in Water. Chapter 33, Ann Arbor Science, 1981.

⁴Sauter, A.D., "Love Canal Study, Analytical Procedures for Organic Compounds in Soil and Sediment, June 1981.

DEVELOPMENT OF A CONFIRMATORY ASSAY FOR IVERMECTIN IN ANIMAL TISSUE USING MS/MS TECHNIQUES; J.R.B. Slayback¹, P.C. Tway², G.S. Rahn¹, R.K. Isensee¹, G.V. Downing², Oneida Research Services¹, New Hartford, NY 13413 and Merck, Sharp & Dohme Research Laboratories², Rahway, NJ 07065.

The avermectins are a new family of antiparasitic compounds which are active broad spectrum agents at very low doses. Ivermectin is a mixture of ivermectin homologues and is comprised of not less than 80% 22,23 dihydro-avermectin B_{1a} and not more than 20% 22,23 dihydroavermectin B_{1b}. Bio-availability and pharmacokinetic studies of ivermectin in animal tissue have been performed using a highly specific and sensitive chemical derivatization/LC/fluorescence assay. Because of regulatory chemical requirements a confirmatory assay was sought which preferably would yield high chemical specificity concomitant with providing positive structural information for unambiguous drug residue identification.

In the course of this work to develop a confirmatory assay for the drug in tissue, numerous analytical and MS techniques, targeted at either the intact ivermectin molecule or a structurally significant portion of the molecule were attempted. Techniques, such as FD/MS, FAB/MS, and LC/MS on the intact or aglycone portion of the molecule did not have the required sensitivity. While GC/MS analysis of the sugar moieties afforded the necessary sensitivity, the ions were not sufficiently specific. An assay based on the sugar moieties was not practical as these sugars are too volatile to carry through an assay cleanup procedure. Only solid probe/EI/MS analysis on the intact molecule had sufficient sensitivity and yielded sufficient structural information to meet regulatory requirements, but despite extensive sample cleanup, even higher resolution solid probe insertion MS (10,000 resolution at 10% valley) did not afford the necessary specificity.

Within the past several years, a newer form of MS has received much attention. This technique is based on tandem MS (MS/MS) and utilizes several stages of mass separation "on-the-fly" to improve specificity and sensitivity of detection. MS equipment utilizing MS/MS technology is centered around both magnetic sector and quadrupole-based instruments. Applications including quantitative GC/MS/MS in a pharmacokinetic assay, the use of direct insertion MS/MS in structure analysis of synthetic medicinals, and the role of MS/MS in drug metabolite screening have been published. However, to date, few studies have been reported specifically on the application of MS/MS to pharmaceutical-related problems.

In this paper we report on the development of a confirmatory assay for ivermectin employing direct exposure-positive ion-chemical ionization MS/MS methodology.

MS/MS analysis of ivermectin was performed on a Finnigan MAT 4500-based triple stage quadrupole system equipped with a Finnigan MAT direct exposure probe. The ion source was run in the direct inlet, chemical ionization mode (CI) using ammonia as reagent gas. In the MS/MS mode, chemically pure argon was used as the collision gas. Due to the energy dependence of MS/MS spectra in quadrupole instruments a separate set of experiments were performed wherein the collision energy and pressure were varied from 10-30 volts and 0.08-0.26 Pa, respectively. Extraction and tissue cleanup procedures were as previously published (Tway, et al., J. Agric. Food Chem., 29, 1981).

The analytical approach used in the development of a final protocol in complex mixture MS/MS assays is usually an iterative process. Key

variables which affect the decision making process are the complexity of the matrix, the detection limit required, and the accuracy/precision of the measurement.

Using this approach, MS spectra of ivermectin were obtained using direct exposure probe-ammonia positive ion chemical ionization. The molecular ion adduct (892) was also base peak and represents the $M+NH_4^+$ ion of the 22,23 dihydroavermectin B_{1a}. M/z 878 represented the $M+NH_4^+$ species of the 22,23 dihydroavermectin B_{1b}. Under these inlet/ionization conditions, the spectra reveal that significant fragmentation (thermal, ion-molecular) of the intact ivermectin structure did not occur.

Using the iterative process, MS/MS instrument parameters were found which when used in conjunction with the extraction/clean-up methods afforded the required detection limit and chemical specificity. Best results were obtained by using a programmed RF mode and selectively monitoring the 892+-567+ transition. Chromatograms of a 10 ppb ivermectin standard, 38 ppb ivermectin in liver (S:N 60:1) and a control liver sample were generated. Control liver samples consistently showed no chemical interference. Liver samples, however, which had not been taken through the preparative LC column clean-up step had large chemical interferences in the MS/MS analysis. The lowest level of reliable detection was 5-10 ppb.

This confirmational method developed for ivermectin analysis in liver tissue by combining wet chemical methods with MS/MS produced a highly sensitive and specific assay. Since virtually all other MS techniques failed, this work clearly demonstrated the utility and the incremental power of the tandem MS technique.

RPA 13 | MASS SPECTROMETRIC STUDY OF THE ELECTRON IMPACT IONIZATION OF CF_4 ; K. Stephan, H. Deutsch, T.D. Märk; Inst. f. Experimentalphysik, Leopold Franzens Universität, A 6020 Innsbruck, Austria

Although a number of experimental studies on the electron impact ionization of CF_4 has been reported, there remains considerable interest in the quantitative determination of partial and total ionization cross section functions, especially in view of the fact that recently properties of CF_4 have become of fundamental interest in the frame of plasma etching.

A double focussing mass spectrometer in combination with an improved electron impact ion source /1/ was used to measure relative partial ionization cross section functions and ratios for the production of CF_3^+ , CF_2^+ , CF^+ , C^+ , F^+ , CF_3^{2+} , CF_2^{2+} and CF^{2+} . Absolute partial and total ionization cross sections were determined by two different normalization methods. Using n-th root extrapolation the appearance potential of the various singly and doubly charged ions was determined. In addition, the unimolecular dissociation process $CF_4^+ \rightarrow CF_3^+ + F$ was investigated using the technique of decoupling the acceleration and analyzer fields. The various results are compared with previous measurements and theoretical calculations.

Work supported by Österr. Forschungsfonds, S-18/05 and 08 /1/ K. Stephan, H. Helm and T.D. Märk, J.Chem. Phys.,73(1980)

RPA 14 | PROPERTIES OF N_4^+ ; T.D. Märk, K. Stephan, J. H. Futrell, A.W. Castleman, Jr., H. Helm; Inst. f. Experimentalphysik, Leopold Franzens Universität A 6020 Innsbruck, Austria

Using a molecular beam-electron impact ionization - double focussing mass spectrometer apparatus the appearance energy for the reaction $(N_2)_2 + e \rightarrow N_4^+ + 2e$ was measured to be 14.80 ± 0.20 eV. From this value the exothermicity of the reaction $N_2^+ + N_2$ to form N_4^+ was found to be 0.9 eV in good agreement with previous determinations by other methods.

In addition the unimolecular dissociation process $N_4^+ \rightarrow N_2^+ + N_2$ was investigated using the technique of decoupling the acceleration and analyzer fields. Besides collision induced dissociation (with a cross section of $4 \cdot 10^{-17}$ cm² at 3 keV collision energy), the existence of a metastable dissociation process with a metastable to stable ion ratio of $5 \cdot 10^{-4}$ in the time window between 2.6 and 8.6 μ s was observed.

A comparison of these results with recent results of quantum mechanically calculated potential energy curves of $N_2 - N_2^+$ renders the conclusion that the collinear $C_{\infty v}$ geometry is the most likely configuration of N_4^+ and that the mechanism responsible for the metastable decay is electronic predissociation.

Work supported by Österreichischer Forschungsfonds, Projekt S-18/05 and S-18/08

REACTIONS; M.J. HENCHMAN, Dept. of Chem., Brandeis Univ., Waltham, MA 02254, J.F. PAULSON, Air Force Geophysics Laboratory (AFSC), Hanscom AFB, MA 01731, D. SMITH and N.G. ADAMS, Dept. of Space Research, Univ. of Birmingham, Birmingham B15 2TT, U.K.

Data are reviewed from our laboratories for the rate constants and product distributions of solvated-ion molecule reactions, as a function of solvation number. How do solvate molecules participate to influence the course of the reaction?

For proton-transfer reactions (from a cation or to an anion), just enough solvate water molecules are transferred efficiently to render the reaction exoergic, e.g., $\text{H}_3\text{O}^+(\text{H}_2\text{O})_2 + \text{NH}_3 \rightarrow \text{NH}_4^+ \cdot \text{H}_2\text{O} + 2\text{H}_2\text{O}$. In contrast, for nucleophilic displacement reactions, solvate water molecules are not transferred efficiently and solvation reduces the rate constant, e.g. $\text{OH}^-(\text{H}_2\text{O})_2 + \text{CH}_3\text{Br} \rightarrow \text{Br}^- + \text{CH}_3\text{OH} + 2\text{H}_2\text{O}$.

For solvate molecules to separate from the site of positive or negative charge, energy is required and this expenditure will influence the reaction. For proton transfer, solvate molecules can simply move with the charge, as the proton is transferred. For nucleophilic displacement, the charge is translocated through the backbone of the intermediate: concomitant transfer of solvate around the periphery of the intermediate is not so facile.

OBSERVATION OF LARGE "MAGIC NUMBERS" FOR THE CLUSTERING OF NITROGEN MOLECULES AND ARGON ATOMS*; R. J. Beuhler and L. Friedman; Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973.

Expansion of gases mixed with seed ions through a supersonic nozzle has been shown to be a simple means of forming cluster ions in the molecular weight range extending up to 100,000. The techniques pioneered in this laboratory, including low frequency quadrupole mass spectrometry and high voltage post acceleration detection of ions, allow rapid analysis of these large cluster ions. With Argon or Nitrogen in the concentration range 5-10% in Helium carrier gas at 77°K, mass distributions were observed which were not smooth but had local maxima corresponding to "magic numbers" of atoms or molecules. A comparison of the Nitrogen and Argon cluster distributions showed that these "magic numbers" for either the number of Nitrogen molecules or Argon atoms in the cluster are virtually identical, and correlated well with the packing of spheres into closed-shell icosahedra. Large maxima were observed at the following magic numbers: 309, 561, and 923, corresponding to the completion of the 4th, 5th, and 6th shells respectively. Smaller peaks corresponding to differences of 17 to 20 monomer units were observed between the magic number peaks, and may indicate the formation of small neutral species or "sub clusters" which then aggregate on the ionic species during expansion. Further implications of these observations to the dynamics of the clustering process will be discussed.

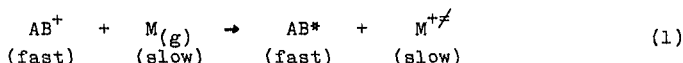
*Research performed at Brookhaven National Laboratory under Contract with the U. S. Department of Energy.

NEUTRALIZED ION-BEAM SPECTROSCOPY

G.I. Gellene, N.S. Kleinrock, R.F. Porter

Department of Chemistry, Cornell University, Ithaca, NY 14853

Neutralized ion-beam spectroscopy¹ is a technique for studying modes of energy disposal in a species produced in an electron transfer process:



where AB^+ is any diatomic or polyatomic ion and M is a thermalized target atom. The state of AB^* produced is strongly dependent on the ionization potential of the target. In this laboratory the metals Cs, K, Na, Ca, Mg, and Zn, spanning a range of ionization potentials of 3.9 eV to 9.4 eV, have been employed. Further the state of AB^* can be characterized as dissociative, predissociative, or stable by observing the angular distribution of its neutral beam profile ($\text{I}(\Theta)\text{Vs } \Theta$). Dissociative states ($\tau < 10^{-13}\text{s}$) are identified by structureless broadening of the beam profile, while production of a stable state ($\tau > 10^{-5}\text{s}$) gives rise to a beam profile with a spacial distribution typical of the primary ion beam. Predissociative states can be identified in some cases by structure in the broadened beam profile reflecting the presence of a predissociative barrier.

Information obtained by this technique can be divided into five areas:

I. Excited products exhibiting radiative emission

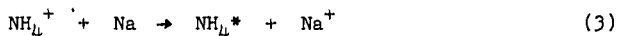
In a combined optical spectroscopy and beam scattering study of the reaction



radiative emission is observed from electronically excited products.² The predominate radiating products are excited metal ions when targets of relatively high ionization potentials (Mg and Zn) are used, while excited molecular hydrogen is produced when K is the target. A major conclusion of this work is the demonstration that in the range of ion velocities studied ($3\text{--}7 \times 10^7 \text{cm/sec}$), reaction (2) occurs under near resonant conditions with vertical transitions.

II. Neutrals produced in metastable ground states

The stability of the ammonium radical $(\text{NH}_4)^{\cdot}$ ³ was determined from measurements of the kinetic energy released in its fragmentation products following formation in a fast electron capture process:

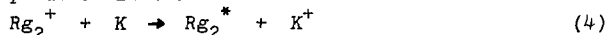


Scattering profiles for heavy (NH_3) and light (H) dissociation products were obtained from beam measurements with 5-16 keV NH_4^+ ions. The existence of a predissociative barrier in the ground state of the radical is inferred from the structure and scattering continua in H atom profiles. The radical is bound

with respect to a potential minimum but all of the isotopic species NH_4 , NH_3D , NH_2D_2 , NHD_3 undergo rapid loss of H atoms and have ground states lying above their dissociation limit. The radical ND_4 has unusual stability with its ground state lying close to or below its dissociative limit.

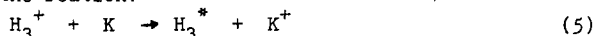
III. Neutrals produced in metastable excited states

The rare gas dimers produced in the reaction:



are formed primarily in their excited excimer states. While excited singlet states are expected to rapidly radiate to the essentially repulsive ground state ($\tau_{\text{rad}} \sim 10^{-8}\text{s}$), excited triplet states will survive longer. For $\text{Rg} = \text{He}$, Ne , and Ar some metastable triplet states of Rg_2^* survive after the $\sim 1\text{ }\mu\text{s}$ flight time and are detected.

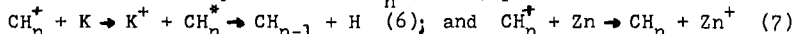
A second interesting example of this case is provided by triatomic hydrogen radicals produced in the reaction:



Herzberg has recently identified emission spectra from several excited Rydberg states of H_3^+ and his analysis of the spectra indicates that the first two excited Rydberg states, the $2s$, $2A_1'$ and the $2p$, $2A_2''$, are predissociative. As predissociation of the $2p$, $2A_2''$ state occurs through the species of a rotation, it is rigorously forbidden for the non-rotating molecules. Neutral beam profiles obtained following reaction (5) show that a fraction of the H_3^* produced does not dissociate prior to detection. These molecules are identified as the non-rotating metastable $\text{H}_3^+(2p, 2A_2'', N=K=0)$.

IV. Neutrals produced in stable ground and excited dissociative states

By employing a target of an appropriate ionization potential in reaction (1) it is possible to produce neutrals primarily in either their ground or excited state. The small hydrocarbons CH_n ($n=1-4$) produced in the reactions:



are examples of this type of molecule.⁵ The energy released in reaction (6) can be used to locate the excited state, while reaction (7) can be used to determine the existence of a bound ground state for species of unknown stability.

V. Neutrals produced from a mixture of ion isomers

Upon ionization, some species are produced in a mixture of isomeric states. Since in general, the different isomers will be associated with different energies, beam profiles obtained with an appropriate target will also be different and can serve as "finger prints" of the isomers. This is the case for the ethyl cation⁶ which was found to be in an equilibrium mixture of its bridged and classical structures.

References:

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The negative ion-neutral collision processes,



are of considerable interest because of their importance in the chemistry of the upper atmosphere¹. Molecular beam studies are highly effective in elucidating the mechanics and dynamics of such ionic collision processes, although very little information bearing on the collision dynamics of negative ion-neutral reactions has actually been obtained. Thus far, only one such study, concerned with the reaction $O^- + H_2 \rightarrow OH^- + H$, has been reported in the literature², and this reaction was also studied previously as a function of incident ion energy, with somewhat conflicting results³⁻⁵. No such information has been reported for reaction (3). In order to better understand these important O^- interactions, we have reinvestigated these processes more extensive in the low interaction energy regime, using both a tandem mass spectrometer and a recently developed crossed ion-molecular beam apparatus.

An in-line tandem mass spectrometer and a crossed ion-molecular beam apparatus were used for the present experiments. The tandem mass spectrometer has been described in detail previously⁶. The crossed ion-neutral beam apparatus utilized here is designed to study the dynamics and energy transfer characteristics of ion-neutral scattering processes⁷. The first stage mass spectrometer and deceleration lens produce a mass and energy-analyzed ion beam, which is crossed at 90° by a chopped supersonic molecular beam. The product ions formed from interaction of these two beams are then energy and mass analyzed and counted by a secondary analyzer which rotates in the horizontal plane about the scattering center, from -10° to $+60^\circ$. The projectile ion, O^- , was produced in these studies by electron impact on N_2O . Presumably, O^- is formed by dissociative electron attachment and is believed to be formed in the ground state⁸.

1. Measurements of Total Cross Sections

Excitation functions (that is, cross section as a function of ion kinetic energy) were measured for reactions (1), (2), and (3). The results indicate that, in the near thermal energy region, reaction (1) is the dominant reaction channel, but as the energy of the system increases, reaction (2) becomes dominant, and reaction (3) also becomes more significant. Similar results were obtained for the reactions of O^- with D_2 . No isotope effect was observed for the associative detachment reaction, (1), as indicated by the fact that the rate coefficients for the reactions of O^- with both H_2 and D_2 , to yield $H_2O + e$ and $D_2O + e$, were equal, within the experimental uncertainty. An isotope effect of about 2 was observed for reaction (2), the production of OH^- being favored over production of OD^- . The rate coefficients measured for reaction (2) are in excellent agreement with those determined from drift tube experiments⁹. For reaction (3), thresholds at 0.58 ± 0.1 and 0.62 ± 0.1 eV were observed for the H^- and D^- product channels, respectively, and the cross sections of both reactions increase rather sharply from the thresholds, attaining a maximum of $0.8 \text{ \AA}^2$ at 0.9 eV for H^- , and a maximum of $0.7 \text{ \AA}^2$ at 1.2 eV for D^- . To our knowledge, this is the first reported measurement of this excitation function for this reaction channel.

2. Collision Dynamics

Differential cross sections for reaction (2) were measured at several different collision energies, at various angles with respect to the collision center. At each angle, the product ion energy distributions were also measured by repetitively scanning a retarding potential energy analyzer. The energy distributions of the product ions (OH^- and OD^-) were determined by using a least squares best fit to the retarding potential curves, followed by a differentiation of this curve. A contour diagram, plotted in Cartesian velocity space,¹⁰ where each curve represents a constant product probability $P(V_x, V_y, V_z)$ was then constructed. For a reaction which involves a long-lived intermediate complex mechanism, the product contour is expected to be roughly centered around the center of mass. In the present study, a typical contour diagram obtained for reaction (2) at a relative energy 1.8 eV is shown in Fig. 1. This diagram shows that the product contour is centered in a region which is located forward of the center mass, indicating that the lifetime of any H_2O^- intermediate complex which is formed is relatively short. The potential energy surfaces for the reactions studied here have been calculated by Taylor et al.¹¹ The latter results show that there is a well in the H_2O^- surface, which lies above the well in the surface for H_2O . This implies that H_2O^- is unstable with respect to detachment. Apparently the lifetime of the immediate H_2O^- complex

is less than several rotation periods for reaction (2) at relative energies 1.8 eV, and thus, this reaction proceeds via a direct mechanism in this energy regime. Similar results were obtained for the reaction of $O^- + D_2$. The present results differ somewhat from those of Johnson et.al.², who reported that at energies below 2.5 eV, the product distribution is centered about the center of mass.

This work was supported by the U.S. Dept. of Energy under contract No. DE-AC02-80ER-10668 with Wright State University.

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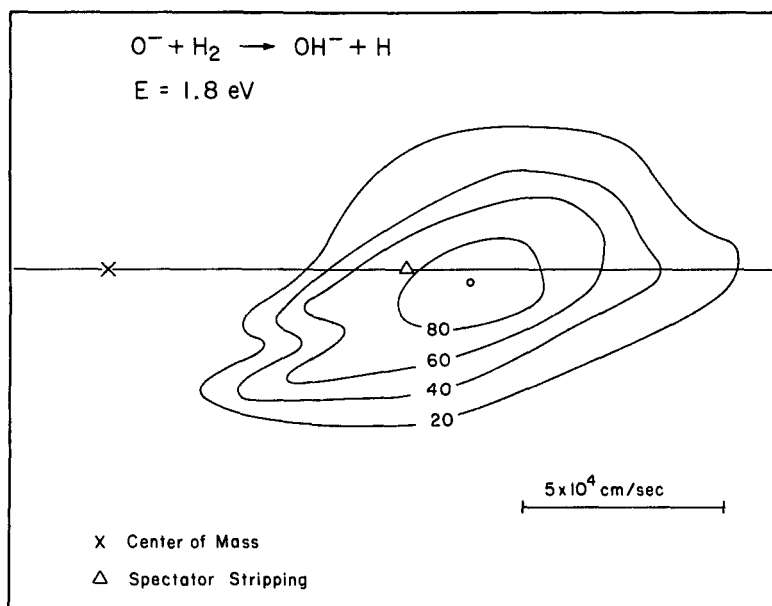


Figure 1. Cartesian Contour Of Product Intensity For The Reaction $O^- + H_2 \rightarrow OH^- + H$ At A Relative Energy Of 1.8 eV (X- Center Of Mass, Δ-Spectator Stripping); The Contours Are At 20% Intervals With Peak At 100.

RPA 19 | $\text{OH}^-(\text{H}_2\text{O})_{0,1,2} + \text{CH}_3\text{X} (\text{X} = \text{Cl}, \text{Br})$; EFFECT OF HYDRATION ON NUCLEOPHILIC DIS-
PLACEMENT AND ITS COMPETITION WITH PROTON TRANSFER;
M.J. HENCHMAN, Dept. of Chemistry, Brandeis Univ.,
Waltham, MA 02254, P.M. HIERL, Dept. of Chemistry,
Univ. of Kansas, Lawrence KS 66045 and J.F. PAULSON,
Air Force Geophysics Laboratory (AFSC), Hanscom AFB,
MA 01731

Nucleophiles can undergo both proton transfer and nucleophilic displacement; but displacement occurs only when proton transfer is endoergic. For OH^- and methyl chloride (bromide), nucleophilic displacement alone is seen at thermal energies: $\text{OH}^- + \text{CH}_3\text{X} \rightarrow \text{CH}_3\text{OH} + \text{X}^-$. However, at relative energies above the threshold for proton transfer, proton transfer competes effectively with nucleophilic displacement: $\text{HO}^- + \text{CH}_3\text{X} \rightarrow \text{HOH} + ^-\text{CH}_2\text{X}$. Proton transfer within the intermediate, to give $\text{HOH} \cdot ^-\text{CH}_2\text{X}$, is exoergic and can occur at all energies; to complete the reaction, which is endoergic, energy must be provided to separate the products. Hydration of the nucleophile, $\text{OH}^-(\text{H}_2\text{O})_n$, $n = 1, 2$ quenches the proton-transfer channel, because the hydration causes the proton-transfer within the intermediate, to give $(\text{HOH})_{n+1} \cdot ^-\text{CH}_2\text{X}$, to become endoergic.

The products seen for the nucleophilic displacement reaction in the gas phase $\text{OH}^-(\text{H}_2\text{O})_n + \text{CH}_3\text{X} \rightarrow \text{X}^- + \text{CH}_3\text{OH} + n\text{H}_2\text{O}$ contrast with those in solution $\text{X}^-(\text{H}_2\text{O})_n + \text{CH}_3\text{OH}$ and illustrate the difference between solvated-ion reactions in the gas phase and those in solution.

PYROLYSIS OF IONS. PROTONATED ETHERS. L. Wayne Sieck and Michael Mautner (Meot-Ner)
National Bureau of Standards, Washington, D.C. 20234.

The thermal activation and subsequent unimolecular decomposition kinetics of some protonated ethers have been investigated by variable-temperature pulsed high pressure mass spectrometry. The basic method involves creation of the protonated ether, $(ROCH_3)^+$, thermal activation by multiple collision with a bath gas (usually cyclohexane), and subsequent time-resolved analysis of the rate of elimination of CH_3OH ; $(ROCH_3)^+ \rightarrow R^+ + CH_3OH$ (k_d). Experimentally, either of two types of kinetics are observed (see Fig. 1). Case I indicates irreversible production of an unreactive fragment ion FH^+ . Case II involves reversible kinetics. In Case II, the fragment ion produced by pyrolysis can protonate the parent ether. A chain reaction results, and a steady-state is achieved at longer reaction times.

In Case II, the rate constant is evaluated either during the approach to the steady-state, or after the steady-state has been achieved. The latter requires that the rate constant for $FH^+ + M \rightarrow MH^+$ (k_2) is known. k_d is then given by

$$k_d = \frac{k_2[M]}{(MH^+/FH^+)_{ss}}$$

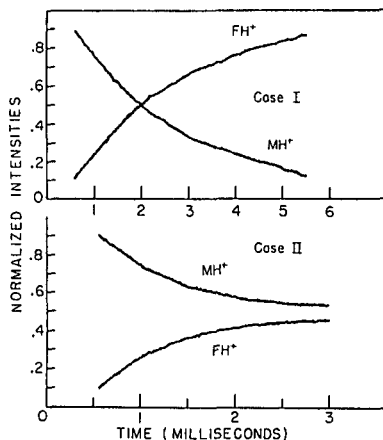


Figure 1

Activation Energies. In simple di- and tri-ethers, with cyclohexane as the bath gas, the high pressure limiting rate constant, k_d , can be measured over a substantial temperature range (Fig. 2). Then, using the formulation $k_d = A \exp(-E_a/RT)$, the true activation energy and A factor for the decomposition can be defined from an Arrhenius plot (Fig. 3). For $(CH_3OCH_2)_2H^+$, $E_a = 21.1 \text{ kcal}\cdot\text{mol}^{-1}$ and $\log A = 11.1$; for $[(CH_3OCH_2CH_2)_2O]^+$, $E_a = 30.7 \text{ kcal}\cdot\text{mol}^{-1}$ and $\log A = 13.7$. To the best of our knowledge, these are the first measurements of this type ever reported for the gas phase decomposition of cations at the high pressure limit.

Activation Efficiencies. In $t\text{-C}_4\text{H}_9\text{OCH}_3$, the dissociation is so rapid that a limiting high pressure value for k_d cannot be obtained; $(t\text{-C}_4\text{H}_9\text{OCH}_3)^+ \rightarrow t\text{-C}_4\text{H}_9 + CH_3OH$. In fact, at temperatures $> 515\text{K}$, k_d is linear with pressure up to 0.5 - 0.6 torr, and the system is at the low pressure limit. Under these conditions, one can evaluate the efficiencies of various bath gases as thermal activators. The relative values of k_d /pressure at constant temperature (518K in Fig. 4) define the relative activation efficiencies. This is true if the low pressure limiting rate constants for all of the various bath gases exhibit the same temperature dependence, which is apparently the case. After correcting for differences in collision rates among the various diluents, the following relative thermal activation efficiencies are obtained ($\text{CO}_2 = 1.00$): $\text{c-C}_6\text{D}_{12}$, 3.89; $\text{c-C}_6\text{H}_{12}$, 3.76; C_2H_6 , 1.79; N_2O , 1.14; CO_2 (1.00); CH_4 , 0.62; Xe , 0.61; Kr , 0.55; CO , 0.55; and N_2 , 0.40. Unfortunately, polar bath-gases cannot be studied due to successive clustering reactions.

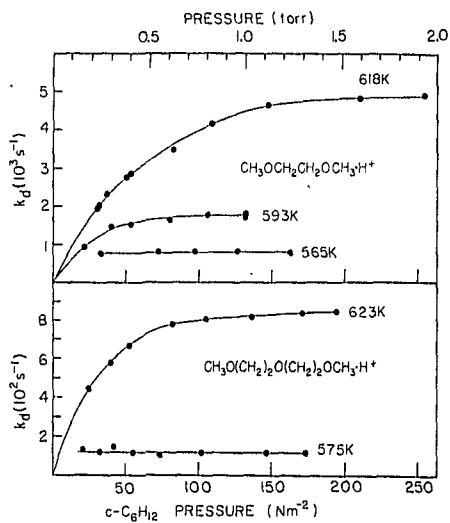


Figure 2

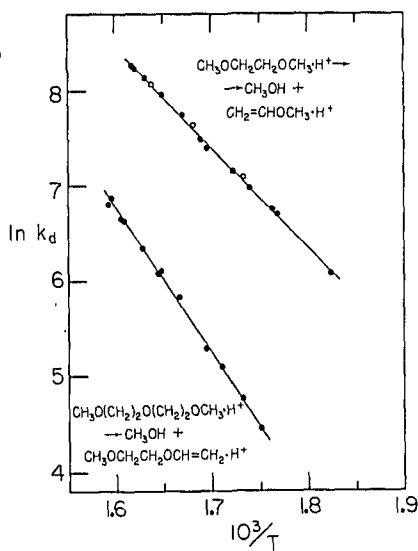


Figure 3

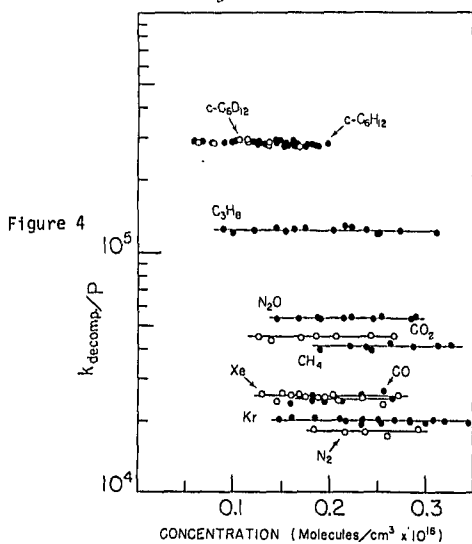


Figure 4

This work was supported by the Office of Basic Energy Sciences,
U.S. Department of Energy.

In dimers $BH^+...A$, an inverse linear correlation is found between the bond dissociation energy ΔH_D^0 and the difference ΔPA between the proton affinities of the proton donor B and the proton acceptor A. The correlation applies for 48 $NH^+...O$ dimers including a series with varying A and constant B, i.e., hydrates of ammonium and pyridium ions ($NH^+...OH_2$); a series with varying B and constant A, i.e. complexes $CH_3NH_3^+...O$; and other dimers with ΔPA values varying from 9 to 70 kcal mol⁻¹, and bond energies from 27 to 12 kcal mol⁻¹. The correlation $\Delta H_D^0(\text{kcal mol}^{-1}) = \Delta H_D^0(0) - b\Delta PA$ for $NH^+...O$ bonds yields the parameters $\Delta H_D^0(0) = 30.0 \text{ kcal mol}^{-1}$ and $b = 0.26$. Analogous linear correlations are observed for $NH^+...N$ bonds in a set of 8 ammonium ion dimers, and for $-OH^+...O-$ bonds in a set of 21 oxonium ion hydrates, with parameters $\Delta H_D^0 = 23.2$, $b = 0.25$, and $\Delta H_D^0 = 30.4$, $b = 0.30$, respectively. Analogous linear correlations apply also for $NH^+...S$ and $SH^+...O$ bonds. The correlations are in accord with trends predicted by ab initio calculations of Desmeules and Allen. The correlations are reliable predictors of $BH^+...A$ energies within experimental error limits.

TABLE I
 Correlation Between ΔH_D^0 and ΔPA for $-XH^+...Y-$ Bonds.
 Parameters for the Least-Squares Lines $\Delta H_D^0 = a - b \times \Delta PA$.^a

Bond	a	σ_a^b	b	σ_b^b	$\sigma_{\Delta H_D^0}^b$	N ^c	coefficient ^d
NH ⁺ ...OH ₂ ^b	30.0 ± 1.5		0.26 ± 0.03	1.0	19	0.923	
NH ⁺ ...O	28.3 ± 0.3		0.23 ± 0.01	1.1	48	0.979	
NH ⁺ ...N	23.2 ± 0.7		0.25 ± 0.05	1.2	8	0.897	
OH ⁺ ...OH ₂	30.4 ± 0.4		0.30 ± 0.01	0.8	20	0.979	
SH ⁺ ...OH ₂	22.7 ± 0.3		0.26 ± 0.01	0.3	4	0.998	
NH ⁺ ...S	22.3 ± 0.9		0.26 ± 0.03	0.8	5	0.970	

- (a) All enthalpies in kcal mol⁻¹.
 (b) Standard deviation of intercept (σ_a), of slope (σ_b), and standard deviation ($\sigma_{\Delta H_D^0}$) of experimental ΔH_D^0 values from calculated values based on least-squares equation.
 (c) Number of points in correlation.
 (d) Correlation coefficient.
 (e) Based on hydrates 1-19, Table I.

Deviations from these correlations may occur due to structural effects. For example, the hydration of the protonated amino acid derivative in $\text{CH}_3\text{COH}^+\text{NHCH}(\text{CH}_3)\text{COOCH}_3 \cdot \text{H}_2\text{O}$ is weakened ($\Delta H_D^\circ = 12.4 \text{ kcal mol}^{-1}$) vs. that of a comparable amide $\text{CH}_3\text{COH}^+\text{N}(\text{CH}_3)_2 \cdot \text{H}_2\text{O}$ ($\Delta H_D^\circ = 16.5 \text{ kcal mol}^{-1}$), due to the partial blocking of the ion- H_2O hydrogen bond by the intramolecular bond.

The above correlations can predict the solvation enthalpies of oxium ions by one solvent (e.g., H_2O) molecule. As to multiple solvation, the relation between enthalpies of solvation of onium ions BH^+ by one water molecule, $-\Delta H_{0,1}^\circ$, and by four water molecules, $-\Delta H_{0,4}^\circ$, is constant for most onium ions. These relations, in conjunction with the correlation between $\Delta H_{0,1}^\circ$ and the proton affinity difference $\Delta \text{PA} = \text{PA}(\text{B}) - \text{PA}(\text{H}_2\text{O})$, allows the prediction of the total four-molecule specific hydration energy $-\Delta H_{0,4}^\circ$ for all onium ions within the experimental accuracy of $\pm 3 \text{ kcal mol}^{-1}$. Specifically, the ratio of the integrated hydration energy by the first four H_2O molecules (i.e., the specific hydration energy) to the attachment energy of the first H_2O molecule, $H_{0,4}^\circ/H_{0,1}^\circ$ is $2.8 \pm .1$, for all oxonium ions and monoprotonic ammonium and pyridinium ions, and $3.1 \pm .1$ for polyprotonic ammonium ions. Further, the differences between the total four molecule hydration enthalpies $\Delta H_{0,4}^\circ$ of various BH^+ ions are equal to the differences between aqueous water hydration enthalpies for most ions. In other words, for these ions the variation in bulk solvation enthalpies is determined completely by the variation in specific hydrogen bonding interactions.

In summary, the correlations between $\Delta H_{0,1}^\circ$ and ΔPA predict the enthalpies of solvation of onium ions by one H_2O molecule. This value in turn predicts the enthalpy of solvation by four H_2O molecules. The differential four-fold solvation enthalpies are then good predictors of differential bulk solvation for many onium ions. Thus for many onium ions the differential bulk solvation enthalpies may be estimated quantitatively on the basis of the proton affinities. For ions that do deviate from these correlations the deviations can be used to identify structural effects on ion solvation.

RPA 22 | INTERACTIONS OF Fe^+ , Ni^+ , AND Co^+ WITH
CYCLIC COMPOUNDS: THE STRUCTURE OF $\text{CH}_3\text{-Fe}^+\text{-CH}_3$
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The reaction of Fe^+ with alkanes normally involves cleavage of carbon-carbon bonds. However, Fe^+ shows anomalous reactivity with cyclo-alkanes. Five and six carbon cyclic alkanes react exclusively through a hydrogen loss mechanism. Eight and nine carbon alkanes react by cleavage of C-C bonds with loss an alkene moiety. Both reaction pathways contribute in the reaction with cyclo-alkanes with ten carbons or more. Collision-induced decomposition studies of Fe^+ -cycloalkane complexes, generated by the reaction:



produce comparable results.

Fe^+ normally undergoes oxidative addition in reaction with alkanes by insertion into a C-C bond with an accompanying β -hydrogen shift. If it is assumed that the basic structure of the intermediate, $\text{RCH}_2\text{-Fe}^+\text{-CH}_2\text{R}$, is linear with Fe-C bond lengths of about 2.0 Å, the following anomalous results of the cyclic alkanes can be explained. The formation of this intermediate with small (5 and 6 carbons) cyclo-alkanes is prevented by ring strain. Fe^+ insertion into intermediate size (8 or 9 carbon) cyclo-alkanes occurs but a β -hydrogen shift to the metal center is impeded by restriction of rotation about the α - β carbon-carbon bond, inhibiting hydrogen loss. Neither mechanism is constrained in larger cyclo-alkanes intermediates and both hydrogen and alkene loss are observed.

The reactions of Ni^+ and Co^+ differ in product distributions and may produce intermediates of different structure.

ION CHEMISTRY OF *n*-BUTANOL

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While the ion chemistry of simple aliphatic alcohols has been investigated extensively, little work has been carried out thus far on *n*-butanol. We report here on the temporal variation of major ion abundances in the *n*-butanol system at 8×10^{-5} Torr, the observation of ions with *m/z* values in excess of that of the protonated dimer, and slow infrared multiphoton induced dissociation (IRMPD) of protonated dimers of *n*-butanol observed in the pressure range $2-8 \times 10^{-5}$ Torr.

Observations were made using a quadrupole ion store (QUISTOR) mounted in tandem with, and abutting the source of, a quadrupole mass filter (QXK400, Vacuum Generators). The apparatus has been described extensively elsewhere [1-3]. Low intensity (8W) CW CO₂ laser radiation at 944 cm^{-1} was chopped and directed radially through the centre of the ring electrode of the QUISTOR. Pulse sequences for ion creation, irradiation, ejection and detection have also been described previously [1,3].

n-Butanol at 8×10^{-5} Torr was subjected to electron impact for 90 μs ; ions were stored in the QUISTOR and sampled for periods of up to 200ms. Ion profiles which represent the temporal variations of the percentage ion composition are shown in Fig. 1.

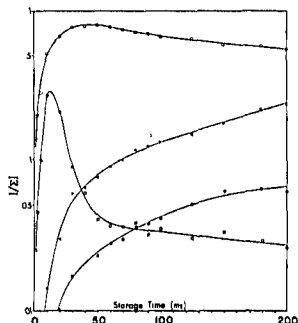
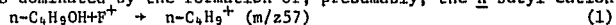
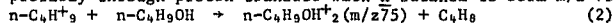


Fig. 1 Abundance of selected ions formed by ion/molecule reactions of *n*-butanol stored for 200ms in a QUISTOR. O, *m/z* 57 butyl cation; Δ , *m/z* 149 protonated dimer; $\bullet$, *m/z* 131 dehydration product of *m/z* 149; $\square$, *m/z* 75 protonated *n*-butanol. 550V (q-p), 1.25 MHz, 8×10^{-5} Torr.

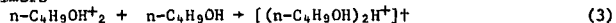
The system is dominated by the formation of, presumably, the *n*-butyl cation, *m/z* 57.



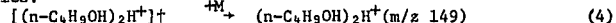
where F⁺ represents fragment ions of *m/z* 39, 41, 43, 55, and 56. The maximum relative abundance of *m/z* 57 was reached at 50ms of storage, and thereonafter declined slowly and continually, probably through proton transfer with *n*-butanol to form *m/z* 75



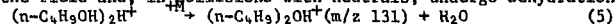
Reaction 2 is slightly endothermic. Protonated *n*-butanol attained a maximum intensity after 15ms of storage, then decreased relatively rapidly due to the formation of protonated dimers



which are then collisionally (or radiatively) stabilized. † denotes a vibrationally excited species.



Protonated dimer ions were equal in intensity to that of *m/z* 57 at 500ms and, at longer storage times, became dominant. A fraction of dimer ions may acquire translational energy from the field and, in collisions with neutrals, undergo dehydration to form *m/z* 131.



Though this process is normally endothermic, *m/z* 131 accounted for 6% of the observed ion intensity after 200ms of storage. Minor stable ions of *m/z* 87, 115, 117, 119 and 175 were observed at long storage times.

An APPLE IIe computer interfaced with an ISAAC has been used for signal averaging, and has been applied initially to the detection of ions in the *n*-butanol system with *m/z* greater than 149. Several ion species were observed in the range *m/z* 155-300. Peaks at *m/z* 223 and 297 may be due to the protonated trimer and tetramer, respectively. Evidence

for the identification of m/z 223 as the protonated trimer and m/z 205 as its dehydration product was obtained by IRMPD.

IRMPD of protonated dimers of n -butanol, $(n-C_4H_9OH)_2 H^+$, m/z 149, requires 131 kJ mol^{-1} [4] when the dimers are fully stabilized; thus the absorption of at least 11 photons at 944 cm^{-1} is necessary for dissociation in the absence of collisional and radiative quenching. Slow IRMPD has been demonstrated to be a sensitive probe of the lowest E_a pathway of a species.

Within the QUISTOR, ions migrate to the centre from whence they are extracted efficiently and analyzed or are dissociated by a single pass of the CO_2 laser. Protonated dimer ions formed from n -butanol at 8×10^{-5} Torr are readily susceptible to MPD as shown in Fig. 2.

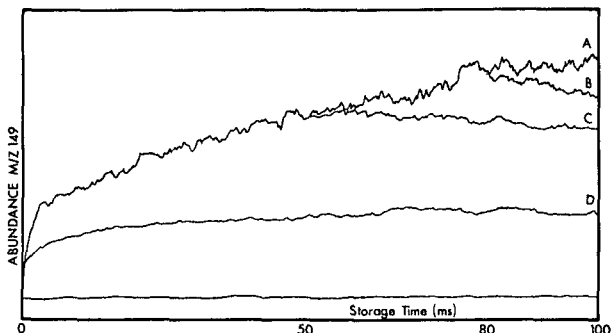
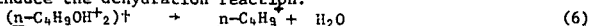


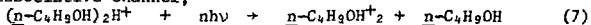
Fig. 2 Single ion monitored profiles of protonated dimer ions of n -butanol, m/z 149, during 100ms of storage. A, abundance of protonated dimer arising from thermal (or dark) reactions; D, abundance of protonated dimer under continuous laser irradiation; B and C, abundance of protonated dimer with laser irradiation during the final 20ms and 50ms of storage, respectively. The laser was operating at 944 cm^{-1} and 8.9 W

The fraction of m/z 149 ions dissociated in each of the three periods of irradiation increased from 0.08 for 20ms, 0.17 for 50ms to 0.57 for 100ms. Similarly the rates of dissociation increased in the ratio 1:2:5.4 for the above irradiation periods. Thus it is seen that MPD of nascent ions is more facile than for stabilized ions.

Irradiation during 20ms following 80ms of ion genesis and stabilization resulted in 32% decrease in m/z 149 and a corresponding increase of m/z 75. There was no evidence for photoreaction pathways corresponding to either dehydration or loss of alkene from the protonated dimer. The absence of these photodissociative pathways in the n -butanol system contrasts sharply with the behaviour observed in C_3 and other C_4 alcohols [5]. Irradiation during 50ms following 50ms of ion genesis produced enhancement of both m/z 75 and m/z 57. That m/z 57 is derived from m/z 75 was demonstrated by irradiation with concomitant resonance ejection [6] of m/z 149. Thus vibrational excitation of protonated n -butanol may induce the dehydration reaction.



Multiphoton absorption by excited nascent protonated dimer ions can lead to dissociation via pathways other than that of the lowest activation energy [7]. Continuous irradiation yielded enhanced intensities of m/z 75 and 57 as anticipated. We conclude that there is but one photodissociative channel,



for stabilized protonated dimers and that the photoproduct, protonated n -butanol, has insufficient internal energy for reaction 6 to occur. This conclusion is supported by the observation of reaction 7 when Argon/ n -butanol mixtures in the ratio 10:1 were irradiated.

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THE REACTIONS OF VINYL IONS WITH METHANE

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Isotope labelling studies of the reaction of methyl cations with ethylene, at a barycentric energy of 1.8 eV, have suggested that the reaction proceeds via a long-lived collision complex,¹ although, in sharp contrast to the case of the reaction of silyl ions with ethylene,^{2,3} no $C_3H_7^+$ was detected. The explanation advanced for the failure to observe the $C_3H_7^+$ collision complex was that, no matter how low the kinetic energy, the nature of the potential energy profile assured that the collision complex contained sufficient excitation energy to make the lifetime significantly shorter than the transit time of the apparatus. This explanation suggested that the same $C_3H_7^+$ collision complex might be directly detectable in the reaction of vinyl ions with methane.

All experiments were carried out in an ion-beam-scattering gas apparatus that has been described previously.³ $C_2H_3^+$ and $C_2D_3^+$ from ethylene and ethylene-d₄, respectively and in the kinetic energy range of 0.7 - 6.0 eV were reacted with CH_4 , CD_4 and $^{13}CH_4$ in the pressure range of $0.6 - 9.0 \times 10^{-3}$ torr. The reactions observed and their relative yields at an energy of 0.7 eV and 0.6×10^{-3} torr are shown in Table 1 for the specific case of $C_2H_3^+ + CH_4$.

Table 1

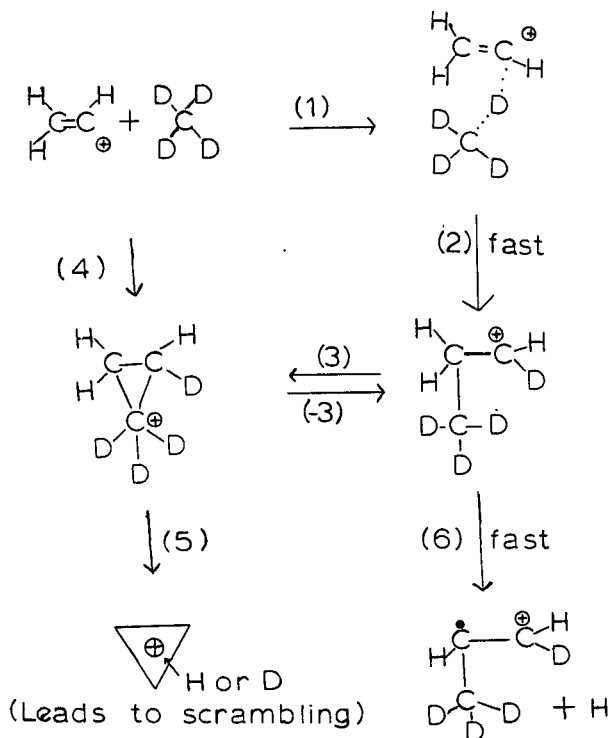
REACTIONS BETWEEN $C_2H_3^+$ WITH CH_4 AT 0.7 eV (C.M.) AND $P_{CH_4} = .6\mu$

<u>RXN</u>	<u>Extent (%)</u>	<u>ΔH_{298}° (kcal)</u>
$C_2H_3^+ + CH_4 \rightarrow CH_3^+ + C_2H_4$	9	+23
$\quad \quad \quad + CH_4^+ + C_2H_3$	6	+90
$\quad \quad \quad + CH_5^+ + C_2H_2$	53	+25
$\quad \quad \quad + C_3H_3^+ + 2H_2$	9	cyc = 6, n = 30
$\quad \quad \quad + C_3H_4^+ + H_2 + H$	2	+70
$\quad \quad \quad + C_3H_5^+ + H_2$	19	-25
$\quad \quad \quad + C_3H_6^+ + H$	3	+30

As may be seen in Table 1, the principal reaction is proton transfer to form CH_5^+ . As indicated by isotope labelling experiments, both this reaction and hydride transfer to form CH_3^+ appear to proceed predominantly by direct processes not involving a $C_3H_7^+$ intermediate; however, a small but discernible amount of CH_3^+ product seems to arise from such a collision complex, as indicated by the $C_2H_3^+ + ^{13}CH_4$ experiments and the process involved must be the reverse of the major reaction observed in the reaction of methyl ions with ethylene,¹ namely (1).

The principal products formed from the unimolecular dissociation of the $C_3H_7^{++}$ intermediate are $C_3H_5^+$ and $C_3H_3^+$ with smaller amounts of $C_3H_6^+$ and $C_3H_4^+$. Isotope labelling studies show that the only H-atoms that can be lost from the complex as atomic hydrogen are those that originate in the $C_2H_3^+$ reactant. Hydrogen originating on the methane molecule are never lost as atomic hydrogen. On the other hand, molecular hydrogen loss is attended by very considerable scrambling of H and D in the labelled experiments.

These facts require a mechanism in which atomic hydrogen elimination occurs very promptly after formation of the $C_3H_7^+$ intermediate but before any scrambling of H, D and ^{13}C has occurred. A mechanism in accord with these facts is as follows:



The addition of (4), namely the direct formation of the symmetrical $C_3H_7^+$ intermediate that leads to the extensive scrambling of H and D, is added to satisfy microscopic reversibility. That is, the study of the reaction of methyl ion with ethylene⁺ indicated the direct formation of the symmetrical $C_3H_7^+$ intermediate with the major products being formed being $C_2H_3^+ + CH_4$.

1. R. N. Abernathy and F. W. Lampe, *Int. J. Mass. Spectrom. Ion Phys.* **41**, 7 (1981).
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ION/MOLECULE REACTIONS IN ALLENE BY HIGH PRESSURE PHOTOIONIZATION
MASS SPECTROMETRY

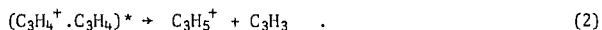
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Département de chimie, Université Laval, Québec, Canada

Ionic processes in allene were studied extensively during the last decade [1-5]. However, the pressure dependence of these reactions was investigated up to 30 millitorrs only. The purpose of the present investigation is to extend the measurements of the ionic processes as a function of the pressure by two orders of magnitude, up to 3 Torr.

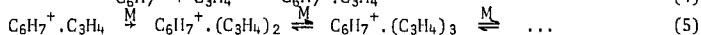
In order to guarantee that the ion/molecule reactions observed have the parent-ion as the only precursor, the $C_3H_4^+$ species was formed by photoionization at 10 eV. At this energy the allene parent ion does not fragment further. In the pressure range of the present investigation the formation of the long lived collisional complex is the obvious first process:



This collisional complex can be either stabilized (a minor reaction channel), or decomposes into $C_6H_7^+$ or $C_3H_5^+$ species:

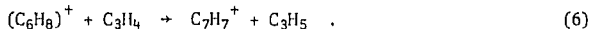


The process (2) is not a very important reaction path accounting for ~6% at low pressure and diminishing with increasing pressure as inferred from the fractional intensity of its solvate $C_6H_9^+$. On the other hand the process (3) is the main reaction path observed in allene, accounting for more than 50% of all ions formed. The $C_6H_7^+$ species solvates further in a well characterized sequence of processes, which with the exception of the second solvation step, are governed by chemical equilibrium:

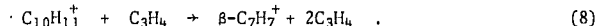
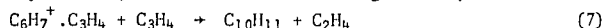


where M stands for the neutral allene molecule.

The next in importance family of ions is $C_7H_7^+(C_3H_4)_m$. The $C_7H_7^+$ species is formed in the following reaction:

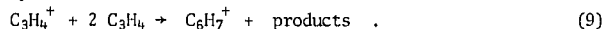


The $C_7H_7^+$ species issued from process (6) clusters further giving $C_7H_7^+(C_3H_4)_m$ ions, where $m = 1-3$. However, this sequence of solvation reactions having as nucleus the $C_7H_7^+$ species formed in process (6) seems to diminish in importance with pressure. With increasing pressure, starting at about 0.2 Torr, two structurally new species having the same mass values as $C_7H_7^+$ and $C_7H_7^+ \cdot C_3H_4$ are formed. They are differentiated from the initial ones by their inability to solvation. Tentatively we assumed that these structurally different species are formed in collisionally induced processes, where isomerization changes take place:



The main feature of these $\beta-C_7H_7^+$ and $C_{10}H_{11}^+$ species is their inability to solvate further. They accumulate in the system.

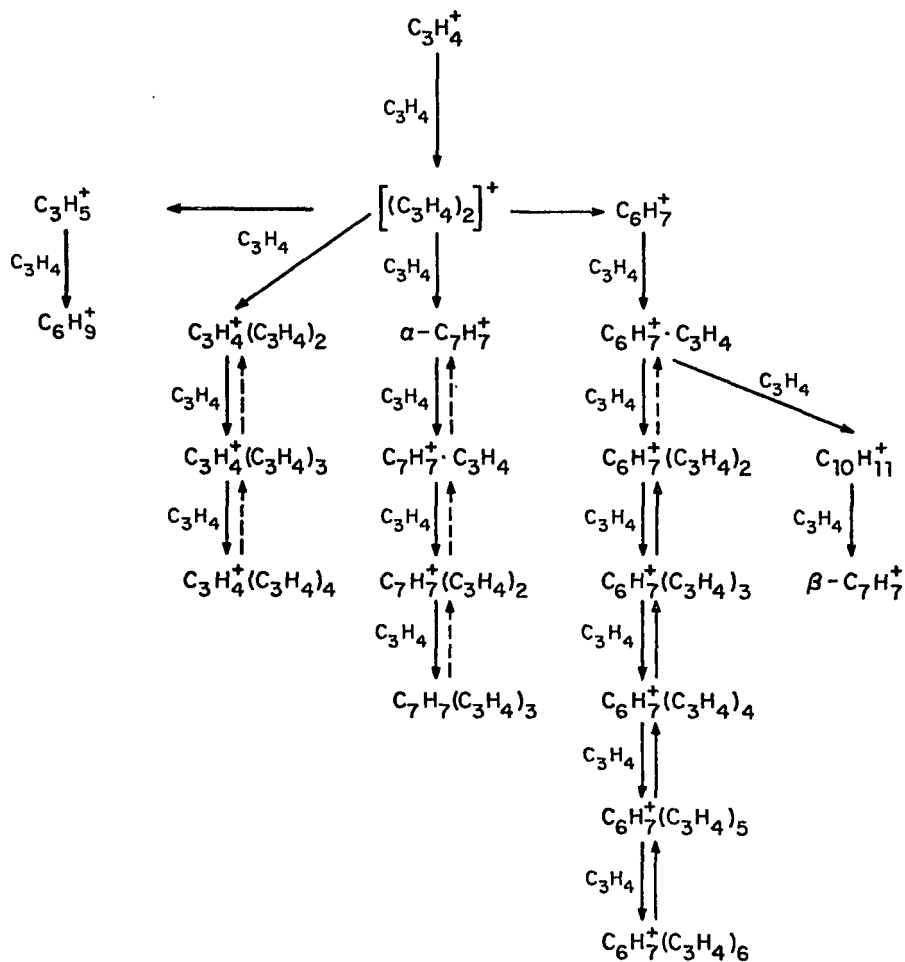
A preliminary kinetic treatment points out that the bimolecular process (3), which was investigated at low pressure ($p < 0.02$ Torr), [5], does not account for the fractional intensities measured at higher pressure ($p > 0.1$ Torr). We have to assume that $C_6H_7^+$ species is also formed in a termolecular process:



Preliminary calculations show that the kinetic rate constant of reaction (9) is very high, of the order of $10^{-25} \text{ cm}^6 \cdot \text{molec}^{-2} \cdot \text{s}^{-2}$.

In fig. 1 we present the overall reaction scheme of ionic processes in allene.

Fig. 1 - Ion/molecule reactions in allene at high pressure.



References

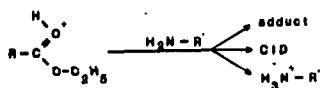
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SELECTIVE ADDUCT ION FORMATION IN THE COLLISION CELL OF A TANDEM QUADRUPOLE MS/MS

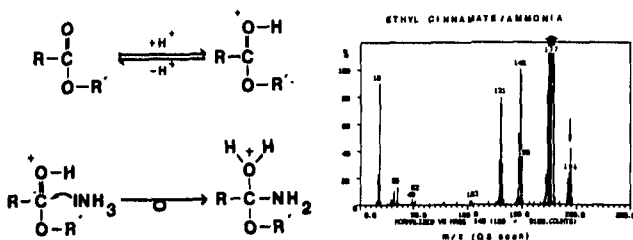
J.P. Schmit, P.H. Dawson, and N. Beaulieu; Health and Welfare Canada, H.P.B., Pharm. Chem. Div., Ottawa, K1A 0L2 and National Research Council of Canada, Div. of Physics, Ottawa, K1A 0R6

Low energy tandem quadrupole MS/MS is particularly well suited to study ion molecule reactions and especially adduct ion formation. By controlling the parent ion energy and the target thickness, we should be able to study the physico-chemistry of adduct ion formation with a neutral target inside a collision cell. To verify this hypothesis we have undertaken to study ion molecule reactions between a series of reactants, mainly esters, and ammonia or methylamine as neutral nucleophilic target. The ionization source was of the atmospheric pressure chemical ionization type and as such yields protonated species except for benzene and aromatic like compounds.

Three main competitive reaction channels were observed: first, proton interchange with the target, second, collision induced dissociation, and finally adduct ion formation.



where R' is either H or CH₃, while the esters are ethyl acetate, propionate, butyrate, isobutyrate, α-hydroxy-isobutyrate, 4-chlorobutyrate, and cinnamate, or methyl benzoate.



Depending on the very nature of the parent ion species and the nucleophilic target, proton interchange or adduct ion formation is predominant.

Proton interchange was found to be the main reaction channel for the unsubstituted esters. Nevertheless, noticeable and reproducible amounts of adduct ion, ranging from 0.01% of the total ion number for ethyl acetate to 0.7% for ethyl isobutyrate, were observed after optimization of the target thickness.

The yield of adduct ion formation for the substituted esters were found to be strongly influenced by the very nature of the substituent and the stability of the parent ion. The hydroxylated ester yields a higher amount (11%) of adduct ion than the chlorinated one (2.5%), while ethyl cinnamate exhibits more than 7%.

The optimum target thickness varies from one compound to another ranging from 80×10^{12} to more than 250×10^{12} molecules/cm². For benzene and ethyl cinnamate, the optimum thickness was not reached in the condition used.

The optimum parent ion energy, expressed as center-of-mass energy, was found to vary between 0.01 and 0.03 eV depending on the compound under study. It is noticeable that for a given compound the usable energy spread is very narrow, usually less than 0.1 eV. Surprisingly, to optimize the output from the collision cell the adduct ion and the protonated target (ammonium or methylammonium) require the use of a drawing potential ranging from about 0.4 volts (protonated species) to 21 volts (adduct ion).

PARENT ION	ENERGY	ADDUCT ION	m/z	YIELD ^a
ACETATE				
$\text{C}_2\text{H}_3\text{O}_2^+$ 0 8 2	→ 0.01 eV	$\text{C}_4\text{H}_5\text{O}_2\text{N}^+$ 4 12 2	108	0.0135
PROPANOATE				
$\text{C}_3\text{H}_5\text{O}_2^+$ 0 11 2	→ 0.01 eV	$\text{C}_5\text{H}_7\text{O}_2\text{N}^+$ 5 14 2	128	0.0045
BUTYRATE				
$\text{C}_4\text{H}_7\text{O}_2^+$ 0 13 2	→ 0.03 eV	$\text{C}_6\text{H}_9\text{O}_2\text{N}^+$ 6 18 2	134	0.0785
ISOBUTYRATE				
$\text{C}_4\text{H}_7\text{O}_2^+$ 0 13 2	→ 0.03 eV	$\text{C}_6\text{H}_9\text{O}_2\text{N}^+$ 6 18 2	134	0.7375
4-CL-BUTYRATE				
$\text{C}_4\text{H}_6\text{O}_2\text{Cl}^+$ 0 12 2	→ 0.01 eV	$\text{C}_6\text{H}_8\text{O}_2\text{NCl}^+$ 6 18 2	166	2.5 %
α-MO-ISOBUTYRATE				
$\text{C}_4\text{H}_7\text{O}_2^+$ 0 13 3	→ 0.01 eV	$\text{C}_6\text{H}_9\text{O}_2\text{N}^+$ 6 18 3	158	11.2 %
^a OPTIMUM TARGET THICKNESS				

Evidence For The S_N2 Character of the Ion-Molecule Reaction In The Gas Phase Generating $[M+NH_4-ROH]^+$ Ions From Steroid Ethers

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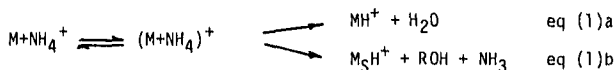
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² Laboratoire De Synthèse Organique, Ecole Polytechnique, Palaiseau Cedex, France

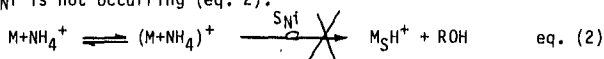
³ NCI-Frederick Cancer Research Facility, Frederick, MD 21701

For several years, the presence of ionic species which are not related to the fragmentation of MH^+ have been observed in CI/NH_4^+ mass spectra of a variety of organic substances. Generally, these particular ions have been identified as products of ion-molecule reactions. In particular, alcohols have been found to produce, under CI/NH_4^+ conditions, $(M+NH_4-ROH)^+$ (thereafter called M_5H^+) which is isobaric with M^+ . Various nucleophilic substitution mechanisms have been proposed for the formation of the substitution ion M_5H^+ observed in either CI/NH_4^+ or DCI/NH_4^+ spectra (1) (2).

The CI/NH_4^+ mass spectra of diastereomeric methyl and benzyl ethers (derivatives of Δ^5 -3-hydroxy cholestane) (Fig. 1) are affected by the configuration (α , β) of the functional group. The use of ND_3 as a reagent gas confirms the introduction of an ammonium group into M_5H^+ . A careful study of the MIKE spectra of appropriate ions shows that the M_5H^+ issued from the ethers having the methoxy or the benzyloxy group with a β -configuration are more stable than those generated by the α -isomers whereas MH^+ ions are found to be more stable when originated from α -isomers [Table 1; eq (1)a and (1)b].



The enhanced stability of $(M+NH_4)^+_{\alpha}$, a precursor of $(MH^+)_{\alpha}$, over $(M+NH_4)^+_{\beta}$ was also clear from the examination of the MIKE data. These observations are explained by the presence of an hydrogen bond (Scheme 1) in the ions with an α -configuration which is impossible for the β -isomers. Also, the loss of ROH from $(M+NH_4)^+$ was not observed in the field free region, which suggests that an S_N1 is not occurring (eq. 2).



The observed differences in stabilities for M_5H^+ and MH^+ issued from diastereomeric couples are not in agreement with an S_N1 mechanism during the formation of M_5H^+ .

The HV scan spectra of the ethylenic carbocations $\{M+NH_4-ROH-NH_3\}^+$ showed a definite inversion in the stabilities of their precursor ions MH^+ [or $(M+NH_4)^+$] and M_5H^+ in relation with their initial configurations. These interesting stereochemical effects, enhanced in the field free region versus the ion source, are explained in terms of the occurrence of a Walden inversion during the reaction between $(M+NH_4)^+$ and NH_3 (Scheme 2). This S_N2 reaction is observed only in the ion source where NH_3 is present and where the ions are thermolyzed. A similar study on the saturated diastereoisomers indicated that the compounds with α -configuration (axial) are more reactive than the β -isomers (equatorial), and that the inversion of stabilities for M_5H^+ and MH^+ in relation to the initial configuration was also observed.

In conclusion, the data indicate that, in addition to the fact that the reactants should have closed proton affinities, the presence of a double bond or of another functional group can be decisive for the visualization of M_5H^+ and of stereochemical effects supporting the S_N2 mechanism during the formation of M_5H^+ ions.

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Figure 1

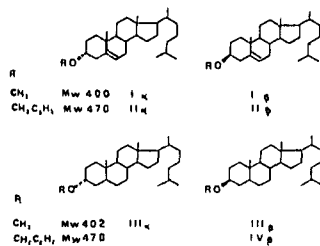
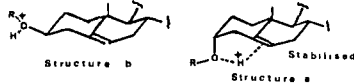


Table 1

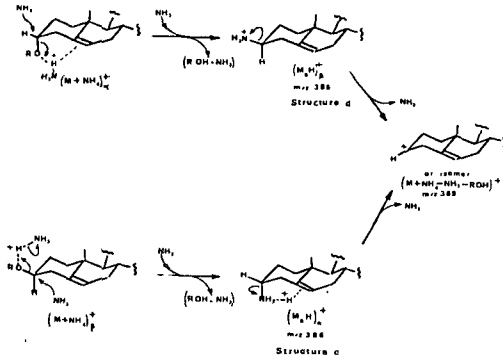
Compounds	MIKE of MH^+		MIKE of M_2H^+	
	m/z of main beam	$(MH-RH)^+$ daughter ion ¹	m/z of main beam	$(M_2H-MH_2)^+$ daughter ion ¹
$\delta_{A-\alpha} OCH_3$	401	0.022	386	0.037
$\delta_{A-\beta} OCH_3$	401	0.127	386	0.004
$\delta_{A-\alpha} OCH_2C_6H_5$	477	0.019	386	0.028
$\delta_{A-\beta} OCH_2C_6H_5$	477	0.102	386	0.002
αOCH_3	403	0.120	388	0.013
βOCH_3	403	0.095	388	0.042

¹ Relative abundances to precursor MH^+ (or M_2H^+). The intensity of main beam was corrected for the natural isotopic contribution.

Scheme 1



Scheme 2



This work was supported by Contract No. NO1-CO-23910, with the National Cancer Institute, NIH, Bethesda, MD 20205.

Double Bond Location By CAD of Fe(Olefin)⁺ Complexes
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The mass spectra of isomeric olefins are often indistinguishable making location of double bonds impossible. Previous attempts using chemical ionization (CI) involving vinyl methyl ether, NO and butadiene as reagents show promise but have certain limitations. Substituted double bonds and cis/trans isomers are often difficult to differentiate. Often peaks indicating the position of the double bond are weak.

A method for double bond location has been developed taking advantage of the oxidative addition reactions of hydrocarbons to gas phase metal ions.¹ The reaction of Fe(CO)⁺, formed by electron impact on Fe(CO)₅ in the CI source, with olefins yields Fe(olefin)⁺ complexes whose collisionally activated decomposition (CAD) spectra allow the double bond position to be determined. The CAD spectrum of Fe(1-octene)⁺ obtained on a Kratos MS-50 triple analyzer instrument² is shown in Figure 1. Loss of C₃H₆ is the dominant CAD fragment but other fragments such as loss of C₅H₁₀ and Fe⁺ are observed. The proposed mechanism to account for the loss of propene is shown in scheme I. The oxidative addition of Fe⁺ to the allylic C-C bond followed by transfer of a β -hydrogen and reductive elimination results in the formation of a bis(olefin) intermediate. Loss of the smaller olefin is preferred giving the Fe(C₅H₁₀)⁺ product.³

To test the generality of the allylic insertion mechanism the CAD spectra of a series of Fe(C₈H₁₆)⁺ complexes of octene and methylheptene isomers were investigated. The predicted losses for the octene isomers are given in reactions 1-4. The partial CAD spectra of the Fe(octene)⁺ isomers are shown in Figure 2. The dominant loss in each CAD spectrum corresponds to the product expected from the cleavage of the allylic C-C bond. If the mechanism is general, the methylheptene complexes should dissociate to give the products shown in reactions 5-8. The predicted losses are in fact the major CAD products observed (See Figure 3).

In addition to the prediction of the major CAD reaction in all of the Fe(C₈H₁₆)⁺ complexes, the cis and trans isomers of 3- and 4-octenes could be distinguished by their CAD spectra. The partial CAD spectra of cis and trans-4-octene are given in Figure 4.

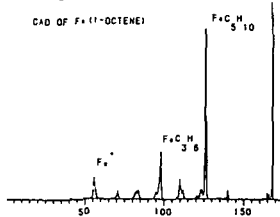
The structure of the products of the allylic cleavage were investigated by the MS/MS/MS technique.⁴ The MS/MS/MS spectrum of the C₅H₁₀ from Fe(1-octene)⁺ compares quite well with the Fe(C₅H₁₀)⁺ formed in the source and with Fe(1-pentene)⁺. The MS/MS/MS of the 2-methyl-1-heptene complex gave a spectrum that is a mixture of Fe(butene)⁺ and Fe(isobutene)⁺ structures (See figures 5 and 6). These results provide further evidence supporting the allylic mechanism.

To test the feasibility of the method for mixture analysis, a 1:1:1 mixture of 1-, 2- and 4-octene was reacted with Fe(CO)₅ in the CI source. The CAD spectrum of the Fe(C₈H₁₆)⁺ mixture is shown in Figure 7. A ratio of 0.95:1.00:0.98 was calculated from the peak heights of m/z 112, 126 and 140.

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Figure 1



SCHEME I

PROPOSED MECHANISM

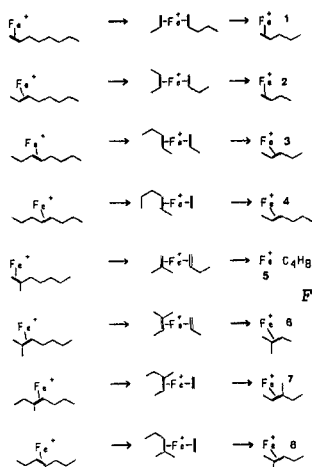
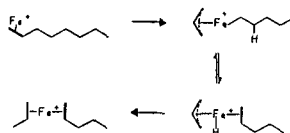


Figure 2

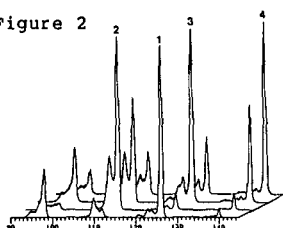


Figure 3

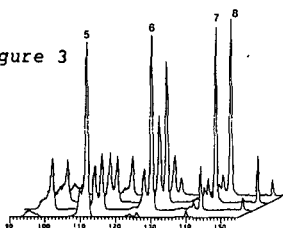


Figure 4

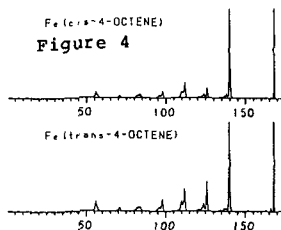


Figure 5

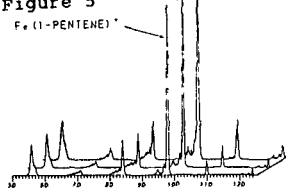


Figure 7

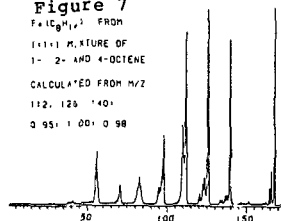
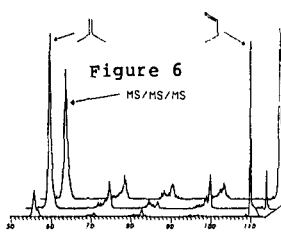


Figure 6

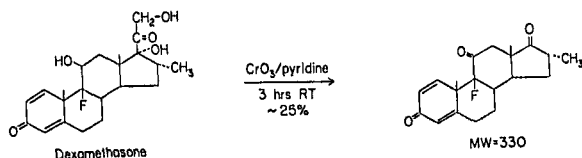


A SIMPLE QUANTITATIVE ASSAY FOR URINARY DEXAMETHASONE BASED ON ELECTRON CAPTURE NEGATIVE CHEMICAL IONIZATION (EC-NCI). G.R. Her and J.T. Watson, Depts. of Chemistry and Biochemistry, Michigan State University, East Lansing, MI. 48824

Synthetic corticosteroids are widely used in human and veterinary medicine. Because of their high potency and therefore low dose, and because of their structural similarity to natural steroids, a selective and sensitive assay is required for fundamental pharmacokinetic studies of these drugs in human and animal. In 1963 Lovelock et. al., found that 4-androsten-3,11,17-trione showed an unusually high electron affinity during electron capture detection in GLC (EC/GC). In this lab, we have observed that 1,4-androstadien-3, 11,17-trione showed ~ 100 times higher response than 1,4-androstadien-3, 17-dione under conditions of electron capture negative chemical ionization (EC/NCI). Based on these observations, a quantitative method has been developed for the determination of dexamethasone in urine samples in the ng/ml range using EC/NCI mass spectrometry.

Experimental:

Synthetic corticosteroids can be easily oxidized to 1,4-androstadien-3,11,17-trione analogs with CrO_3 /pyridine at room temperature as shown below:



EC/NCI-GC/MS was performed using a HP-5985 GC/MS with a custom-built negative ion detector. A 6 ft. 3% OV-1 packed column was used throughout the experiment. The injector, oven, and separator temperatures were 280°C, 260°C, and 280°C, respectively. Methane was used as reagent gas and helium was used as carrier gas. The mass spectrometer was operated at an ion source pressure of 0.2 torr and with an ion source temperature of 120°C.

Collection of urine was made at two hours and then every four hours thereafter following administration of 5mg dexamethasone to a horse.

Results and Discussion:

Since most organic compounds do not have an intrinsic high electron affinity, derivatization is usually necessary in order to enhance their response under EC/NCI conditions. However, in most derivatization reactions, many compounds are derivatized in addition to the compound-of-interest in the real sample analysis; therefore, selectivity is lost. In this method, since only corticosteroids can be oxidized to 1,4-androstadien-3,11,17-trione analogs (high electron affinity structure analogs), the selectivity is high.

EC/NCI spectra of oxidized corticosteroids are very simple. Only M^- at m/z 298 is observed for the oxidized trione product of prednisolone. M^- at m/z 330 and $(\text{M}-\text{HF})^-$ at m/z 310 are observed for the oxidation product of dexamethasone. The sensitivity for oxidized corticosteroids is quite good. When using the technique of selected ion monitoring (SIM) to monitor M^- and $(\text{M}-\text{HF})^-$, 25 pg of oxidized dexamethasone injected onto a packed column gave a signal to noise ratio greater than 2.

A standard curve was prepared from pure dexamethasone mixed in proportion with prednisolone (internal standard) prior to extraction and oxidation. An internal standard is essential because the oxidation reaction is not quantitative. Experimental results were used to construct a standard curve approximating a straight line from 0 to 200ng/ml as shown in Fig. 1. Each point represents duplicate analyses of that particular mixture of prednisolone and dexamethasone. The standard deviation for each point is in the 4% - 8% range. The correlation coefficient is 99.9%.

Selected ion current profiles of the 2-hour and 30-hour horse urine samples are shown in Fig. 2. They represent 143ng/ml and 4ng/ml, respectively, of dexamethasone in the horse urine. The preliminary results demonstrate that the method is rapid, sensitive, and selective enough for use in pharmacokinetic studies employing therapeutic doses of synthetic corticosteroids.

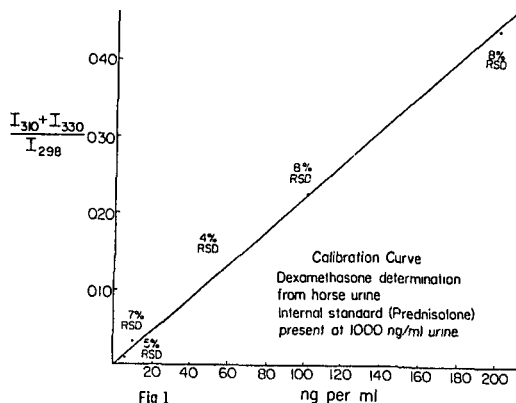


Fig 1

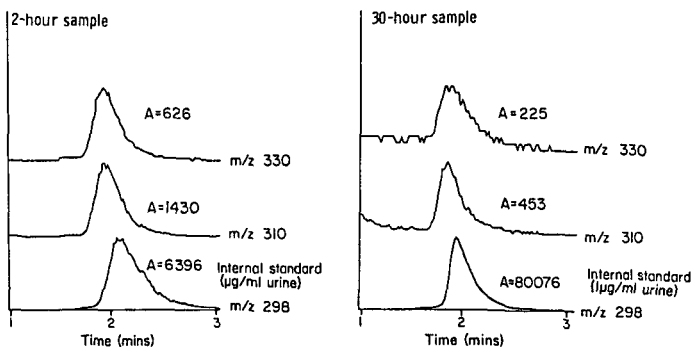


Fig 2. Selected ion profiles at m/z 330 (M^+) and m/z 310 ($M-HF$) $^-$ for dexamethasone and at m/z 298 (M^+) for prednisolone internal standard during analysis of extract of horse urine collected 2 hours and 30 hours after administration of 5mg dexamethasone. Dwell times were 600 ms, 300 ms, and 100 ms for m/z 330, m/z 310, and m/z 298, respectively. Relative peak areas indicated in each case; gain of detector is greater for 30-hour sample which corresponds to 4ng Dex / ml.

Quantitation of Molecular Species of Intact Phospholipids by
Direct-Exposure Chemical Ionization Mass Spectrometry

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Obtaining quantitative data for the molecular species making up natural phospholipid mixtures presents a challenge to the analytical chemist. The usual procedures involve an enzymatic step to produce 1,2 diacyl glycerols from the phospholipids, followed by derivatization and analysis by GC. Analysis of intact phospholipids by direct-exposure CI-MS has proved to be a one-step approach to quantitative information that is applicable to submicrogram quantities of sample. Mixtures of reference saturated phosphatidylcholines (PC) from C_{30} to C_{44} were analyzed by CI-MS. The CI mass spectrum showed a small protonated molecule (MH^+), but the intense peak at $MH-183^+$ containing both acyl groups was used for quantitative measurements. A plot of log area for the $MH-183$ signal vs molecular weight was linear across the range of the data (Figure 1). From these data, response factors for individual components could be calculated. In experiments with PC molecular species having from 0 to 6 double bonds, there was no significant loss of response due to unsaturation. Good linearity and results were obtained for individual PC components in mixtures across the range of 30 ng to 5 μ g.

Preliminary results indicate that di 15:0 PC can be added to a total lipid extract, and the lipid classes can be fractionated on an analytical TLC plate. Amounts as little as 5 μ g of PC can be chromatographed and recovered for MS analysis without selective loss of molecular species.

Natural phospholipid samples contain a few molecular species that have identical molecular weights (e.g., 18:1-18:1 and 18:0-18:2) not resolved by the MS technique. When overlaps occur, they can be resolved by MS/MS daughter spectra of the $MH-183$ ion. The daughter spectra show RCO^+ ions for each fatty acid, and the relative intensities can be used to quantitate the different molecular species.

Table 1 shows the results we obtained for a commercial sample of soy PC using the MS technique and compares them with literature values. These data demonstrate the potential of the direct MS technique in analysis of phospholipid molecular species.

Table 1
Comparison of Composition of Soy PC by MS
with Literature Values

m/z	Species	Wt %	σ	Daughter Ion	Literature*
577	16:0-18:1	2.6	± 0.4		2.6 ± 0.1
575	16:0-18:2	24.8	± 0.7		25.3 ± 0.9
573	16:0-18:3	2.3	± 0.2		2.4 ± 0.1
605	18:0-18:1	0.5	± 0.5		0.5 ± 0.1
603	18:1-18:1	9.6	± 0.5	2.4	0.7 ± 0.1
	18:0-18:2			7.2	6.6 ± 0.6
601	18:1-18:2	14.1	± 0.6	13.4	10.4 ± 0.3
	18:0-18:3			0.7	0.4 ± 0.1
599	18:2-18:2	38.2	± 0.8		42.4 ± 0.5
597	18:2-18:3	6.8	± 0.4		8.3 ± 0.1
595	18:3-18:3	0.6	± 0.3		0.5 ± 0.1

*Myher, J. J. and A. Kuksis, Can. J. Biochem. 60, 638, 1982 (Table 4 B).

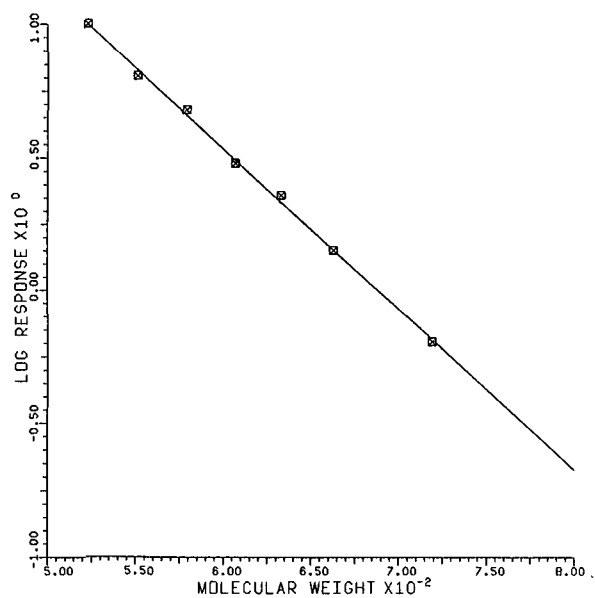


Figure 1. Response relative to di C15:0 PC vs. molecular weight for mixture of saturated PC's.

MASS SPECTROMETRIC QUANTIFICATION OF 25-HYDROXYVITAMIN D₃ IN CHILDREN WITH
HYPOPHOSPHATEMIC VITAMIN D RESISTANT RICKETS (VDRR)

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An abnormality in vitamin D metabolism has been inferred in children with VDRR since the disease is characterized by osteomalacia unresponsive to nutritional amounts of vitamin D. In order to monitor therapy in VDRR patients, we have developed a selected ion monitoring mass spectrometric method for the quantification of 25-hydroxyvitamin D₃, the major circulating metabolite whose concentration in serum is a sensitive indicator of nutritional vitamin D status. The method is rapid, simple, sensitive (detection limit = <100 pg), requires a minimal amount of serum and is quantitative from 1 to at least 100 ng/ml of serum.

An internal standard, 26,26,26³H₃-25-hydroxyvitamin D₃, was synthesized by reacting 26-nor-3 β -hydroxy-5-cholestene-25-one with CD₃MgI to yield 26,26,26³H₃-5-cholestene-3,25-diol which was allylically brominated (1,3-dibromo-5-7-dimethylhydantoin) and dehydrobrominated (trimethyl phosphate) to 26,26,26³H₃-5,7-cholestadien-3,25-diol. Photolysis of the 5,7 diene yielded pre-vitamin 26,26,26³H₃-25-hydroxyvitamin D₃ which was heat isomerized to the vitamin form. ¹³C-NMR confirmed the presence of three deuterium atoms at the 26(27) position in the side chain. Fig. 1 shows high resolution mass spectrometry (Kratos MS-902) of natural and trideutero 25-hydroxyvitamin D₃. The molecular ion (M⁺ = 403) and fragments containing the deuterated side chain (m/z 385, 370, 367, 352, e.g.) are found three mass units higher in the trideutero compound than in the corresponding fragments from the natural compound.

The internal standard (12.5 ng) was added to 0.5 ml or less of serum and extraction was carried out in CHCl₃:MeOH (97.6 $\pm$ 1.8% recovery). After sequential silica and C¹⁸ Sep-Pak purification (85.2 $\pm$ 3.4 and 89.1 $\pm$ 1.8% recovery, respectively) samples were analyzed as trimethylsilyl ethers on a Kratos MS-25S mass spectrometer equipped with a Varian 3700 gas chromatograph. A 24 m x 0.3 mm i.d. fused silica column coated with 0.2% OV-1 was programmed between 180 and 260°, at 5°/min, following splitless injection at room temperature, and the molecular ions m/z 547 for the trideutero internal standard and m/z 544 for the natural compound were measured by selected ion monitoring. A selected ion monitoring trace of 1:10 natural to trideutero standard 25-hydroxyvitamin D₃ is illustrated in Fig. 2a while that of an actual serum sample is shown in Fig. 2b. It is evident that the purification procedure for serum results in a relatively clean chromatographic trace with little interference from contaminating substances. A standard curve covering the range of 1-50 ng was generated each day and, as shown in Fig. 3, variation of the ratio of m/z 547/544 was minimal at each concentration tested.

Multiple determinations (n = 6) of 0.5 ml aliquots from a single sample of the serum of a normal adult gave an intraassay value of 27.5 $\pm$ 1.5 ng/ml. Concentration of 25-hydroxyvitamin D₃ in the serum of normal adults (n = 6) in January 1982 was 18.2 $\pm$ 8.1 ng/ml. Table I shows that duplicate sera from normal children quantified by competitive protein binding and SIM averaged 21.0 $\pm$ 11.7 and 25.0 $\pm$ 10.9 ng/ml, respectively. Values for children with VDRR receiving no therapy (Table II) were consistently lower (12.5 $\pm$ 6.5 ng/ml) than normal. Treatment with dihydrotachysterol or 1,25-dihydroxyvitamin D₃ was without effect on 25-hydroxyvitamin D₃ levels. Average values for children given 25,000 or more units of vitamin D₃ daily were surprisingly low (11.7 $\pm$ 6.7 ng/ml). Subsequent investigation revealed that at least 3 of the children tested were not conforming to therapy.

Conclusion: SIM quantification of 25-hydroxyvitamin D₃ in conjunction with silica and Sep-Pak C-18 purification is a rapid, fairly simple and clinically useful means of monitoring vitamin D₃ therapy and nutritional status.

Acknowledgment: This work was supported in part by NIH Grant R01 HD-13787 and by NIH Division of Research Resources Grant P41 RR-01614.

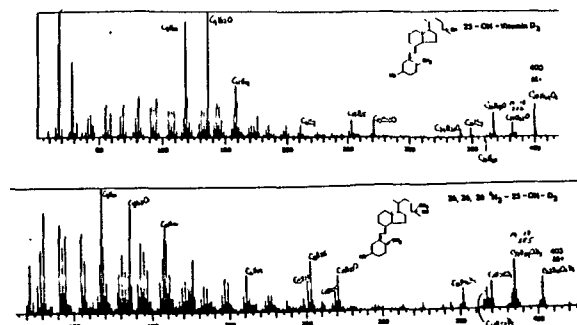


Figure 1

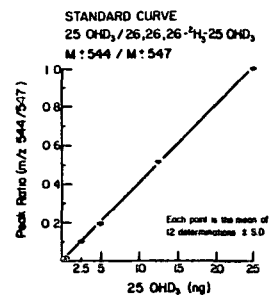


Figure 3

QUANTIFICATION OF 25OH₂ IN SERUM OF NORMAL CHILDREN.
COMPETITIVE PROTEIN BINDING (C.P.B.)
VERSUS SELECTED ION MONITORING (S.I.M.)

	C.P.B.	S.I.M.
	ng/ml	ng/ml
C.M.	27	22
C.S.	15	21
C.S.	14	21
C.H.	25	27
P.H.	53	56
H.M.	4	8
S.T.	19	21
L.G.	19	22
E.F.	20	26
J.L.	21	28
A.J.	14	25
Ave. ± S.D.	21 ± 11.7	25 ± 10.9

Table I

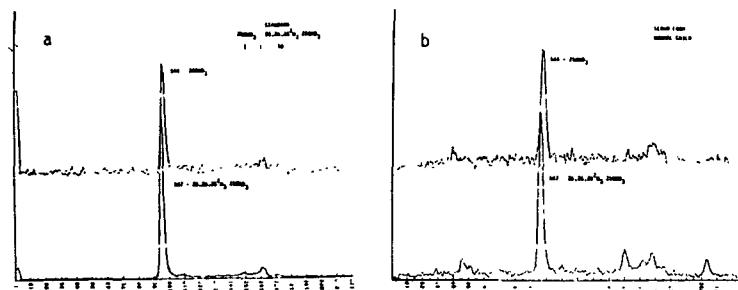


Figure 2

CONCENTRATION OF 25OH₂ IN SERUM OF CHILDREN WITH
HYPOPHOSPHATEMIC VITAMIN D RESISTANT RICKETS ON
VARIOUS THERAPEUTIC REGIMES

	ng/ml
No therapy (7)*	12.5 ± 6.5**
Vitamin D ₃ (6) (25-50,000 U/day)	11.0 ± 6.7
1,25(OH) ₂ D ₃ (2) (1 µg/day)	11.2
Dihydrocholesterol (3)	10.2

* Number in () = total number of samples

** Mean ± S.D.

Table II

QUANTITATION OF LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID
ON SHORT CAPILLARY COLUMNS USING NEGATIVE ION CHEMICAL IONIZATION MS
WITH OXYGEN 18 INTERNAL STANDARDS

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5-Hydroxyeicosatetraenoic (5-HETE) and leukotriene B₄ (LTB₄) are oxidative metabolites of arachidonic acid from the action of the enzyme 5-lipoxygenase (1). They have been implicated as mediators of the inflammatory response and also possess potent chemotactic and chemokinetic properties toward the polymorphonuclear leukocyte (2). We have been interested in measuring these compounds which are present only in picogram amounts in certain biological systems, and have therefore undertaken a study on the negative chemical ionization behavior of suitable derivatives as well as development of stable isotopes for quantitative mass spectrometry.

The stable isotope, oxygen 18, was incorporated into synthetic 5-HETE by five cycles of methylation followed by hydrolysis in 0.2N Li¹⁸OH (3). This provided an isotopic internal standard with 82% of the 1,1-¹⁸O₂ species and only .5% of the 1,1-¹⁸O₂ species. Three oxygen 18 atoms were incorporated into Δ⁶-trans LTB₄ by hydrolyzing the epoxide of LTA₄-methyl ester in H₂¹⁸O, and then using the methylation-hydrolysis procedure to incorporate ¹⁸O in the carboxyl moiety (5 cycles). This gave 1,1,12-¹⁸O₃-Δ⁶-trans LTB₄ with 81.1, 17.5, and 1.3 atom % respectively for the tri, di, and mono oxygen-18 species. The atom % of totally unlabelled material was not detectable above instrument background, but from theoretical considerations is less than 0.1%.

The resulting compounds were analyzed by GC-MS on a 5m capillary column after forming the pentafluorobenzyl (PFB) trimethylsilyl (TMS) derivatives. Figures 1 and 2 show the positive ion electron impact and negative ion chemical ionization (CH₃) spectra of 5-HETE. Under electron impact, the positive ion spectra is dominated by ions characteristic of moieties introduced by derivatization (m/z 73 for TMS and m/z 181 for PFB). A weak molecular ion is seen (m/z 572), as well as loss of trimethylsilanol (m/z 482), cleavage α to the 5-oxygen (C₅, C₆ - m/z 369) and apparent cleavage at C-9, C-10 (m/z 421). These ions were all shifted by 4 a.m.u. in the labelled compound, confirming assignments and incorporation of two oxygen-18 atoms. The other possible cleavage ion adjacent to the 5-oxygen atom produces m/z 305 and is not shifted in the labelled compound.

The negative ion (NCI) spectrum was quite striking in contrast, producing mainly the carboxylate anion (loss of PFB; m/z 391) and further loss of TMS-OH. The absence of m/z 181 (pentafluorobenzyl anion) may be explained by several factors. One is the resonance stabilization of the negative charge in a carboxylate anion. Secondly, the leaving benzyl radical is stabilized inductively by the presence of five fluorines. Finally, cleavage in the opposite direction would produce a pentafluorobenzyl anion. The stabilization of benzyl cations in an expanded aromatic tropylium structure (6π electrons - Fig. 3) has long been known. However, this isomer of the benzyl anion is not energetically favored, because it would be antiaromatic (8π electrons).

The calibration line for 5-HETE was linear in an initial investigation at the 1-50 ng level (r = .9999 with a y intercept of .013). In a carrier experiment, 40 pg was detected at an S/N of 15:1. We anticipate good potential in this method for measuring specifically the aforementioned small quantities of these compounds.

Acknowledgement

This work was supported in part by a grant from the National Institutes of Health (HL 25785)

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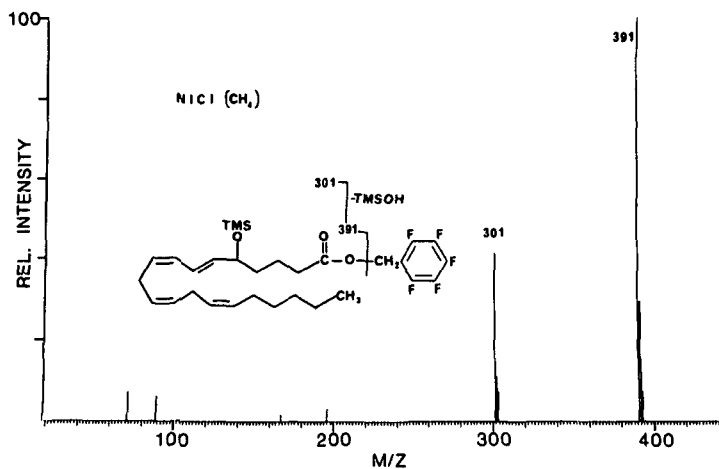
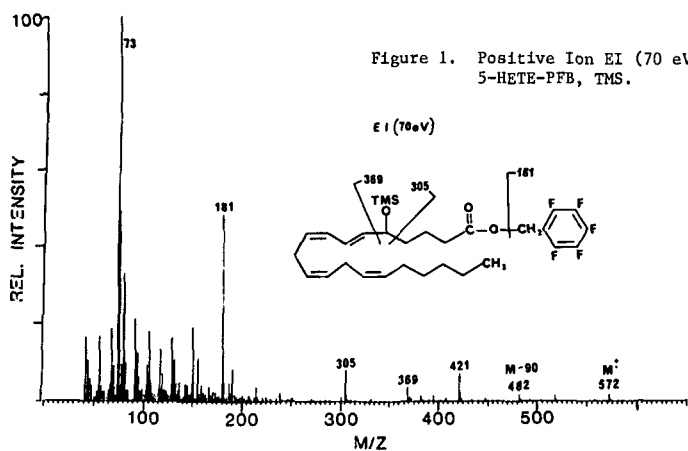


Fig. 3 (4n+2) Rule - π electrons

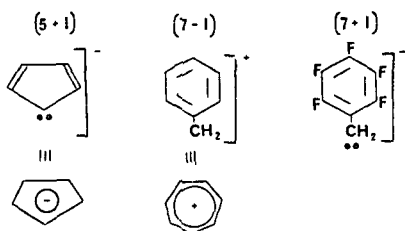


Figure 2. Negative Ion
Cl (CH₃)
5-HETE⁻-PFB,
TMS.

RPB 7 | GCMS ASSAY OF PINEAL SEROTONIN AND ITS
METABOLITES IN RATS EXPOSED TO 60 Hz ELECTRIC FIELDS, B.W.
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west Laboratory, Richland, WA 99352.

There has been considerable speculation concerning possible loci for metabolic, behavioral, and possible teratogenic effects observed in animals exposed to 60 Hz electric fields. Since the pineal is thought to function as a neuroendocrine transducer, the metabolism and function of this gland has been monitored for changes in exposed versus control animal populations. Over the past three years we have developed and applied GCMS assays using selected ion monitoring with an internal standard to the measurement of derivatized pineal serotonin, melatonin and five methoxytryptophol in exposed and sham exposed rat pineal glands. The assay has been applied to rapidly prepared crude pineal extracts, and affords excellent sensitivity and specificity. Data from these assays have shown that the normal circadian rhythms in pineal serotonin and melatonin were essentially abolished by exposure of the animals to electric field intensities as low as 1.5 kV/meter. These findings have given some fundamental insight into possible mechanisms for the action of electric fields on the central nervous system.

In order to facilitate the analysis of an increasing number of samples in this program, we have initiated the design and construction of a fully automated, quadrupole based GC/GC/MS system. Performance of the CG/CG/MS will be discussed and contrasted to that of the high performance double focussing magnetic based GCMS system currently in use.

RPB 10 | THERMOCHEMICAL PROPERTIES OF SOME GASEOUS
METAL MONOXIDE IONS; Edmond Murad; Air Force Geophysics
Laboratory, Hanscom AFB, MA, 01731.

The dissociation energies of a number of metal monoxide ions have been obtained directly by measuring the thresholds for endothermic reactions of metal ions with various neutral molecules. The experiments were conducted on an apparatus consisting of a 2.5-cm, 90° radius of curvature mass spectrometer coupled in tandem to a quadrupole mass filter via a stationary target collision chamber. The metal ions were generated by surface ionization and the kinetic energies of the primary ions were obtained by retarding potential analysis. Among the monoxide ions studied were AlO^+ , BaO^+ , CaO^+ , and SrO^+ . In addition, threshold information for the reaction of Ca^+ and Sr^+ with water and dissociation energies of the respective ions were obtained. Cross sections for the reactions and thermochemical data for the ions will be presented.

DEVELOPMENT OF A GCMS ASSAY FOR CATECHOLAMINES IN BIOLOGICAL FLUIDS.

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Catecholamines are physiological and clinically important compounds. They occur normally at low levels in body fluids: plasma, pgml^{-1} range and urine, ngml^{-1} range.

Quantitation by GCMS of catecholamines in body fluids is difficult because (1) analysis has to be performed near the detection limit of the instrument; (2) complications in isolating the minute amounts caused by oxidation of the catechol function at alkaline conditions and losses due to irreversible adsorption; (3) derivatization of the chemical complex compounds with catechol, phenolic and aliphatic hydroxyl groups and primary and secondary amino groups.

We have attempted to develop an analytical method which reduces most of the above mentioned problems. This has been achieved by derivatization of the catecholamines in the buffered aqueous sample by reaction with methyl chloroformate¹. The reaction with phenolic hydroxyl groups and primary amino groups takes place virtually instantaneously at pH 7.2. The reactivity of secondary amines depends on its alkyl substituent. The reaction can be enforced by increasing the pH of the medium. This can safely be done because the catechol function has already been protected.

The methyl formate derivatives are highly lipophilic and can be easily extracted from the aqueous medium into ethylacetate (recovery >99%). Further derivatization of the free aliphatic hydroxyl group was best performed with tert.-butyldimethylsilylimidazole. This reagent also completely exchanged the O-methyl formate groups. The resulting O-tBDMS-N-methyl formate derivatives of catecholamines are well resolved on a OV-1701 fused silica capillary column. The EI mass spectra are strongly dominated by α -cleavage of the hydroxy-ethylamino chain. The intensity of this ion (m/z 481) represents approximately 20% of the total ion current.

The detection limit of the GCMS (Jeol JMS-D-100) was 5 pg injected ($S/N = 3$), which enabled the determination of 50 pgml^{-1} in biological samples. The analytical recovery was better than 90%. The calibration curve was linear over the range studied ($0.1\text{--}100 \text{ ngml}^{-1}$).

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METHOD

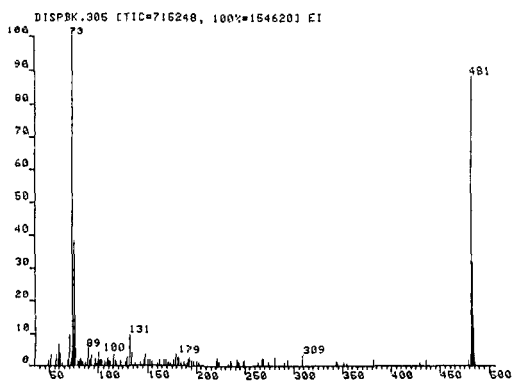
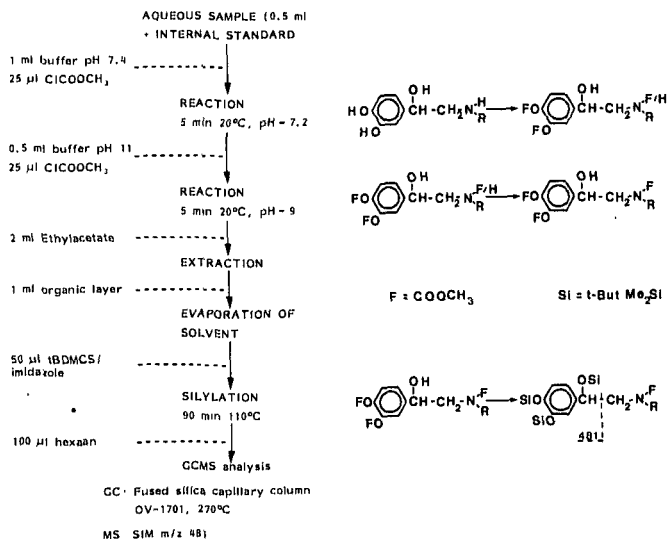


Fig.1 EI mass spectrum of Norepinephrine-O-tBDMCS-N-methyl formate

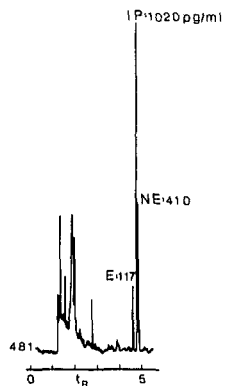
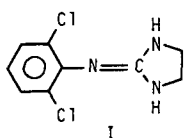


Fig.2 Sensitivity

CLONIDINE ASSAY BY SELECTED ION RECORDING-CAPILLARY GAS
CHROMATOGRAPHY-MASS SPECTROMETRY.

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Assays for the important antihypertensive drug clonidine, 2-(2,6-dichlorophenylimino)-2-imidazolidine (I) have been developed by different laboratories over the last eight years. A comparative study [1] of



these assays has appeared from which one can conclude that even now there is need for a reliable instrumental alternative to the method most frequently used i.e. radioimmunoassay. The new assay is based on capillary GCMS of underivatized I together with a deuterated internal standard and selected ion recording of $(M + H)^+$ -ions formed by ammonia

chemical ionization. Previous clonidine assays [2, 3] by GCMS had to rely on the artifact-prone on-column methylation [4] with trimethyl-aniliniumhydroxide for acceptable chromatography.

Starting with 2 ml of alkaline plasma and 10 ng ml^{-1} of clonidine- D_4 an extract of I in ethyl acetate is obtained which is purified by an additional acid base distribution. The final extract is evaporated to dryness in a stream of nitrogen and reconstituted in $20 \text{ } \mu\text{l}$ ethyl acetate. $1 - 2 \text{ } \mu\text{l}$ are injected into the capillary GC ($25 \text{ m} \times 0.31 \text{ mm}$ i.d. CPSil 5 or CPSil 5 CB; splitless injection at 70° C , ballistically heated to 230° C). Under these conditions I elutes from the column at approximately 205° C with a peak width at half height of $1.5 - 2 \text{ sec}$. The Finnigan 4021 GCMS-computer system distributes the total scan time of 0.35 sec between the signals m/z 230 and 234 at a ratio of 2:1. I-supplemented plasma samples ($0.15 - 1.25 \text{ ng ml}^{-1}$ and 10 ng ml^{-1} of clonidine- D_4) were used for calibration purposes. Calibration curves were computed by linear regression of measured peak height ratios on concentrations of I ($r = 0.99$).

The precision of the assay was 3.6% at the 0.50 ng ml^{-1} - and 5.0% at the 2.50 ng ml^{-1} -level ($n = 16$). Analysis of variance done on data at the 2.50 ng ml^{-1} -level shows that the sample work-up contributes $\geq 80 \%$ to the total variance.

0.15 ng ml^{-1} of I x HCl could be determined with a signal/background ratio of 3:1.

Plasma samples were analysed which had been supplemented with I x HCl at various concentrations unknown to the analyst. The determined concentrations deviated from the "true" concentrations by -2.7 %. The assay was used to monitor I after single oral doses of 150 µg clonidine x HCl. Excellent agreement between I-concentrations determined by the new GCMS-assay and by radioimmunoassay [1] from these "real life"-samples was observed.

While the new method based on GCMS (20 plasma samples per working day) can not compete with the radioimmunological assays for speed and lower limit of detection, it represents a reliable instrumental alternative for cross checking purposes once clonidine has to be assayed by two independent procedures.

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RECENT DEVELOPMENTS IN SSMS

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Spark source mass spectrometry is still a useful tool for simultaneous semi-quantitative determination of trace impurities in solids. We want to describe some modifications of the standard MS 7 apparatus, microdensitometer and the improvements observed.

. The modification and control of the R.F. spark circuit (damped discharge mode) and a convenient counter electrode system are used for localized analysis. Some applications for determination of trace level impurities in thin film (electronic devices) and microsamples analysis (corrosion, geological and biological samples) will be shown.

. The influence of fringing magnetic field is known and has been increased by the adaptation of a Hall probe control system. So, we have built a complete internal shield of the optic from source to magnet ; the improvement observed at low masses is very important for non selective transmission and focalisation.

. The standard M D₃ (CDC) microdensitometer has been completely modified, all electronic, mechanic and optical density measurement devices are changed. An APPLE II mini computer is designed to control the microdensitometer measurements, calibration and data reduction. A fast reading of each exposure is stored and then only intensities of useful mass are calculated ; the photoplate calibration program makes a dialog between operator and the theoretical determination. A continuous control of the computer by operator allow erroneous data to be eliminated at various stages of the processing even with complex lines.

RELATIVE SENSIBILITY COEFFICIENTS FOR ALCALI HALIDES IN SPARK MASS SPECTROMETRY

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As other analytical mainly spectroscopic methods spark source mass spectrometry lifes the problem of sensibility coefficients. The classical application of SSMS to elemental analysis makes this point of real interest. This coefficient can be briefly resumed as the ratio of measured to real concentration. Its relative value being used ratioing the concentration of an element A to another element X in a matrix Z

$$(S \frac{A}{X})_Z = \frac{C_A/C_X \text{ meas.}}{C_A/C_X \text{ real}} Z \quad (1)$$

Three factors are mainly involved in this value:

I) ion production in the spark source II) ionic transmission and discrimination
III) photoplate detection and reading of resolved ionic intensities

Factor II can be experimentally normalized for the various elements in a comparative study with careful instrumental procedures.

Factor III is usually encircled via the various handling ways of the photoplate detection. Ion production seems than to be the critical one:

mechanisms of vacuum discharge play an important role and eg (1) can be written as

$$(S \frac{A}{X})_Z = k (\text{atomization, vaporization})^a (\text{ionization})^b \quad (2)$$

Sensibility coefficient of alcali metal (A) to halogen (X) using electrodes of homogeneous mixtures of alcali halides (AX) mixed at 5 at % A with carbone graphite (Z) have been measured using AEI MS 702 R Mass spectrometer, photoplate Ilford Q₂ detection and Jarell Ash ID 57 microdensitometry. Parameters for sparking and careful readings of blackenings have been described elsewhere (¹)

The classical formula (3) has been used.

$$(S \frac{A}{X})_Z = \frac{(E_i Mc)_X}{(E_i Mc)_A} F \quad (3)$$

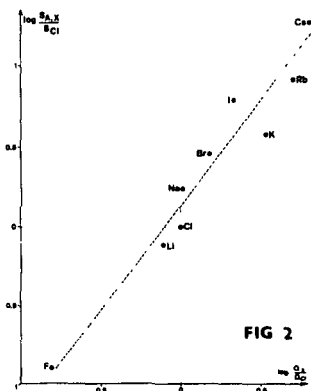
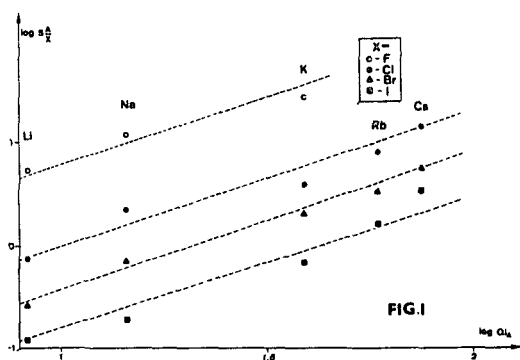
E: Experimental exposure of the blackening reference point divided by the half height width of the ionic density profile.

i: isotopic abundance

c: atomic fraction

M: appartent mass

F: mass, energy, polycharge correction



The physical significance of these values (2) clearly appears in the evolution versus the metal ionization cross section values (9) as plotted in fig. 1; this parallel evolution for the alkali metals in the various halides leads to a single common straight line after arbitrary normalization to chloride (sensitivity coefficient equal to 1,0) as in fig. 2.

The simple relationship

$$\frac{S_{X,A}}{S_{Cl}} = \frac{Q_{X,A}}{Q_{Cl}}^{1,39} \quad (4)$$

holds for the alkali halides studied and can be extended to other similar data obtained by Sasamoto et al. (3). Other ionization cross section values can be selected from literature data and Konishi et al. (4) obtained a rather similar equation with exponent 1,6.

Other factors are than of lower importance.

1) Polycharge ion rate is rather irreproducible; statistical analyses of the alkali halides give negligible value for Li and Na than rising for heavier metals ($K=0,04$, $Rb=0,05$, $Cs=0,09$) negligible value for F than rising for heavier halogenes ($Cl=0,22$, $Br=0,24$, $I=0,30$). These results can be taken into account for calculation with eq (3).

2) Atomization term can be approached via the element sublimation heat ΔH_S .

These two effects can complete eq (4).

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Int. J. Mass. Spectrom. Ion. Phys., 9 (1982) 33

FORMATION OF MOLECULAR IONS DURING SPARKING OF BINARY ALLOYS

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Molecular ions appear with very weak intensities in spark source mass spectra, and they have received little attention so far, being commonly considered as interferences in the spectra. Our research is aimed at studying their appearance characteristics in vacuum spark discharge. Series of Cu-Al, Cu-Ag and Cu-Au alloys were sparked in an AEI 702-R mass spectrometer under standard conditions⁽¹⁻³⁾. Photoplate detection was used in the first two series, whereas molecular ions were registered electrically in copper-gold alloys. Most of the information obtained concerning molecular ion production mechanism was gained by:

i) examining the evolution of molecular ion intensities as a function of alloy composition (for this purpose, intensities of the ions were corrected by an appropriate detection factor and normalized so as to take into account the obvious dependence upon composition (fig. 1 and 2).

ii) studying the influence of some discharge parameters, such as spark voltage or electrode gap width. The electrical detection mode was particularly helpful in monitoring these parameters.

Formation mechanism of molecular ions

The complexity of the processes occurring in a spark discharge makes any simple model of molecular ion production somewhat unrealistic. However, two mechanisms already proposed in earlier works⁽⁴⁾ can be summarized as:

(a) formation in the spark plasma; (b) direct ejection from the solid.

A number of results leads us to consider the second mode as the dominant one. Molecular ions tend to disappear when the electrode gap is reduced. Their intensities also decrease as the average mass of particles in the plasma becomes higher (see, e.g., fig.2). In addition, an explosive discharge, as occurs in the copper aluminium sample of 44 % at. Al composition, gives rise to an overall sharp decrease of molecular ion intensities (fig. 1).

Whether ejection from the solid corresponds to vaporization of neutral species (with a further ionization) or to sputtering processes can be decided taking into account the following observations: pulse length and repetition frequency, which influence the temperature of the solid and its vaporization rate, have no effect on molecular ions. On the contrary, their appearance is enhanced by increasing spark voltage. Finally, reducing intensities to ionic fractions (i.e., dividing intensities by an estimated value of the total charge registered) smooths Cu_2^+ and CuAu^+ curves (fig. 2b) so that the molecular

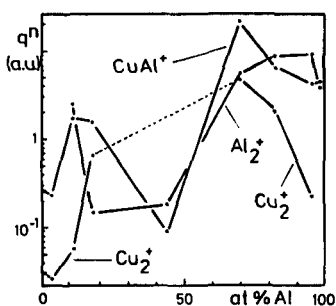


Fig. 1. Normalized intensities of CuAl^+ , Al_2^+ and Cu_2^+ ions in Cu-Al alloys.

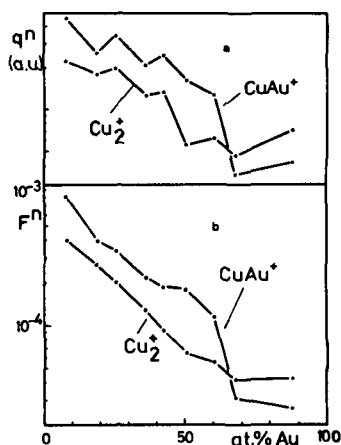


Fig. 2. (right). Normalized intensities(a) and ionic fractions(b) of CuAu^+ and Cu_2^+ ions in Cu-Au alloys.

ion energy reflects in a manner that of the monatomic ions of the plasma. All these facts support a predominance of sputtering processes in the production of polyatomic ions.

In conclusion, molecular ions detected in sparking binary alloys may be considered as originating mainly by sputtering processes due to the ions of the discharge plasma. The first series of results shows that production of molecular ions through addition reactions as proposed by Ramendik and Derzhiev⁽⁵⁾ is not of major importance in our experiments. Of course, different mechanisms may be found in sparking other types of materials⁽⁶⁾.

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- (4) J. Franzen, in A.J. Ahearn (ed.), *Trace Analysis by Mass Spectrometry*, Academic Press, New York, 1972, p. 27.
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- (6) A. Cornu, private communication;

EFFECT OF SPARK DAMPING ON THE SSMS SPECTRUM
OF SOLAR GRADE POLYCRYSTALLINE SILICON

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During the development step of a new process to produce photovoltaic silicon, the elemental concentration range of most impurities sought corresponds well to the capabilities of SSMS.

Nevertheless, solid polycrystalline silicon, likely because of some crystalline effects, is often uneasy to spark, and gives rise to numerous molecular ions Si_n^+ of strong intensity, which complicate the analysis. In the same way, sparking of raw material powders, mixed with high purity graphite to meet the electrode requirements, produces many Si_nC_m^+ ions, which overload the mass spectrum.

The occurring of molecular ions on the photoplate can be reduced by spark damping, as noticed in 1977 by BERTHOD* on copper and steel samples. This alteration consists in setting the spark at a damped discharge mode instead of an oscillating mode. This is easily done with an AEI MS 702 spectrometer by mounting 2 resistors of suitable value (between 500 and 600 Ω) in the high voltage supply circuit of the electrodes, between each of them and the secondary of the Tesla transformer.

Figure 1 illustrates the change in aspect of the spectrum of a solid polycrystalline silicon sample, before and after damping. The relative intensity of $^{28}\text{Si}_n^+$ molecular ions is reduced by a factor of 10 to 1000, according to the value of n. In addition, Si_n^{2+} doubly charged molecular ions, which are always visible in the solid silicon spectrum before damping, are no longer detected after damping.

Figure 2 reveals the influence of damping on the relative intensity of $^{28}\text{SiC}_n^+$ ions, measured on the spectrum of a powdered sample mixed with graphite.

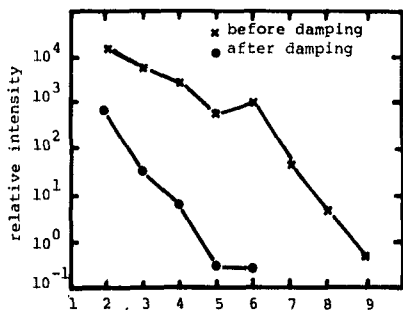


Fig.1 - Relative intensity of $^{28}\text{Si}_n^+$ ions in solid polycrystalline silicon.

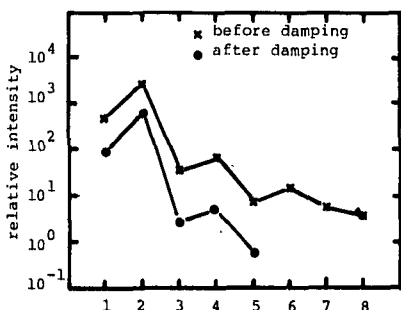


Fig.2 - Relative intensity of $^{28}\text{SiC}_n^+$ ions in silicon powder mixed with graphite.

The decrease in the number and the intensity of molecular ions result in an improvement of the resolving power and the detection limit for strongly interferenced elements, such as magnesium, calcium, chromium, iron, nickel, cobalt, strontium in solid or powdered silicon. The table shows the detection limits attained on these elements before and after damping.

* BERTHOD, Thesis, 1977, GRENOBLE.

	SOLID SILICON		SILICON POWDER	
	before damping	after damping	before damping	after damping
Ca			5	0.1
Ti			0.4	0.05
Cr			2.5	0.1
Fe			5	0.2
Ni	1.5	0.3		
Co	12	0.04		
Zn			0.6	0.05
Sr	5	0.05		
Y	0.4	0.04		
Ce	30	0.2		

Detection limits of strongly interferenced elements
p.p.m., atomic.

Analysis of both raw material powder and solid samples taken at various levels in the ingots reveal the purification and segregation effects obtained by melting and controlled cooling (table below).

As a conclusion, spark damping allows to improve the help provided by SSMS in some steps of a photovoltaic silicon elaboration process. In addition, the analytical results are more rapidly available than by any other method.

	RAW MATERIAL POWDER	INGOT (SOLID SAMPLE)
B	43	20
F	20	0,05
Na	11	0,7
Mg	8	0,1
Al	75	0,5
P	12	1,5
Cl	2	0,4
S	7	0,07
K	4	(0,04-0,2)
Ca	5	0,05
Ti	1	0,05
Cr	5	0,04
Mn	0,3	0,03
Fe	4	1
Ni	3	0,8
Cu	16	0,1
Zn	1,5	0,1

RPR 15

CONTROL OF SSMS APPARATUS WITH
A PERSONAL COMPUTER

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BP N°6 92260 FONTENAY AUX ROSES - FRANCE

The activities of our SSMS Lab about analytical allowances are characterized by the diversity of received samples.

In order to improve the analytical result, while avoiding automation's constraints, we have developed some control gears of our apparatus.

We describe here the electronic and physical improvements of a double focusing mass spectrometer, Thomson THN 212, made so as to use either electrical or photographic detection. The spectrometer's control (spark parameters, magnetic field, ion current, instantaneous readout or integration) is achieved by a personal computer.

We present also the interface allowing connection between our microdensitometer (Joyce Loebl MK 3) and a minicomputer. When the program is running, the operator chooses the lines to be measured; the computer controls the densitometer's mechanism gets data and assumes data treatment, including calculation and plotting of calibration curves. An analytical report is printed at the end of densitometry.

All the devices presented can be driven either by Hewlett-Packard HP 9815 or HP Series 80 (85-86-87).

A HIGH SENSITIVITY PROCEDURE FOR CHROMIUM USING SILICA GEL-BORIC ACID IONIZATION ENHANCEMENT; K. A. Brletic, L. J. Powell, E. L. Garner; National Bureau of Standards, Center for Analytical Chemistry, Inorganic Analytical Research Division, Washington, DC 20234.

A previously reported thermal ionization isotope dilution procedure for chromium (1) required a minimum sample loading of 3-5 micrograms. This relative high sample size requirement in combination with poor ionization efficiency and large analytical blanks limited the application of this technique to the trace level. Since many of the samples of interest contain chromium at or near the ultra-trace level, the insensitivity of this method became a major hindrance to high accuracy concentration measurements at this level. A new procedure which has improved the instrumental sensitivity by more than a factor of 50 has been developed. This method involves the sequential drying of silica gel, chromium (III) nitrate and boric acid onto a rhenium filament. The filament is then transferred to a nitrogen atmosphere where it is heated to 830 °C, using an optical pyrometer to allow precise reproduction of the filament temperature from sample to sample. This procedure yields ion currents of $3\text{-}5 \times 10^{-11}$ A for an optimal sample loading of 250 nanograms of chromium. Minor modifications to the previously reported chemical separation procedure coupled with increased sensitivity of the mass spectrometric procedure has led to a 5 fold reduction in the variability of the analytical blank, which has greatly enhanced the ability to analyze nanogram quantities of chromium.

This procedure has been applied to a wide variety of matrices including environmental, biologicals, and metals. Table 1 shows the results obtained for Cu X, one of the NBS Standard Reference Materials (SRM's) in the Copper Benchmark Series. Samples 1 and 12 represent different ends of the copper bar; whereas compositions 1 and 2 are composite samples from the bar and, as a result, are in good agreement. Table 2 shows the results from six bottles of SRM 1643b, Trace Elements in Water. The data are in good agreement and that would be expected for a homogeneous matrix such as water. Note that the level of chromium in the copper samples is at the 200 microgram per gram level whereas the chromium in the water is less than 20 nanograms per gram. This wide range of concentration (four orders of magnitude) clearly demonstrates the applicability of this technique to a large spectrum of samples.

Table 1. Chromium in Cu X

<u>Sample No.</u>	<u>µg Cr/g</u>
1	215.6
12	217.2
Comp 1	216.0
Comp 2	216.5

Table 2. Chromium in SRM 1643b, Water

<u>Sample No.</u>	<u>Concentration ng Cr/g</u>
1	18.71
2	18.68
3	18.75
4	18.57
5	18.63
6	18.84

Mean = 18.7
S.D. = 0.1 (0.5%)

- (1) Dunstan, L. P. and Garner, E. L., "Trace Substances in Environmental Health-XI," D. D. Hemphill, ed., 334-337, 1977.

DETERMINATION OF SELENIUM AND TELLURIUM IN NBS COPPER BASE SRM's BY SSMS ISOTOPE DILUTION.
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Analytical Chemistry, Inorganic Analytical Research Division, Washington, DC 20234.

The concentrations of selenium and tellurium were determined by isotope dilution spark source mass spectrometry in four NBS copper base Standard Reference Materials (SRM's), SRM C1251-C1253 and SRM 454. Samples were spiked with ^{82}Se and ^{125}Te , dissolved in HNO_3 in an open beaker, evaporated to dryness and redissolved in 5 N HCl. Selenium and tellurium were pre-concentrated and isolated from the copper chloride solution by reduction with hypophosphorous acid to the elemental form along with a gold carrier (1). This precipitate was centrifuged, washed, dried, and then mixed with 30 mg of 99.9999% of pure gold powder in a Wig-L-Bug mixer. Flat electrodes were pressed from the gold and the Se and Te isotopic ratios measured on the SSMS using electronic detection. Table 1 gives the values for Se and Te as determined by SSMS isotope dilution.

Table 1. Se and Te in Copper Standards

SRM	Ave. Conc. of Se		Ave. Conc. of Te	
	$\mu\text{g/g}$	S.D.	$\mu\text{g/g}$	S.D.
1251	11.3	0.83	14.3	0.92
1252	53.7	0.62	49.4	0.81
1253	167	7.8	196	3.7
454	479	7.6	27.2	0.2

The Se values determined on all four SRM's were found to be higher by an average of 20% compared to those values reported by other techniques. In order to test for errors arising from selenium losses prior to spike equilibration, SRM 454 (with the largest difference) was analyzed using a sealed tube technique. The spiked copper sample, the spike calibration mixture, and even the natural selenium used for the spike calibration were all dissolved and equilibrated in sealed Carius tubes held at 200 °C for 16 hours. The results of the sealed tube analysis and open beaker analysis of Selenium in SRM 454 are given in Table 2. The close agreement between these two techniques indicates that the original open beaker analysis was not subject to losses which affect analytical accuracy.

Table 2

Bottle	Se in SRM 454	Se in SRM 454
	Sealed Tube ($\mu\text{g/g}$)	Beaker ($\mu\text{g/g}$)
1	481	482
2	478	481
3	473	476
4	491	474
4	476	
2	478	
Average = 480		479

- (1) P. J. Paulsen, L. P. Dunstan, and M. G. Diaz, 25th Ann. Conf. of Mass Spectrometry and Allied Topics (1977), pp. 703-704.

RPB 18 | RAPID ELEMENTAL ANALYSIS USING A PLASMA
DISCHARGE MASS SPECTROMETER
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Winsford, Cheshire, CW7 3 BX, U.K.

A cold cathode glow discharge has been used as the ion source for a magnetic sector mass spectrometer. Such an instrument should prove to be an attractive alternative to the spark source type of mass spectrometer.

The use of various types of glow discharge ion sources has been suggested by a number of workers. The principal of operation is that the sample to be analysed forms the cathode of an abnormal glow discharge in a gas such as argon. Positive gas ions are accelerated towards the sample in the cathode fall region and impact with the sample at energies of a few hundred eV. Sample material is sputtered mainly as neutral species which diffuse through the discharge and are ionised by collision with gas and electrons. Positive ions are extracted from the discharge region through a small slit and are focussed into a sector mass spectrometer.

As an ion source the glow discharge retains the advantages of the spark source, i.e. a small range of relative sensitivity factors, small matrix effects and bulk sampling. In addition the ions produced have a much lower energy spread permitting the use of simpler ion optics, and high speed magnetic 'peak jumping' type operation.

Trace element analysis of a number of different types of material have been carried out which show that detection levels for most elements are in the range 10-100 ppba with spectra obtained in a matter of minutes.

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THE THERMODYNAMIC PROPERTIES OF THE RU-O SYSTEM

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Ruthenium dioxide is noted for its high electrical conductivity which is comparable to that of the noble metals. Because of this important property, RuO_2 has found its way into the fabrication of conductive glasses. RuO_2 may undergo significant decomposition during the firing process which may seriously limit its usefulness for very high temperature glasses. The only previous work on RuO_2 was above 1400°K (1). These studies have shown that RuO_2 decomposes to the metal and O_2 gas, where the O_2 pressure varied from 0.001 to 1.0 atmosphere. Our studies were concerned with the low temperature region from 900 to 1400°K , the range where these conductive glasses are prepared.

Two forms of RuO_2 were studied. The first was a powder from A. D. Mackay Chemicals with an average particle size of $12\ \mu\text{m}$. This powder was shown by X-ray diffraction to be stress-free and had a nearly perfect tetragonal lattice structure. The second was a single crystal synthesized and fully characterized in our laboratories. The RuO_2 samples were heated in a quartz-lined molybdenum Knudsen cell. The cell temperature was measured by a Pt-10Rh vs Pt thermocouple. The modulated molecular beam was electron impact ionized at 30 eV and mass analyzed in a quadrupole mass spectrometer. An IBM S/1 computer was employed for control of the temperature and mass spectrometer and also for measurement of the ion signals (2).

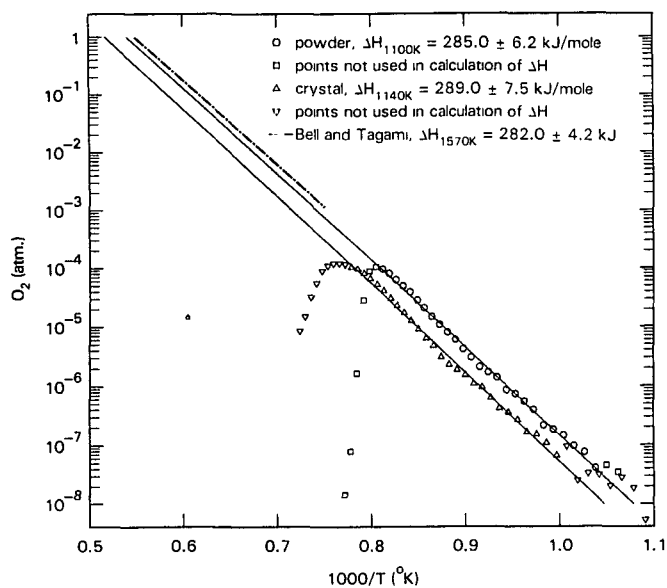
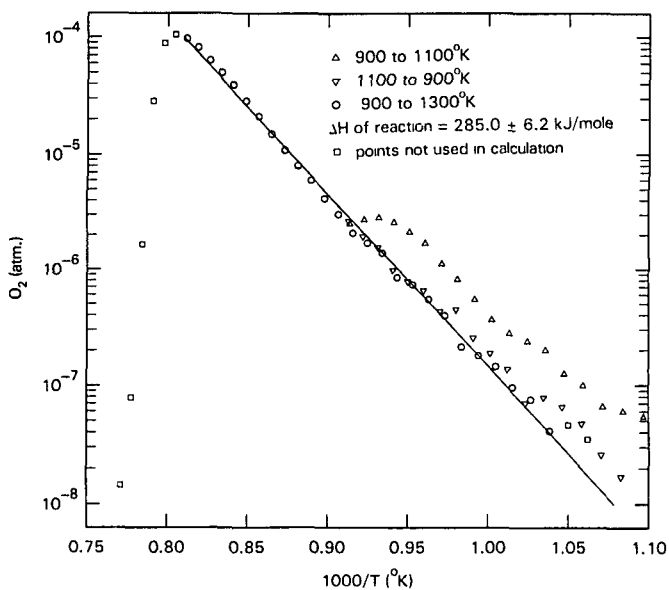
Both the powder sample and the single crystal were heated from 300 to 1400°K at $4^\circ/\text{min}$. We observed during the first heating of the powder sample that the O_2 pressure initially desorbed at a rate higher than the equilibrium pressure. This excess pressure indicated the powder has a stoichiometry somewhat greater than two. This excess oxygen was removed in the second runs on both the powder and crystal samples by first heating to 1100°K , cooling to 900°K followed by the final heating to 1400°K . These latter results for the powder sample are shown in Fig. 1.

Two different sample sizes were studied for both the powder (6.07 and 16.2 mg) and the single crystal (10.95 and 84.6 mg). The weight loss agreed remarkably well with the value predicted by the decomposition reaction: $\text{RuO}_2(\text{s}) = \text{Ru}(\text{s}) + \text{O}_2(\text{g})$. The values range from 24.71 to 25.45% while the predicted value is 24.05%. The higher value determined experimentally can be attributed to small amount of both H_2O and CO_2 which also desorbed. The O_2^+ intensities were converted to pressure by integration of the ion signals and relating that to the weight loss measurement. Also by integrating the ion signal during the first heating up to 1100°K we were able to determine the amount the powder sample is off stoichiometry. The powder sample composition is $\text{RuO}_{2.005}$.

The two runs for the powder overlay each other precisely. This is also true for the single crystal experiments. However, the O_2 pressure is about three times lower for the crystal sample compared with the powder. This cannot be attributed to sample size since no difference was observed with an increase in size of 8 times. The only reasonable explanation is that the fine powder is inherently more unstable than the single crystal material. For the four runs, we found the heat of decomposition of RuO_2 to be $288 \pm 8\ \text{kJ mole}^{-1}$. This result is in excellent agreement with the transpiration measurements of Bell and Tagami (1). Our results for the powder and crystal samples are compared with those of Bell and Tagami in Fig. 2.

1. W. E. Bell and M. Tagami, J. Phys. Chem. **67**, 2432 (1963).

2. M. A. Frisch and W. Reuter, J. Vac. Sci. Technol. **16**, 1020 (1979).



HOW START THE BREAKDOWN IN THE RF SPARK SOURCE OF A MASS SPECTROMETER - A NEW APPROACH

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The main difficulties in obtaining quantitative results in SSMS are involved by the lack of knowledge of the phenomena which produce the ions in the high voltage and high frequency spark. Very few modifications of the basic system have been performed during the last decade, the spectrometer being used in "good standard conditions" allowing the best apparent constancy of several parameters ensuring the best transmission of the ions compatible with the best mass resolution. This lack of improvement is certainly responsible of the decline observed in the field.

The true mechanism of the spark's beginning known from the works of Honig¹, Evans and Morrison², Franzen³ may be certainly found in the interaction of the electronic current induced by field emission.

In order to approach the mechanism of the initiation of the breakdown and then the creation of different ion species, we have studied the voltages and the electronic currents involved in the initiation of the breakdown by the spectroscopy of hard X-rays produced by the electrons accelerated between the electrodes.

For each choice of the maximum RF voltage, the spark is initiated by narrowing the electrodes and then separating them in order to reduce the production of ions (controlled by the monitor of the mass spectrometer) to a constant level which is as low as possible. From this moment, no readjustment of this maximum gap between electrodes is made during the recording of the full X-ray spectrum. If the stability of the discharge is not sufficient to collect the full spectrum is one run, the spectrum is erased and the procedure is begun again, more than 20 trials are sometimes necessary. Under these conditions each detected photon has been produced in the same geometry of the experiment.

The spectroscopy of the X-rays emitted during the spark have been interpreted as a composition of 3 different electronic emissions at high voltages. The first, at the highest voltage produced by field emission in the secondary coil of the RF (open) circuit, is responsible for less than 1 % of the total electronic current and is attributed to field emission between "flat" electrodes. A second characteristic voltage, lower than the first, arises when the spark is initiated also by field emission at a sharp point : it corresponds up to 10 % of the current-voltage diagram for these two processes. The third, nearly independent of the applied RF voltage, follows one of these two first and gives rise to more than 90 % of the current (from thermoelectronic origin) which initiates the "heating" of the electrodes and the start of the ion emission⁴.

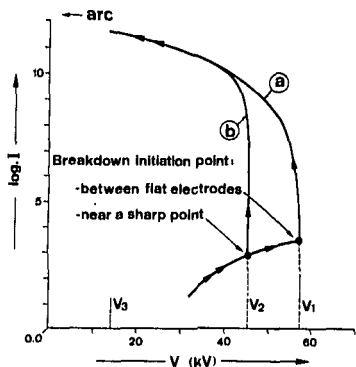


Fig. 1 : Current-voltage diagram of field emission at maximum and reduced voltages V_1 and V_2

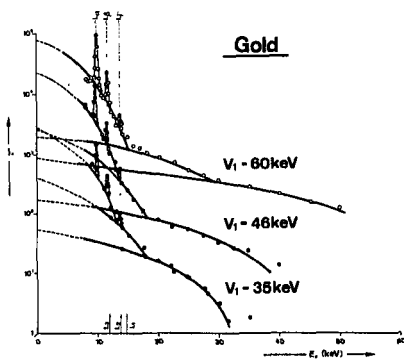


Fig. 2 : X-ray spectra recorded during sparking of gold at three different RF voltages.

The shape of a bremsstrahlung spectrum produced by monoenergetic electrons eV_0 on a material of atomic number Z is linear and given by

$$I_x(v) = Kiz (v_0 - v) \quad (I)$$

where K is a constant independent of V_0 , $iz = q$ is the total electronic charge involved and v is the maximum photon frequency related to the maximum electron energy by

$$hv_0 = eV_0 \quad (I)$$

For harmonic oscillation of the high voltage, induced in the third phenomenon :

$$I_x(v) = \frac{2}{\pi} Kq_3 v_3 \left[\sin(\cos^{-1} \frac{v}{v_3}) - \frac{v}{v_3} \cos^{-1} \frac{v}{v_3} \right] \quad (II)$$

Calculation of the X-ray spectra is in fairly good agreement with the experimental spectra. Cut off of the third characteristic voltage is also explained by the ratios of characteristic X-rays lines of elements used as electrodes. Figure 2 illustrates the situation for gold sparked at 3 different V_{RF} voltages. Relative intensities of X-ray lines confirm the cut-off at V_3 .

The appearance in the X-ray spectra, recorded in a well-defined geometry of the electrodes, of intense characteristic hard X-ray lines indicates that electronic vacancies are created in the deepest shells of the atom. These highly excited states must give rise to polycharged ions. Correlation of X-ray intensities with relative intensities of the polycharged ions collected on the photoplate of the mass spectrometer is reported on Figure 3. The number of ions shearing a high charge is, in each case, much greater when the geometry of the electrodes is set so as to increase the X-ray emission (large gap). It seems, therefore, that an important fraction of the polycharged ions produced in the spark are generated from the solid itself during the beginning of the breakdown procedure. This last observation takes also into account the fact that the production of X-rays and polycharged ions is more probable when the pulse duration is reduced from 200 μs to 25 μs , but for the same number of ions in the mass spectrometer.

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- 4.- G. Demortier, L. Lamberts, International Journal of Mass Spectrometry and Ion Physics, 31 (1979) 135-150.

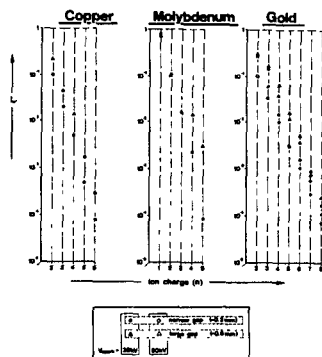


Fig. 3 : Intensities of polycharged ions for different conditions of sparking.

CHARACTERIZATION OF GLUCURONIDES BY THERMOSPRAY LC-MS

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The thermospray LC-MS technique is used in this work for the direct analysis of a class of non-volatile polar substances. Glucuronides were chosen as our class for investigation because they are formed in the metabolism of most xenobiotic substances and because glucuronidation is the most common mammalian conjugation pathway. In this work we report on the separation and positive and negative ion mass spectra of three model glucuronides. We also report on the separation and mass spectra of diastereomeric glucuronides of propranolol formed by *in vitro* incubation of racemic propranolol with immobilized rabbit glucuronyltransferase.

Unlike many of the currently available interfaces, the thermospray ionization interface allows 100% of an aqueous eluent to enter the mass spectrometer at flow rates up to 1.5 ml/min. In addition, when the mobile phase contains a volatile buffer (e.g., ammonium acetate or formic acid) in solution, no external ionizing source is required for detection of solutes.

The design and operation of the interface was essentially the same as that reported previously (1) except that a cold finger trap (charged with a dry ice-acetone mixture) was placed between the mechanical pump and source to eliminate the daily changing of pump oil. Briefly, buffered solvent pumping through a heated vaporizer forms a jet of small droplets, a small percentage of which have net positive or negative charges. The droplets totally disappear in crossing from the vaporizer to the pumping port due to a combination of solvent evaporation and charge density induced disruption. Ions of both solute and solvent are formed in a ratio approximating that seen in chemical ionization.

Positive and negative ion mass spectra are presented for the four glucuronides in Tables I and II, respectively. Molecular ion species are observed in the positive ion spectrum as either the protonated or ammonium ion (from buffer) adduct, and with greater intensity than (M-H)⁻ ions in the negative ion spectra. Sensitivity for the molecular ion in the positive ion mode for a full scan is in the 10-100 pmole range. Fragment ions occur at masses corresponding to protonated or ammoniated aglycones and to ammonium attachment to the sugar portion of the molecule. It should also be noted that the ammoniated sugar fragment ion (mass 194) is formed from each of the four compounds studied here and may represent a class characteristic ion for thermospray analysis of glucuronides.

TABLE I

Positive Ion Mass Spectra of the four Glucuronides

Compound	glucuronide ions (RI)	aglycon ion (RI)	sugar fragment ions (RI)		other ions (RI)
p-nitrophenyl-β-D-glucuronide	333* (see Table II) (69)	157* (20)	194* (100)	177 [†] (3)	149 (4)
4-methylumbelliferyl-β-D-glucuronide	370* 352 [†] (52) (16)	177 [†] (100)	194* (84)	177 [†] (100)	
8-hydroxyquinoline-β-D-glucuronide	322 [†] (74)	146 [†] (100)	194* (48)	177 [†] (3)	304 (4)
propranolol-β-D-glucuronide	436 [†] (100)	260 [†] (90)	194* (7)		418 (7)

TABLE II

Negative Ion Mass Spectra of the Four-Glucuronides

Compound	glucuronide ions (RI)	aglycon ion (RI)	sugar fragment ions (RI)	other ions (RI)
p-nitrophenyl- β -D-glucuronide	314 [¶] (100)	138 [¶] (17)	175 [¶] (5)	
4-methylumbelliferyl- β -D-glucuronide	351 [¶] (62)	175 [¶] (100)	175 [¶] (100)	
8-hydroxyquinoline- β -glucuronide	320 [¶] (100)		175 [¶] (100)	
propranolol- β -D-glucuronide	434 [¶] (100)			

* ammoniated ion

† protonated ion

¶ hydride loss ion

When racemic propranolol was incubated with immobilized rabbit liver glucuronyltransferase and a portion of the extract was injected onto the LC-MS, the following RIC and UV traces were obtained (Figure 1). As can be seen in Figure 1 there appears to be little loss in resolution when going from the UV detector (280 nm) to the mass spectrometer.

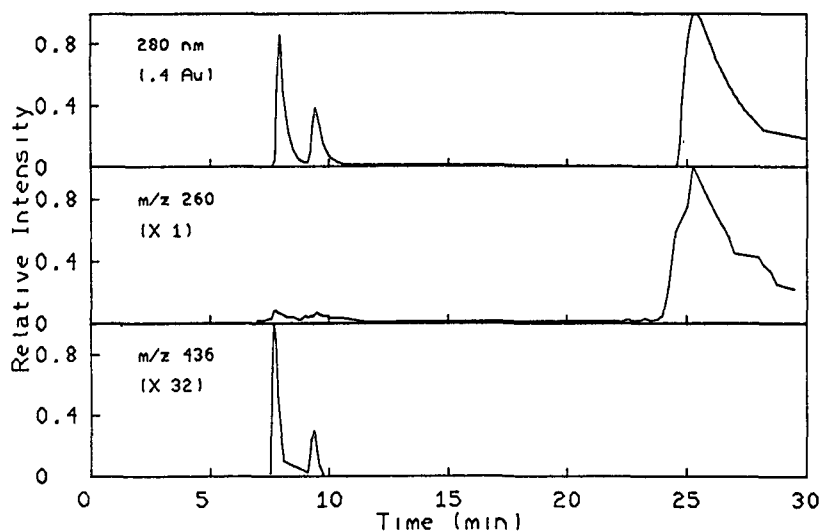


Figure 1: Separation of diastereomeric glucuronides of racemic propranolol and propranolol; 47.5% MeOH/52.5% (0.05 NH_4OAc), 1 ml/min.

(1) C.R. Blakley and M.L. Vestal, *Anal. Chem.*, **55**, 750 (1983).

ANALYSIS OF MODIFIED NUCLEOSIDES BY COMBINED LIQUID CHROMATOGRAPHY - "THERMOSPRAY" MASS SPECTROMETRY; C. G. Edmonds and James A. McCloskey Departments of Medicinal Chemistry and Biochemistry, University of Utah, Salt Lake City, Utah 84112

Transfer RNAs are the functional intermediaries of protein biosynthesis at the ribosome. These relatively small nucleic acids, typically containing 70 to 80 nucleotide units are particularly characterized by a complex secondary structure and a high proportion of post-transcriptionally modified residues. The simplest modifications arise from methylation of the base or the 2'-OH of the ribose moiety but an increasing number of so-called hyper-modifications are now recognized which arise from much more elaborate transformations. A specific modified residue, of the more than 50 so far identified, may occur once-per isoaccepting tRNA molecule and that molecule may typically contain 5-15 such residues. Thus, the investigation of modification of known and unknown structure in tRNAs requires a multicomponent analysis where the compounds of interest may constitute 1-2 percent of the mixture and where total sample is normally limited (1-100 μ g). In this context, direct analysis of nucleoside mixtures obtained by enzymatic hydrolysis by combined liquid chromatography mass spectrometry offers significant advantages over conventional chromatographic methods which rely solely on chromatographic mobility for identification, or over MS and GC/MS procedures which require preparation of volatile derivatives of frequently sensitive chemical entities.

We present examples of the utility of a combined LC/MS instrument operating on the "thermospray" principle in such investigations. Details of the instrument configuration and performance (1,2) and of the "thermospray" ionization process (3,4) have appeared elsewhere. With the exception of the most complex modifications, the $8H_2^+$ and MH^+ ions predominate in the positive ion "thermospray" mass spectra of nucleosides. At lower levels of sample cationized alkali metal adduct ions may be observed. The appearance of the mass spectrum is generally somewhat dependent on sample concentration, with MH^+ ion abundance improving at higher levels. Guanosine and its derivatives have so far proven conspicuous by their low MH^+ ion responses though these are compensated by higher BH_2^+ abundance. Instrument performance, both in respect of mass spectral appearance and sensitivity, is a sensitive function of solvent flowrate and composition and the temperature chosen for operation of the vaporizer and transfer line. Buffers of 0.1M ammonium acetate at pH 5.6 with MeOH as organic modifier in the range of 0-20% at flowrates of 1.5 ml/min with vaporizer temperatures of 255-275°C have proven optimum and typically afford measured sensitivities of the order of 10⁻¹¹ coulombs/g of adenosine at m/z 268, the MH^+ ion. Isocratic conditions or solvent gradients (typically over 5-15% MeOH) are accommodated and provide useful multicomponent separations with conventional reverse phase columns.

Samples of tRNA are hydrolyzed by a two-stage process employing ribonuclease T₂ followed by a mixture of alkaline phosphatase and snake venom phosphodiesterase. The resulting nucleoside mixtures are examined directly without further preparation. Protein and constituents of enzyme preparations provide a significant background in the mass spectrometer. However, a UV absorbance monitor in series to the mass spectrometer provides a useful adjunct for registration and qualitative assessment of the chromatogram and interpretation of the mass spectra obtained. Figure 1 shows such a UV absorbance chromatogram obtained during the full-scan LC/MS analysis of 10 μ g of a hydrolysate of tRNA^{Val} of rabbit liver. This tRNA was selected as an analytical model for the assessment of modification arising in tRNA^{Val} of rat ascites hepatoma cells (5).

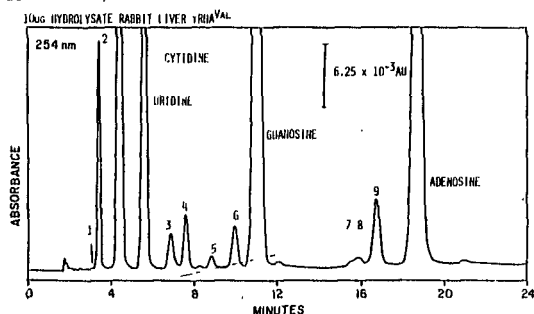


Figure 1: UV absorbance chromatogram for LC/MS analysis of rabbit liver tRNA^{Val}. LC conditions: 300 x 4 mm 10 μ μ Bondapak C-18; 0.1M NH_4OAc , pH 5.6; solvent gradient 5-15% MeOH, 0-15 min, isocratic thereafter; 1.5 ml/min.

Table 1 summarizes the mass spectrometric data obtained on all the modified nucleosides detected.

TABLE 1 SALIENT LIQUID CHROMATOGRAPHIC AND "THERMOSPRAY" MASS SPECTROMETRIC DATA ON THE MINOR NUCLEOSIDES DETECTED IN THE HYDROLYSATE OF RABBIT LIVER tRNA^{Val}.

COMP	K'	$\frac{A_{254NM}}{A_{280NM}}$	ASSIGNMENT	MASS SPECTRAL DATA				
				MH ⁺ M/Z(%)	MNA ⁺ M/Z(%)	MK ⁺ M/Z(%)	BH ⁺ M/Z(%)	OTHER IONS M/Z(%)
1	0.77	-	Dihydrouridine (1)	247(100)	269(39)	285(14)	115(-)	
2	0.88	2.49	Pseudouridine	245(100)	267(44)	223(81)	113(-)	
3	2.89	6.31	1-Methyladenosine	282(6)	-	-	150(100)	
4	3.26	0.66	5-Methylcytidine	258(2)	280(2)	296(2)	126(100)	
5	4.03	1.04	2'-O-Methylcytidine	258(6)	-	-	112(100)	164(23) 182(23)
6	4.60	2.38	7-Methylguanosine Inosine	298(-) 269(-)	-	-	166(81) 137(100)	150(15) 154(20) 168(22) 259(5)
7	7.8b	2.5	1-Methylguanosine	298(12)	-	-	166(100)	
8	8.14	2.8	2'-O-Methylguanosine	294(-)	-	-	152(100)	
9	8.49	1.79	N ² -Methylguanosine	298(1)	-	-	166(100)	

A separate MID experiment, confirming each of the modified nucleosides identified above is shown in Figure 2.

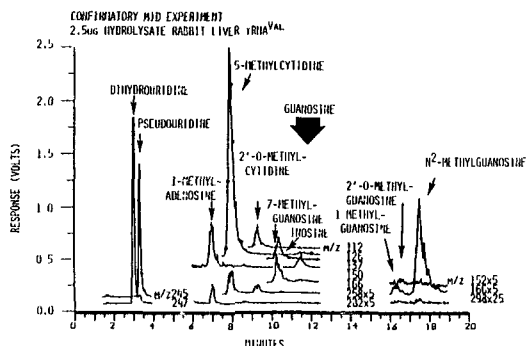


Figure 2: MID ion current profiles for the LC/MS analysis of rabbit liver tRNA^{Val}. LC conditions: same as Figure 1.

Combined liquid chromatography-"thermospray" mass spectrometry is providing a useful tool for the analysis of mixtures of nucleosides derived from tRNAs. Efforts to improve and refine instrument performance continue.

Acknowledgment: We are pleased to acknowledge the continuing contribution of our principal instrumental collaborator, Dr. M. L. Vestal, University of Houston. Dr. Susumu Nishimura, National Center Research Institute, Tokyo, Japan, provided tRNA samples and important biochemical expertise and is gratefully acknowledged.

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ANALYSIS OF PERBENZOYLATED NEUTRAL GLYCOSPHINGOLIPIDS BY AMMONIA CI LC/MS

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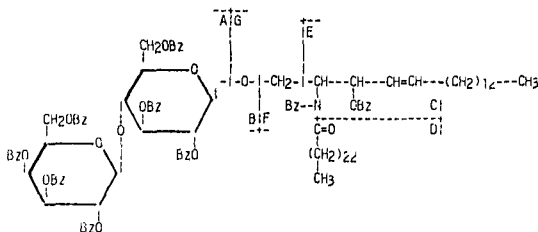
HPLC analysis of perbenzoylated glycosphingolipids with 230 nm detection, a procedure developed in this laboratory, is utilized routinely for assay of glycosphingolipids from a variety of tissues. While this procedure is highly sensitive it provides only chromatographic retention time as an indication of glycolipid composition. Our objectives in this study have been to define conditions for obtaining useful mass spectra of perbenzoylated neutral glycosphingolipids from a moving belt transport type LC/MS interface. Off-line and/or on-line mass spectrometric analysis of these high molecular weight thermally labile derivatives would facilitate the characterization of many tissue glycosphingolipids.

We have obtained spectra of a number of benzoylated GSL standards including glucosyl-N-stearoyl-DL-dihydrosphingosine, glucosyl-N-lignoceroyl-DL-dihydrosphingosine, bovine brain nonhydroxy fatty acyl galactosylceramide, bovine brain hydroxy fatty acyl galactosylceramide, and lactosyl-N-lignoceroyl-DL-dihydrosphingosine. Spectra were acquired with a Finnigan 4000 mass spectrometer under ammonia CI conditions as follows: ion source temperature 230°C, ion source pressure 0.45 Torr, electron energy 15 eV, LC/MS evaporator temperature 300°C. Approximately 2 µg of each derivative was applied directly to the belt in chloroform solution. Fragment ions from the ceramide, fatty acid, long chain base and carbohydrate moieties were identified. The hydroxy and nonhydroxy fatty acyl containing GSL produced distinct fragmentation patterns presumably due to the presence of the N-benzoyl group in the nonhydroxy fatty acid GSL. Spectra obtained with mixed galactosylceramides from bovine brain contain ions that reflect the ceramide composition of the mixture.

A proposed fragmentation pattern for perbenzoyl lactosylceramide is shown in Figure 1 and the spectra obtained for perbenzoyl N-lignoceroyl-DL-dihydrolactosylceramide is shown in Figure 2. Most of the major ions for all of the derivatives could be rationalized from the proposed fragmentation pattern. The proposed ion compositions for the benzoylated glycolipids analyzed are given in Table 1.

We conclude from these studies that the inertness of the polyimide belt and the relatively rapid thermal desorption of the sample from the belt makes it possible to obtain useful CI spectra of these derivatives.

Figure 1



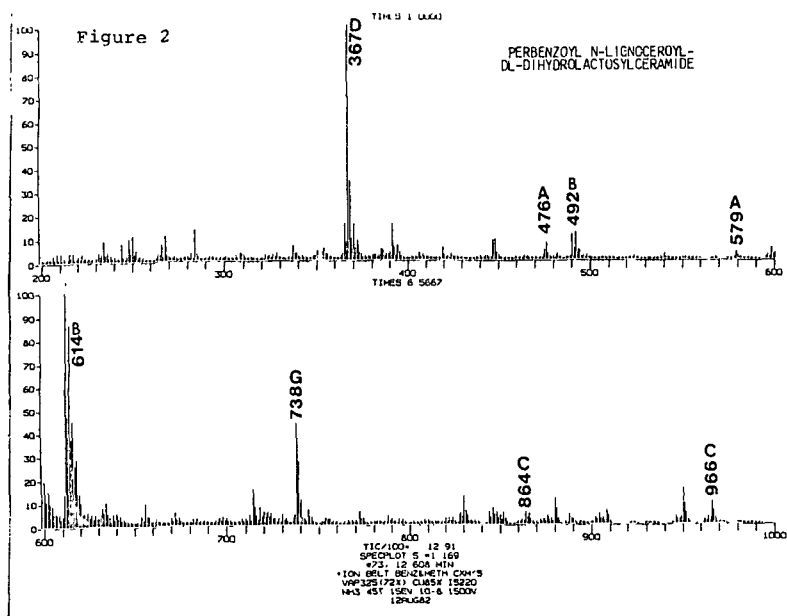


Table 1
PROPOSED ION COMPOSITIONS FOR BENZOYLATED
GLYCOSPHINGOLIPIDS

MOLECULAR WEIGHT	GALACTOSYL CERAMIDES		LACTUSYL CERAMIDE		
	FATTY ACID - SPHINGOSINE				
	18:0-018:0	24:0-018:0	BOVINE BRAIN		
			NFA 90%	HFA 1451	1911
FRAGMENT IONS	1353	1437	1435		
HEXOSE					
A + K15	598	598	598	598	598
A	579	579	579	579	579
A + K14 - OBZ	476	476	476	476	476
A - 2 OBZ	351	351	351	351	351
B + K15	614	614	614	614	614
B + K14 - OBZ	492	492	492	492	492
PSYCHOSINE					
C + 2H + NH5 - OBZ	986	986	986	-	-
C + H - OBZ	966	966	966	-	966
C + H + NH5 - 2OBZ	864	864	864	-	864
FATTY ACID					
D + K12	283	367	367	-	367
D + H	268	352	352	-	-
CERAMIDE					
E - CBZ - BZ	518	602	600	-	600
F - CBZ - BZ	532	616	614	630	616
F + H - OBZ	-	-	-	726	-
G + H - OBZ	654	738	736	-	738

GLYCOSPHINGOLIPIDS BY AMMONIA CI-MS VIA A MOVING BELT LM/MS INTERFACE;

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Glycosphingolipids (GSL) are thermally labile, nonvolatile naturally occurring compounds of considerable complexity due to heterogeneity in both the lipid and carbohydrate moieties. In an effort to develop procedures that will provide simultaneous class separation, lipid structural information and quantitation, we have evaluated the Finnigan moving belt LC/MS interface as an inlet to the Finnigan 4500 MS.

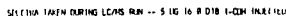
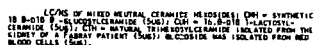
Figure 1 shows the total ion current and several selected ion chromatograms obtained by LC/MS of a mixture of 5 ug each of 1) synthetic glucosyl-N-stearoyl-D,L-sphinganine (CMH), 2) lactosylceramide (CDH) obtained from blood plasma, 3) trihexosyl ceramide (CTH) obtained from the kidney of a Fabry patient, and 4) globoside from human red blood cells. LC was conducted with a 2mm ID X 15cm column packed with 3µm spherical silica. A methylene chloride-methanol-water solvent gradient was run from (93:6.5:0.5) to (65:31:4) in 5 min with a 10 min hold at the final conditions. The flow rate was 0.4 ml min⁻¹. Ammonia (0.7 torr) CI was used with the ion source at 150°C. The LC/MS evaporator was at 330°C and the clean up heater at 250°C.

Figure 2 shows the spectra obtained from the lactosylceramide, globoside and sulfatide peaks in this run as examples of the fragmentation patterns obtained. The base ion in CDH spectra is m/z 180 and is derived from the galactose or glucose moiety. Its dehydration and NH₄⁺ ions are also seen at m/z 162 and 198 respectively. An analogous set of ions for lactose are seen at m/z 324, 342 and 360. A [M+H]⁺ ion is present at m/z 862. The CMH also gave a [M+H]⁺ of similar intensity. Ions for the ceramide moiety are present at m/z 502, 520 and 538, and those that identify the fatty acids are present at m/z 240 and 256. An ion that is characteristic of the sphingosine portion is seen at m/z 264.

The fragmentation pattern for globoside shows many similarities to those for CMH, CDH, and CTH except for the relatively intense base ion at m/z 204 and its NH₄⁺ adduct at m/z 221. The [M+H]⁺ ion could not be observed, if present, because the high MW of this compound exceeds the mass range of our instrument. The ceramide and fatty acid ions are reduced in intensity due to the variety of fatty acids present in this compound. The outstanding feature of this spectra is the efficiency with which the ion at m/z 204 carries the charge which results in the diminution of all other ions relative to the spectra of the other GSL. When a spectra was obtained for the desialoated ganglioside (AGM1), Cer-glu-gal-NAoal-gal, where the positions of the neutral and amino sugar in the terminus were reversed from that of globoside, the m/z 204 ion was present but greatly diminished, and di- and trisaccharide ions became more prominent, as did all other fragments.

A spectra was also obtained from a CMH containing hexose sulfate (sulfatide) with good results. The fragmentation pattern was similar to the CMH, CDH and CTH except that the base ion was relatively intense and appeared at m/z 162. Presumably this ion results from removal of H₂SO₄ from the protonated hexose sulfate ion.

The spectra we obtained for all of the GSL demonstrate that LC/MS with a moving polyimide belt transport interface is useful for separation and structure determination of complex glycosphingolipids. The inertness of the polyimide belt and the relatively rapid thermal desorption of the sample from the belt makes it possible for useful CI spectra to be obtained for these labile compounds. Considerable information concerning the carbohydrate composition and sequence for each GSL was obtained and was probably limited by the mass range of our instrument. The composition of the major fatty acids and long chain bases in the ceramide portions of these glycolipids are also apparent from the spectra. Further developments of this methodology are expected to include capability for direct analysis of biological extracts for structure, composition and quantitation of these presently difficult to analyze compounds.



LC-MS BY THERMOSPRAY SAMPLE DEPOSITION AND LASER DESORPTION

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We are currently investigating the potential of combined LC-LDMS. Our goal is to develop an instrument for separation and identification of a wide variety of nonvolatile, thermally labile biomolecules which are currently difficult to analyze by conventional mass spectrometric techniques.

The laser desorption LC-MS interface utilizes a thermospray vaporizer for removing the LC solvent while depositing the effluent components onto a moving stainless steel belt sample transport system. Travelling into the source, the samples are ionized with submicro-second laser pulses from a Q-switched Nd:YAG laser. A cylindrical lens is used to produce a line image across the full width of the belt. With the exception of a boxcar integrator to process the time dependent signal, the instrument is a conventional scanning quadrupole mass spectrometer. Scan rates of 100 amu/sec are routinely used with laser repetition rates of 100 Hz.

Thus far, we have obtained cationized molecular ions from a wide variety of nonvolatile biomolecules including nucleosides, nucleotides, saccharides, amino acids, and peptides up to 1200 amu. Excellent sensitivity is obtained with sample surface coverages down to 50 ng/cm², and characteristic mass spectra are routinely obtained from sample injections in the range of 10 ng to 1 µg. Generally, the more polar or ionic a sample is, the better its LD ionization efficiency. The optimum power density for sample ionization is $\sim 1 \times 10^8$ W/cm².

Both negative and positive molecular ions are obtained from most samples. With nucleosides, MK⁺ and (M-H)⁻ ions are the most abundant molecular ions. Large low mass background levels (<150 amu) from salt cluster ions are a continuous problem; alkali salt impurities are practically unavoidable, and these salts have a much greater ionization efficiency in the LD process than do organic sample molecules.

In the LC-MS interface, the advantage of thermospray deposition approach is that most of the solvent is already vaporized before the sample is deposited onto the belt. Unlike conventional moving belt interfaces, our samples are deposited onto the belt under vacuum, and strip heaters for solvent removal are unnecessary. Using water only, flow rates up to 2 ml/min can be easily accommodated. The LDMS-RIC peak widths are virtually identical to those obtained with a UV detector at belt speeds greater than 0.25 cm/sec. In comparison with the electrospray sample deposition method, the thermospray sample deposition method is about 40% as efficient but further improvement is expected.

LC/MS OF PESTICIDES

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The inlet system of a Finnigan 3300 chemical ionization mass spectrometer, previously modified for negative chemical ionization, was modified to accommodate a Hewlett-Packard LC/MS interface, and a desolvation chamber was mounted on the source. A Waters HPLC system, consisting of two Waters 600A pumps, and a Perkin Elmer LC-55 UV detector, and Brownlee C-8 columns were used for the separations.

Five classes of triazine herbicides were studied by combined LC/MS (1). Separations were done on a reversed-phase C-8 column, using acetonitrile:water (45:55) as the mobile phase, followed by in-line UV and mass spectral analysis. Positive and negative ion mass spectra were recorded. For most of the triazines both positive and negative ion spectra obtained with the direct liquid introduction LC/MS probe interface gave molecular ion information. Most of the triazine studies were more sensitive in the positive ion mode than in the negative ion mode.

A series of organophosphorus pesticides were studied by combined HPLC/NCI-MS. LC separation was done on a reversed-phase C-8 column, using acetonitrile:water (60:40) as the mobile phase (2). The negative ion mass spectra obtained under these LC/MS conditions are very simple and are very similar to those reported for methane-enhanced negative ionization. Molecular ions are generally not present in either mode, but intense fragment ions containing useful structural information are usually observed.

Preliminary studies on several organophosphorus pesticides indicate that NCI source temperature effects and spectra obtained with some chlorinated solvent modifiers are similar to reported CI-attachment NCI mass spectra obtained with CH_2Cl_2 as the reagent gas (3), although more fragmentation is generally observed.

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EI SPECTRA FROM A DLI LC/MS INTERFACE?: OPTIMIZATION OF THE MASS SPECTROMETER

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Extranuclear

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In 1968, Tal'rose et al¹ first introduced a liquid solution into the vacuum system of a conventional EI mass spectrometer. Flow rates, however, in this initial work were limited to the nanoliter range. In 1973 Baldwin and McLafferty² reported on the direct introduction of liquid solutions into a CI (chemical ionization) mass spectrometer. This approach to LC/MS interfacing has received considerable attention. However, due to the simplicity of the resulting spectra, little or no structural information is provided. Christiansen et al³, using a continuous preconcentrator LC/MS and a relatively tight EI ionizer have reported spectra of mixed EI-CI character at flow rates of 2.5 to 15.0 $\mu\text{l}/\text{min}$. The purposes of this work are two-fold: (1) to optimize the operating conditions and source design for the LC/MS interface; (2) to determine the feasibility of using the SIMULSCAN[®] tandem CI/EI source for obtaining EI spectra from this interface.

DATA:

Solvent Ions

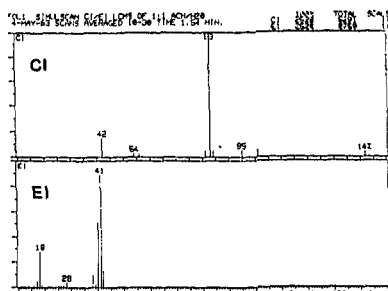


Fig.1

Bis(2ethylhexyl)phthalate

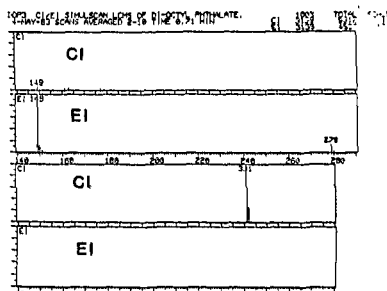


Fig.2

Desolvation temp. : 165C

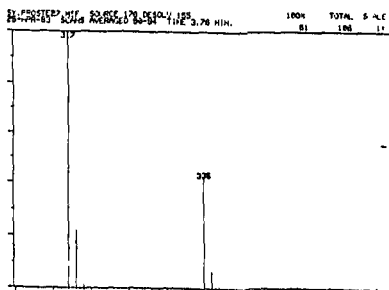


Fig.3

Desolvation temp. : 200C

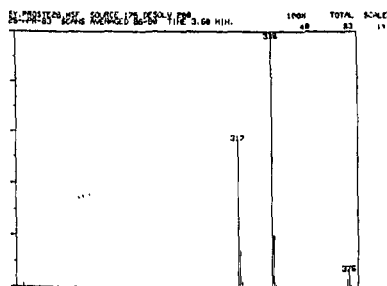


Fig.4

Response vs. Desolvation temp.

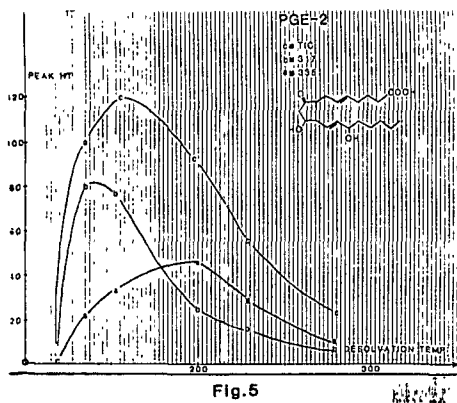


Fig.5

RESULTS:

Experiments with removeable CI ion volumes having different ion exit apertures verified that the 0.040" (1 mm) exit yielded the best results for CI or simultaneous CI/EI operation. Figure 1 shows the solvent related ions from a SIMULSCAN acquisition of a 60:40 AcN/H₂O solvent system. Note the cluster ions in the CI spectrum and the lack of even an (M+H)⁺ ion for AcN or H₂O in the EI spectrum. Figure 2 shows similar results for a 75 ppm solution of bis(2-ethylhexyl) phthalate introduced as a steady state (split ≈ 100:1) under the same conditions. Note that the (M+H)⁺ ion at m/z 391⁺ is the base peak in the CI spectrum, while in the EI spectrum m/z 149⁺ is the base peak and there is no ion at m/z 391⁺.

Experiments with the temperature of the desolvation chamber indicate that the optimum temperature is probably dependent on the solvent system and the analyte. Fig. 3 is the CI spectrum of prostaglandin E2 introduced through the LC/MS probe in 60:40 AcN/H₂O with the source at 170°C and the desolvation chamber at 155°C. The ions at m/z 335⁺ and m/z 317⁺ correspond to (M+H-H₂O)⁺ and (M+H-2H₂O)⁺. All parameters are the same for Figure 4, except that the desolvation chamber is at 200°C. The ion at m/z 376⁺ corresponds to (M+H+AcN-H₂O)⁺. Figure 5 summarizes the response data as a function of desolvation chamber temp. Note that the TIC, m/z 317⁺, and m/z 335⁺ curves all maximize at different temperatures.

CONCLUSIONS:

- EI spectra can be obtained with a DLI type interface and a SIMULSCAN tandem CI/EI ionizer.
- Optimum CI exit aperture is apparently 0.04" or less for CI only or SIMULSCAN operation.
- Optimum desolvation temperature can be both solvent and solute dependent, but appears to be at least 50 degrees above the highest boiling solvent.

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²M.A. Baldwin and F.W. McLafferty, Org. Mass Spectrom. 7: 1111 (1973).

³R. Christiansen, et al: Anal. Chem. 53, 1981, 171-174.

FOURIER TRANSFORM ION CYCLOTRON RESONANCE
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The title subject is a technique which allows all of the experiments of conventional ion cyclotron resonance (ICR) mass spectroscopy to be performed with greater speed, greater sensitivity, greater resolution, greater mass range, greater convenience and greater reliability than is possible with conventional technology. Examples of FT-ICR experiments which illustrate these advantages will be described. The history of the title subject from its inception in 1973 to the present will be reviewed. The relationship between FT-ICR and other forms of Fourier spectroscopy will be noted with emphasis on those aspects of FT-ICR which differ and those which do not differ from the corresponding aspects of other applications of FT techniques. The importance of considering these aspects to the development of FT-ICR will be described. Contributions of other forms of FT spectroscopy to FT-ICR and contributions of FT-ICR to other forms of FT spectroscopy will also be mentioned.

WHAT IS THE OPTIMUM MAGNETIC FIELD
STRENGTH IN FOURIER TRANSFORM ION CYCLOTRON RESO-
NANCE SPECTROMETRY? M. Allemann, Hp. Kellerhals,
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Experiments with FT ICR spectrometers are discussed, utilizing magnetic field strengths of at least 4.7 Tesla. Resolution, trapping time, and to some extent mass range are dependent upon strength and homogeneity of the magnetic field. At 4.7 T a resolution of $m/\Delta m = \omega/\Delta\omega = 550,000$ (ω : cyclotron frequency of ion with mass m , $\Delta\omega$: full width at half height of peak) at $m/e = 866$ has been obtained, sufficient for exact mass determination at $m/e > 1,000$. At low masses very high resolution has been observed: at $m/e = 18$, $m/\Delta m > 10^5$. Long trapping times at high magnetic fields open new fields of application. The study of chemically interesting slow ion-molecule reactions becomes possible. Trapping times for ions with $m/e = 78$ of more than 12 hours at 4.7 T have been observed. An important drawback of high magnetic fields is the increase of the frequency range needed to cover a certain mass range. The results obtained with high magnetic field FT ICR spectrometry are compared with results at lower magnetic field and with results of other mass spectrometric methods.

LASER DESORPTION FOURIER TRANSFORM
MASS SPECTROMETRY OF ORGANIC COMPOUNDS
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Pulsed lasers have shown promise as an ionization source for Fourier transform mass spectrometry (FTMS). Laser ionization has been used by Freiser, et al. to generate metal ions for studies of their gas phase reactivity (1, 2) by FTMS methods. Recently, the observation in a Fourier transform mass spectrometer of quaternary ammonium cations and $(M - H)^+$ ions generated by laser ionization of several organic compounds was reported by McCrery et al. (3)

In this paper, we report the observation of ions generated from organic compounds by the use of an excimer laser in conjunction with a Nicolet FT/MS-1000 Fourier transform mass spectrometer. Both $(M + K)^+$ and $(M + Na)^+$ ions are seen in positive ion spectra, as well as ions due to the fragmentation of the cationized compounds. In addition, laser desorption of neutral molecules followed by electron impact ionization of the desorbed neutral molecules can be used to generate both positive and negative ion mass spectra.

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3. McCrery, D.A.; Ledford, E.B. Jr.; Gross, M.L., Anal. Chem., 1982, 54, 1435-1437

ANALYTICAL APPLICATIONS OF FOURIER TRANS-
FORM MASS SPECTROMETRY: APPLICATIONS TO BIOCHEMICAL AND
RELATED RESEARCH; M. E. Castro, T. A. Adams, H. J. Williams,
and D. H. Russell; Department of Chemistry, Texas A&M
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The basic instrumental features of Fourier transform mass spectrometry (FTMS), e.g., mass range and absence of mass discrimination, high mass resolution, variability of operating parameters via computer, and operating speed, make FTMS a very attractive analytical spectrometer. On the other hand, some of the potential problem areas, viz. sensitivity and dynamic range, have not been adequately answered especially for problem solving applications. In our laboratory we are investigating the problem solving utility of FTMS with particular emphasis on biochemical and related problems. In these studies we utilize functional group specific ion molecule reactions, low and high mass resolution gas chromatography-FTMS, collision-induced dissociation (CID), and desorption ionization techniques for non-volatile/thermally labile compounds. Specific examples of these applications will be discussed and where possible the general utility of FTMS will be compared with that of more conventional mass spectrometers.

ANALYTICAL APPLICATIONS OF FOURIER TRANSFORM MASS SPECTROMETRY

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Fourier Transform Mass Spectrometry (FTMS) has three principal advantages as an analytical mass spectrometer. First, it has exceptionally high resolving power under conditions of low pressure and narrow mass range. Second, it possesses the Fellgett advantage which permits observation of all masses simultaneously without the significant ion losses which accompany transmission of ions through sector mass spectrometers. Thus, reasonable sensitivities are achievable without the use of electron multipliers. Third, it has the capability for time resolved measurements which are important in ion chemistry studies and for chemical ionization. Other advantages include the ability to perform the equivalent of MS/MS experiments using ion ejection pulses and pulsed collisional activation.

A significant disadvantage is the low dynamic range of the instrument which is attributed to the fact that present cells work best with low ion densities (ca. 10^5 ions/cm³). Thus, the technique is best suited for acquisition of "sparse spectra", i.e. spectra containing limited numbers of different mass ions.

We have carefully considered the advantages and disadvantages of the technique in order to design methods of sample and ion handling and sample ionization which are compatible with the operational advantages of the technique.

Laser Desorption. "Sparse spectra" are obtained by chemical and desorption ionization methods. Thus, we expect the combination of laser desorption and FTMS will have definite advantages over combinations involving other mass analyzers. Furthermore, the Fellgett advantage permits acquisition of full low resolution spectra for a single shot of the laser. Narrow band observation yields the highest mass resolution detection of laser desorbed ions thus far demonstrated. Recent experiments with a Nd YAG Laser (1 micron) show additional fragmentation of small peptides and carbohydrates when compared to desorption using a CO₂ laser (10 micron). Translational excitation of ions produced by laser desorption

leads to collisionally activated decompositions which have been recently observed in our laboratory.

Pulsed Sample and Reagent Gas Handling. The highest sensitivity experiments involve ionization of the sample at high pressures or by chemical ionization which also may require the presence of higher pressure reagent gases. High cell pressures are also found in GC/FTMS experiments. Yet high resolution mass analysis is best done at low pressures. Both requirements can be satisfied by the use of pulsed valves. For example, pulsed admission of the reagent gas during the ionization pulse leads to rapid proton transfer and production of protonated sample molecules. The valve is then closed, and the reagent neutrals are pumped away so that mass analysis is at low pressure. We have obtained the high resolution CI spectrum of a mixture of thiophene and cyclopentanone, both of nominal molecular weight 84, at a resolving power of 145,000. Furthermore, we find that the sensitivity of the pulsed CI experiment to be at least an order of magnitude better than non-gas pulsed and self- CI experiments conducted by FTMS.

Pulsed introduction of volatile samples also permits acquisition of higher sensitivity high resolution spectra than static pressure experiments.

A pulsed interface for a GC and the FTMS has also been constructed and evaluated. Here the mode of operation is to pulse the GC eluent during the ionization pulse, allow the sample and helium to pump away, and then pulse on the ion detection. For example, we have conducted single ion monitoring experiments in which 20ng of naphthalene was easily detected at a mass resolution of 60,000 using capillary column GC/FTMS (these pulsed valve experiments are more thoroughly described in the paper by Sack and Gross in this volume).

Impulse Excitation and Hyperbolic Cell. We believe the problems of dynamic range and of routine high resolution are exacerbated by inefficiencies in ion excitation and detection and by electric field inhomogeneities. In an attempt to solve these problems, we have developed an impulse or "Dirac" excitation mode and a hyperbolic cell (identical to

the classical Penning trap). The impulse excitation makes use of up to a 250 Volt pulse with a FWHH of 0.5 microseconds and is capable of uniform excitation starting at m/z 50 (at 1.2 tesla). The impulse excitation in a cubic cell gives superior exact mass measurements (errors are 1-2ppm) over a much broader range of ion densities than does the conventional "chirp" excitation in the cubic cell (see paper by Ledford in this volume).

The hyperbolic cell and impulse excitation were also found to produce low mass measurement errors over broad ranges of ion density. Thus, impulse excitation appears to be more generally useful for exact mass measurements than "chirp" excitation.

A more thorough approach for evaluating the impulse excitation and the hyperbolic cell is the method of generating 3-dimensional tuning surfaces whereby peak intensity or similar evaluatory parameter (mass resolution, peak area, isotope ratio, peak position, etc.) was examined as a function of trap voltage and excitation amplitude (see paper by Rempel in this volume). Tuning surfaces have been generated for different numbers of ions in the cell. In general, all surfaces are sharply peaked and fall off rapidly as ion excitation amplitude is increased. In fact, the fall-off occurs long before the amplitude of the ion excitation is sufficient to eject ions from the cell. We believe this indicates that the FTMS analyzer cells now in use are inefficient for detection of ions trapped within. The highest efficiency signal generation was found for the hyperbolic cell and impulse excitation operating at low trapping voltages ($<0.5V$) and low numbers of ions. We propose to investigate other methods of excitation with the hyperbolic cell to overcome the effect of z-axis excitation which may be degrading its performance.

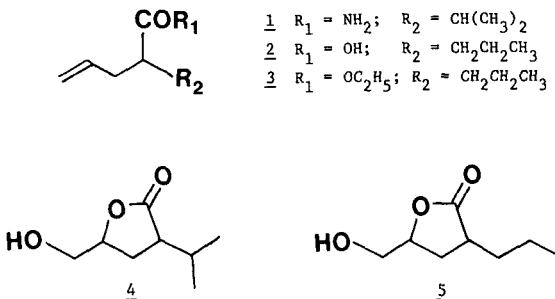
In summary, pulsed ionization, pulsed sample introduction, and pulsed chemical ionization permit more efficient ionization at high pressure and high resolution mass detection at low pressure. New methods of ion excitation (impulse) and ion detection (hyperbolic cell) have definite potential for improving mass measurement, signal-to-noise ratios, and isotopic ratios in FTMS.

STABLE ISOTOPE LABELING STUDIES ON THE OXIDATIVE METABOLISM OF OLEFINIC "SUICIDE SUBSTRATES" FOR CYTOCHROME P-450.

Thomas A. Baillie and Kathryn S. Prickett, Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195.

4-Pentenoic acid and certain derivatives thereof are of interest as inhibitors of specific enzyme systems involved in the biotransformation of both drugs and compounds of endogenous origin. In those examples which have been studied in detail, it appears that irreversible inhibition results from alkylation of the target enzyme following metabolic "activation" of the substrate to a reactive electrophilic species. A case in point is 2-isopropyl-4-pentenamide (allylisopropylacetamide, AIA, 1) which causes destruction of cytochrome P-450 via a "suicide substrate" mechanism. This property of AIA is now known to be associated with the cytochrome P-450-mediated oxidation of the 4,5-double bond, an event which transforms AIA to a chemically reactive intermediate of which a fraction alkylates the prosthetic heme and thereby inactivates the enzyme [1]. The bulk of this intermediate, however, appears to collapse to AIA-4,5-epoxide, a putative metabolite which is further transformed, by an undefined pathway, to 2-isopropyl-4-hydroxymethyl-γ-butyrolactone (4).

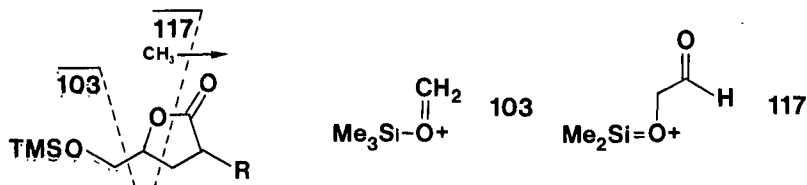
Recently, we have shown that 2-*n*-propyl-4-pentenoic acid (2), a metabolite of the anti-convulsant drug valproic acid, and its ethyl ester (3) also destroy hepatic cytochrome P-450 *in vitro* [2]. Furthermore, we have shown that oxidation of these substrates by cytochrome P-450 leads to the formation of the butyrolactone 2, which is isomeric with the lactone produced from AIA.



Interest in our laboratories is presently focused on establishing the detailed mechanisms by which the above derivatives of 4-pentenoic acid are metabolized to the lactones 4 and 5 with the object of obtaining indirect information on which enzyme systems participate in the respective processes. Tracer studies with the stable isotope ¹⁸O have proved to be especially valuable in this regard for two reasons: (1) oxidation of olefins to the corresponding epoxides is known to be catalyzed by cytochrome P-450, which utilizes oxygen derived from the atmosphere, and (2) cleavage of epoxides to the corresponding vicinal diols results in incorporation of oxygen from water, the site of addition to the oxirane structure being dependent upon whether the hydrolytic reaction is purely chemical in nature or is catalyzed by the enzyme epoxide hydrolase [3]. Thus, by performing metabolic experiments with the parent olefins in environments containing either ¹⁸O₂ or H₂¹⁸O and analyzing the pattern of ¹⁸O incorporation into the products by mass spectrometric techniques, the extent to which chemical and enzymatic processes are involved in the overall oxidation scheme can be determined.

In the present study, metabolic experiments, employing as substrate each of the three olefins 1-3, were performed using hepatic microsomal preparations from rats which had been pretreated with phenobarbital (80 mg kg⁻¹ day⁻¹ for 4 days) to induce liver mixed function oxidase activity. In order to obtain the desired mechanistic information, incubations (15 min at 37°) were carried out either under an atmosphere of ¹⁸O₂ or in a medium enriched in H₂¹⁸O (50 atoms % excess). Following acidification of the incubation products to pH 2-3 with HCl, metabolites were extracted into ethyl acetate and converted to trimethylsilyl (TMS) derivatives for analysis by capillary gas chromatography-mass spectrometry (GC-MS).

Comparison of the low resolution EI mass spectra of the TMS and $[^{2}\text{H}_9]\text{TMS}$ ethers of butyrolactone 5, and an analysis of the high resolution mass spectrum of the unlabeled TMS derivative of this compound, served to establish the identity of prominent, structurally informative fragment ions which could be employed to define sites of ^{18}O incorporation. The ions selected for this purpose, which were also abundant in the spectrum of 4 TMS, were as follows: m/z 103 ($\text{C}_4\text{H}_{11}\text{OSi}$; oxygen from C-5), m/z 117 ($\text{C}_4\text{H}_9\text{O}_2\text{Si}$; oxygen from C-4 and C-5) and the $[\text{M}-15]^+$ ion, m/z 215 ($\text{C}_{10}\text{H}_{19}\text{O}_3\text{Si}$; oxygen from C-1, C-4 and C-5). The origin and structures of the ions at m/z 103 and 117 is as shown below.



Thus, by determining the ^{18}O enrichment of each of the above key fragment ions in the spectra of metabolites formed under $^{18}\text{O}_2$ or in the presence of H_2^{18}O , it was possible to assign unambiguously the site(s) and degree of label incorporation.

When AIA (1) served as substrate, the lactone 4 was formed as the major metabolite and incorporated one atom of ^{18}O per molecule from both the labeled atmosphere and the ^{18}O -enriched medium; in the former case, the ^{18}O in 4 was found to be present exclusively at the C-5 position, while in the latter experiment, only the carbonyl oxygen of the product became labeled with isotopic oxygen. These data are consistent with a mechanism in which the epoxide generated metabolically from AIA is opened via intramolecular attack at C-4 by the oxygen of the amide function and the resulting imino lactone species is hydrolyzed to the observed product. Similarly, the acid 2 was found to undergo metabolism under an atmosphere of $^{18}\text{O}_2$ to give the corresponding *n*-propyl- γ -butyrolactone (5) labeled with one atom of ^{18}O at C-5, while the product formed in H_2^{18}O was unlabeled, as would be predicted if an epoxide-opening reaction analogous to that outlined above was operative. Thus, the epoxides derived from these two olefinic precursors are opened by non-enzymatic, intra-molecular processes, without the intervention of microsomal epoxide hydrolase.

In contrast, similar ^{18}O labeling studies performed with the ethyl ester 3 indicated that the epoxide formed from this precursor is a substrate for epoxide hydrolase, oxygen from the atmosphere being incorporated into the lactone 5 exclusively at the C-4 position. In addition to 5, a "dihydrodiol" metabolite of 3 was identified in microsomal incubations, viz. ethyl 4,5-dihydroxy-2-*n*-propylpentanoate. When 3 was incubated in a medium enriched in H_2^{18}O , this dihydrodiol species incorporated label solely at C-5, a finding which is also consistent with participation of epoxide hydrolase in the metabolism of 3.

It is concluded, therefore, that the mechanisms by which 4-pentenoic acid derivatives are metabolized *in vitro* to substituted γ -butyrolactones is dependent upon the nature of the carboxyl moiety of the parent compound. If this functional group contains an ionizable hydrogen (as in $-\text{CONH}_2$, $-\text{CO}_2\text{H}$), the intermediate epoxide, formed by the action of cytochrome P-450, is opened by way of intramolecular nucleophilic attack at C-4 which leads directly to a cyclized product. When the substrate is devoid of any such ionizable hydrogen, however, (as in $-\text{CO}_2\text{C}_2\text{H}_5$), metabolism to the lactone occurs via an "epoxide-diol" pathway in which hydrolytic cleavage of the epoxide is mediated by microsomal epoxide hydrolase. These examples of the usage of ^{18}O in conjunction with analytical procedures based on GC-MS demonstrate effectively the great utility of this stable isotope of oxygen in mechanistic studies of drug metabolism.

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STUDIES ON THE FORMATION OF A TOXIC METABOLITE OF PULEGONE

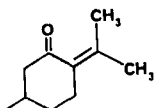
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Department of Medicinal Chemistry, University of Washington,
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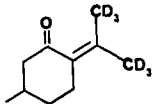
Pennyroyal oil is an essential oil that is obtained by steam distillation of plants of the Labiatae family, in particular Mentha pulegium and Hedeoma pulegioides. Because of its distinct mint-like odor, the oil and some of its constituent terpenes are approved for use in small quantities as flavoring agents in some alcoholic beverages, baked goods, candies, ice creams, and as a fragrance component of some soaps and detergents. The oil has also been used as a folklore medicine for its reputed abortifacient properties, however the large doses that are required can cause severe liver and lung injury in both man and laboratory animals (1,2).

We have determined that R-(+)-pulegone (I) is the monoterpene constituent of pennyroyal oil most responsible for the toxic effects caused by pennyroyal oil (2). More recently, we have shown that oxidative metabolism of R-(+)-pulegone by cytochrome P-450 is required for the pathogenesis of the toxic reaction (unpublished results). Because allylic oxidation is a major pathway of metabolism for many terpenes, we speculated that deuterium substitution for hydrogen in the allylic methyl groups would decrease hepatotoxicity if oxidation of allylic methyl groups was required for the formation of a toxic metabolite.

Therefore, R-(+)-pulegone- d_6 (II) was synthesized by oximation of acetone- d_6 , followed by chlorination of the oxime and ozonolysis to produce the enolate acceptor, 2-nitro-2-chloro-propane- d_6 . This was condensed with the lithio enolate of R-(+)-3-methylcyclohexanone followed by elimination of nitrous acid to yield the desired product, R-(+)-pulegone- d_6 , with 96% total deuterium incorporation.



I



II

Comparative studies of the effects of R-(+)-pulegone and the deuterated analog showed that deuterium substitution for hydrogen in the allylic methyl groups decreased the hepatotoxic effect two to three-fold in male Swiss-Webster mice. This effect was paralleled by a decrease in the rate of formation of a major metabolite of R-(+)-pulegone, menthofuran, by mouse liver microsomes. Results of competitive experiments with incubation of mixtures of R-(+)-pulegone and R-(+)-pulegone- d_6 gave an intermolecular isotope effect of $k_1/k_0 = 1.89$ for the formation of menthofuran as determined by capillary GC/MS and monitoring the ratio of the parent ions of the products, menthofuran and menthofuran- d_4 .

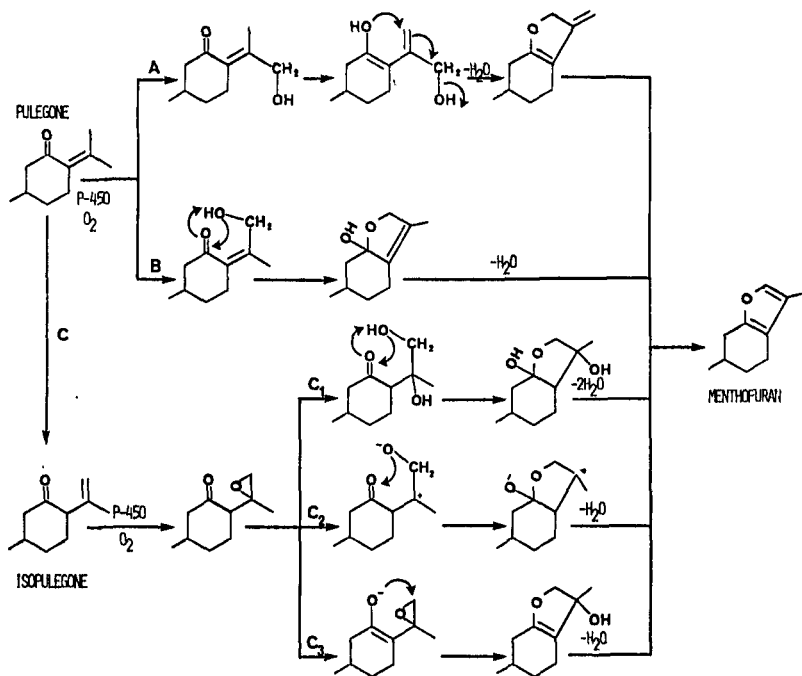
Because menthofuran could be formed from pulegone in a variety of pathways (Figure 1), we carried out incubations of pulegone under atmospheres of $^{18}O_2$ and in $H_2^{18}O$. Mass spectral analysis of the menthofuran that was formed showed that it incorporated at least 92.6% of its oxygen from molecular oxygen, whereas no oxygen-18 was incorporated

from $H_2^{18}O$. Thus, only pathways B and C, are consistent with the data. In separate experiments, isopulegone epoxide was found to be stable under the incubation conditions and did not form menthofuran. Therefore, the data are compatible with pathway B in which pulegone is metabolized by oxidation of the allylic methyl group syn to the ketone function, followed by intramolecular condensation to a hemiketal and subsequent dehydration to menthofuran. Menthofuran has been tested and found to be a potent liver and lung toxin that itself requires further metabolic activation.

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Figure 1. Possible pathways for the metabolism of pulegone to menthofuran.



METABOLISM OF AZINPHOSMETHYL AND AZINPHOS-

ETHYL. Shen-Nan Lin, R.S. Takazawa and S.D. Murphy., Division of Toxicology and Analytical Chemistry Center, University of Texas Medical School, Houston, Texas 77025.

Gas chromatography-mass spectrometry was used to study in vitro metabolism of Azinphosmethyl and azinphosethyl by mice or rat liver homogenates. High performance liquid chromatography was used for purification of biological samples. Organophosphorothioate insecticides are metabolized following multiple metabolic pathways. In this study we found that azinphosmethyl and ethyl homologue are oxidatively desulfurated to the oxons and degraded to benzazimide. The oxons are also degraded to benzazimide, but at a slower rate than the parent insecticides. Benzazimide formation appears to result from breaking the S-C bond to form hydroxymethyl benzazimide with further degradation to benzazimide. Capillary column GC/MS also detected two metabolites containing phosphorous: dimethyl or diethyl phosphorothioic acid, monomethyl or monoethyl phosphorothioic acid. Previously, detection of dialkyl phosphorothioic acid in mice liver homogenates was believed to result from oxidative breakage of the P-S bond of the insecticides. However, we failed to detect mecaptomethylbenzazimide, the metabolite containing the organic moiety resulting from this metabolic pathway. Detection of monoalkylphosphorothioic acid in liver metabolizing systems has not been reported before. The possible metabolic pathways leading to this metabolite are currently under investigation. Oxidative activation of azinphosmethyl in vivo was studied by a combination of HPLC and anti-cholinesterase assay. Plasma levels of the oxons were measured at the time of death after rats were given azinphosmethyl intravenously in Emulfor and DMSO solution. There appeared to be a good inverse correlation between the time of death and plasma content of the oxon. Supported in part by NIEHS Research and Training Grants ES01831 & ES07090.

GC-MS IDENTIFICATION AND QUANTITATION OF
DESIPRAMINE AND ITS METABOLITES IN DISCRETE REGIONS OF THE
RAT BRAIN; N. Narasimhachari, S. Bogema and R.O. Friedel;
Medical College of Virginia, Richmond, VA. 23298.

Groups of Sprague Dawley rats (n=4) were administered intra-peritoneally desipramine hydrochloride 2.5 mg/kg and 5.0 mg/kg for 3 days. Two hours after the last dose on the third day the animals were sacrificed by decapitation, the brains were dissected to separate cortex, cerebellum, corpus striatum and the rest of the brain. The tissues were homogenized with 3 vols of phosphate buffer (pH 7.4, 0.1M) containing 250 ng/ml each of d_4 internal standards of imipramine, desipramine and 2-hydroxy derivatives. The homogenates were centrifuged and processed by the procedure using C_{18} SEP-PAK cartridges.¹ The extracts after evaporation and derivatization into TFA derivatives were quantitated by gc-ms-sim according to the method of Narasimhachari et al.² We have identified desipramine, 2-hydroxy desipramine, desmethyl desipramine and 2-hydroxy desmethyl desipramine in regions of rat brain and found significant differences in the relative concentrations of various metabolites in different regions. The results from the rat brain will be compared with those found in the whole human brain in two autopsy samples from desipramine overdose.

In another experiment, groups of rats (n=5) were administered 5 mg/kg and 10 mg/kg of desipramine for 5 days and the animals sacrificed on the fifth day. Plasma, red blood cells and whole brain samples were analyzed for desipramine and its metabolites. The plasma:brain, RBC:brain, plasma:RBC ratios for desipramine were .086, 0.132, 0.648 for 5 mg dose and 0.101, 0.154 and 0.674 for 10 mg dose respectively.

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THE QUANTITATION OF ALDOPHOSPHAMIDE IN PATIENT PLASMA BY GCMS/SIM/CI; Sharon L. Pallante, Louise B. Grochow, O. Michael Colvin and Catherine Fenselau, The Department of Pharmacology and Experimental Therapeutics and The Oncology Center, The Johns Hopkins Medical Institutions, 725 N. Wolfe St., Baltimore, MD 21205.

While the use of cyclophosphamide [Fig. 1(I)] as an antineoplastic or immunosuppressive agent has been widespread for nearly 20 years, its total metabolism is still under investigation. Even though the drug's mode of action is through alkylation of the nucleic acids of the DNA, the parent compound, cyclophosphamide, does not possess alkylating activity. It must first be oxidized by the hepatic microsomal system to 4-hydroxycyclophosphamide (IIa). This 4-hydroxyCP may then be oxidized to the inactive metabolite 4-ketocyclophosphamide (VI) or, as in the major pathway, may rearrange spontaneously and exist in equilibrium with an open chain aldehyde, aldophosphamide (IIb). The aldophosphamide may then be oxidized to inactive carboxyphosphamide (VII) which decomposes to inactive nornitrogen mustard (V), or it may decompose to the major active metabolite phosphoramidate mustard (III) and acrolein (IV). Quantitation of cyclophosphamide, phosphoramidate mustard and nornitrogen mustard in patient plasma and urine was accomplished by GCMS in 1978. In the last several years emphasis has shifted to the unstable tautomeric intermediates: 4-hydroxyCP and aldophosphamide. Indeed, one or both of these compounds may be the transport mode for the active phosphoramidate mustard. As such their formation may be the rate limiting step in the production of the phosphoramidate mustard, and therefore, knowing the quantities present is essential to understanding the metabolism of the parent drug.

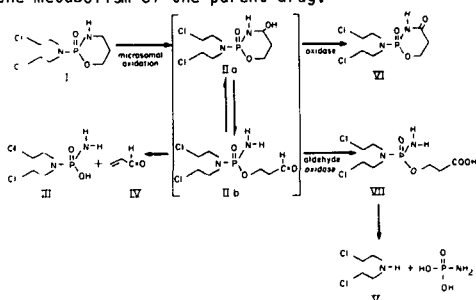


FIGURE 1.

A major roadblock to work with the 4-hydroxyCP/aldo has been instability. We found the T 1/2 of the tautomer pair in plasma at room temperature to be 45 minutes. In addition, degradation could not be stopped by flash freezing. Thus any assays devised for quantitation had to be performed in close proximity to the collection point and each sample processed immediately. Stabilization of the isomers by derivatization to a single aldophosphamide cyanohydrin which contained both populations confirmed the presence of the metabolites in patient plasma in 1977. This same technique has been modified and used for our quantitation assays. The inherent instability of the 4-hydroxyCP also precludes its use as a labelled internal standard. The drawback was overcome by the generation of a d_4 internal standard in situ from a stable d_4 -4-hydroperoxyCP precursor which spontaneously undergoes conversion to the 4-hydroxyCP/aldo in plasma. Figure 2 elaborates the assay method which we have employed. For standard curves, both d_0 - and d_4 -4-hydroperoxyCP are added to 2 ml plasma aliquots. Both compounds rearrange to their respective d_0 - and d_4 -4-hydroxyCP/aldo equilibria. Protein is precipitated from the plasma and NaCN and NaHSO₃ are added. The bisulfite serves as a trapping agent for aldehydes and ketones which are promptly converted to cyanohydrins by the NaCN. An ethyl acetate extract of the mixture is then treated with BSTFA containing 1% TMCS/pyridine for production of the trimethylsilyl derivative. In actual assays, the d_0 -4-hydroperoxyCP is omitted and only the d_0 -4-HOCP/aldo of the patient is measured. The NaCN/NaHSO₃ trapping method is found to be extremely efficient with the 4-HOCP/ Aldo but at the same time very pH dependent. Experiments show a sharp pH profile peaking at pH 8.9 - 9.1. A more acid environment leads to hydrolysis of the cyanohydrin while a more basic one gives rise to loss of chloride.

Selected ion monitoring of the TMS derivative was accomplished using the M-HCl ion sets of both the d_0 and d_4 compounds. These were the base peaks of the GC/CI spectra. A slight overlap of the ion sets is created by the presence of m/e 416 in both the d_0 and d_4 . Such an occurrence is not uncommon when assaying chlorine containing drugs. The effect of this overlap can be seen in our standard curve which is slightly offset. The standard curve is

linear across our range of $0.1 \rightarrow 2 \mu\text{g } d_0 : 1 \mu\text{g } d_4$ with a total assay precision of 5%. All GCMS/SIM/CI-isobutane analyses were performed under conditions given in Figure 4. Concentration of 4-hydroxyCP/aldo was measured as a function of time following 1 hour infusions of cyclophosphamide monohydrate in several patients (Figure 5) -- one patient (X) at 60 mg CP/kg and four patients at 50 mg CP/kg. The lower limit of detection of the aldophosphamide cyanohydrin TMS is 10 ng/ml. The study is ongoing and continues to collect data with reference to differing dose levels.

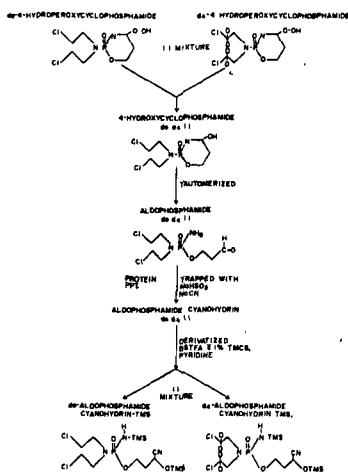


FIGURE 2.

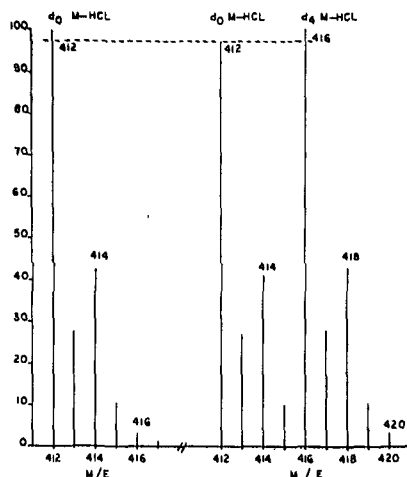


FIGURE 3.

DU PONT DP-102 GC/MS/DS
3% OV-101, 5' x 2 MM. ID.
200+280° IN 18 MIN, THEN +300° IN 5 MIN
SOURCE 200°, INJ 250°, JET 250°
SIM M/E 414+418 WITH 300 MSEC DWELL /AMU
(ISOBUTANE C)

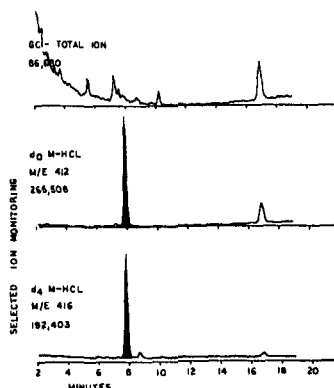


FIGURE 4.

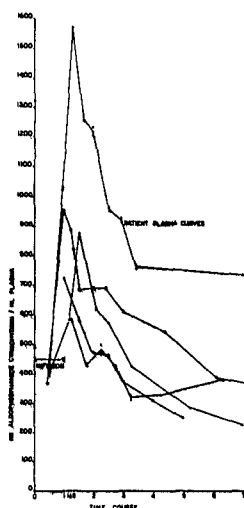


FIGURE 5.

QUANTITATIVE CHEMICAL IONIZATION DETERMINATION OF DEXAMETHASONE IN HUMAN PLASMA BY STABLE-ISOTOPE DILUTION MASS SPECTROMETRY; Y. Kasuya*, J.R. Althaus, J.P. Freeman, and R.K. Mitchum; National Center for Toxicological Research, Division of Chemistry, Spectroscopic Techniques Branch, HFT-154, Jefferson, Arkansas 72079.

The assay of steroid hormones in biological fluids has been greatly simplified with the development of highly sensitive radioimmunoassay techniques (1). However, cross reactivity with other steroids limits the specificity of the technique. HPLC methods are widely used to measure natural and synthetic corticosteroids in biological fluids (2), however, the technique lacks the sensitivity to make accurate measurements of plasma levels in the nanogram to subnanogram range. The use of GC/MS and isotopically labelled diluents (ID) allows subnanogram quantitation with the accuracy enhanced by stable label isotope dilution methodology. This paper describes the GC-ID/MS determination of circulating levels of dexamethasone utilizing dexamethasone M+9 ($^{13}\text{C}_6$, $^2\text{H}_2$) as the isotope diluent to determine the bioavailability of tableted dexamethasone (3).

Isolation of circulating dexamethasone from plasma was carried out using a C_{18} -bonded reverse phase Sep-Pak[®] cartridge followed by normal phase HPLC and collecting the appropriate fraction. An isotope diluent, M+9, was added to the plasma prior to isolation. The tetra-TMS derivative of dexamethasone was prepared by reacting with BSTFA. The methane chemical ionization mode provided the high sensitivity needed for the analysis. The major ions in the methane CI spectrum were m/z 681 (M+1), 665, 691, and 499.

The GC effluent from a 3% SP2100 (2 ft x 3 mm, supelcoport 80-100) column was introduced into the ion source by the direct transfer line of a Finnigan-MAT 4023 GC/MS. Simultaneous monitoring of m/z 681 and m/z 690 (isotope diluent), showed that no endogenous compounds were present in the blank plasma extract which interfered with the assay. Calibration curves obtained over the range 5-200 ng with a constant 20 ng (M+9) spike produced a good correlation of the mixed molar ratio and observed peak area. The relative error was found to be less than 3%. The precision defined by the coefficients of variation for intra-assay analysis of triplicate six 1 ml plasma samples were 3.78% for 2 ng/ml and 1.29% for 5 ng/ml. Inter-assay coefficients of variation were 3.83% and 2.64% at 2 and 5 ng/ml respectively.

Time course dexamethasone plasma concentration profiles of two human volunteers were followed after the oral administration of three 0.75 mg dexamethasone tablets or a single 1.0 mg i.v. dexamethasone phosphate ester dose. The orally administered dexamethasone and i.v. administered dose resulted in half lives, in close agreement (2-3.5 hr). Based upon the area under the plasma concentration time curve (AUC), the bioavailability of the oral dose was 55% and 71% for subjects 1 and 2, respectively.

The GC-ID/MS method described, offered a sensitive and reliable technique to measure nanogram to subnanogram amounts of dexamethasone in human plasma after administration of the drug at clinical levels.

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MASS SPECTROMETRIC ANALYSIS OF CYCLOSPORINE METABOLITES
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The cyclic undecapeptide cyclosporine (M.W. 1202) (insert Fig. 2) is a fungal metabolite with potent immunosuppressive activity. The compound is neutral, rich in hydrophobic amino acids and contains a number of N-methylated peptide linkages. The drug is becoming the agent of choice for the prevention of the rejection of kidney, liver and heart-lung allografts and to prevent or treat graft-vs-host disease in patients who are receiving bone marrow transplants. Cyclosporine is extensively metabolized *in vivo* by the liver into a number of hydroxylated and N-demethylated metabolites. In an investigation of the *in vitro* hepatic metabolism of cyclosporine, we have developed mass spectrometric procedures in order to rapidly elucidate the structure of metabolites. The molecular weight of isolated metabolites can be conveniently determined by FAB-MS (Fig. 1). The structure of altered amino acids can be determined by acid hydrolysis of metabolites followed by GC-MS of their N,O-heptafluorobutyryl isobutyl derivatives (Fig. 2).¹ A typical spectrum is shown in Fig. 3. However, four of the amino acids in the parent drug are N-methyleucines and two are alanines. In order to differentiate metabolism at these sites, limited acid hydrolysis is carried out and the resulting peptide mixture is analyzed by GC-MS according to Blemann² (Fig. 4). The resulting spectra of the derivatized polyamino alcohols derived from amino acids 5,6,7 and 8 and from amino acids 1 and 2 are shown in Figs. 5 and 6, respectively. The spectrum of Fig. 5 was obtained after B₂D₆ reduction while that of Fig. 6 was obtained after B₂H₆ reduction.

¹ S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.* **171**, 195 (1979).

² S. A. Carr, W. C. Herlihy and K. Blemann, *BMS* **8**, 51 (1981).

CYCLOSPORINE A FAB/MS (-) MU 1202.6

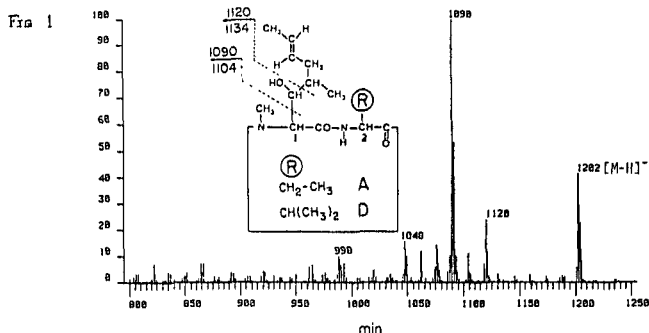
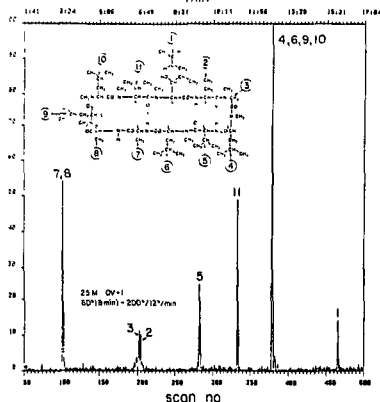
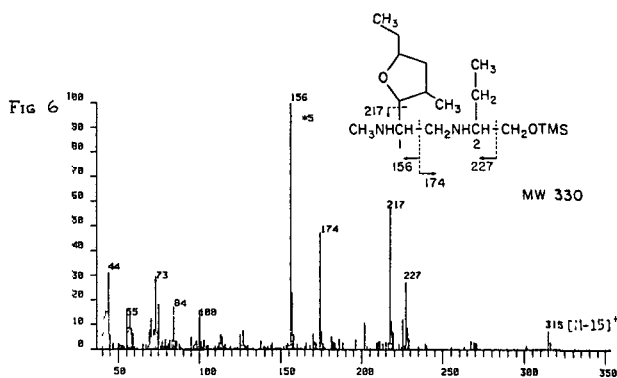
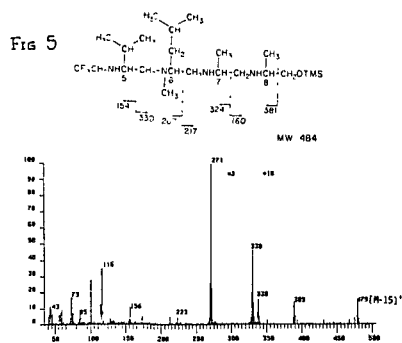
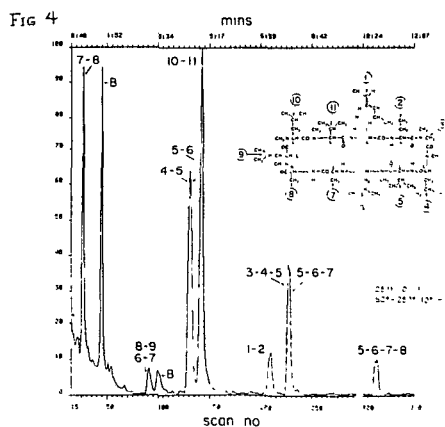
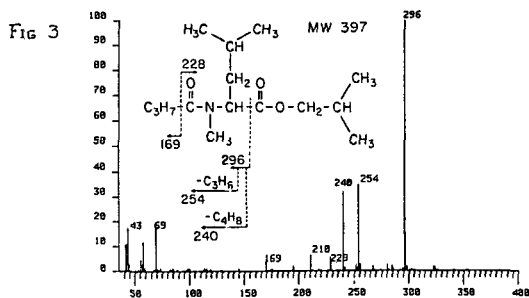


Fig 2





QUANTITATIVE MICRO LC/MS OF DRUGS IN BIOLOGICAL SAMPLES, Jack Henion and Dale Skrabalak, NYS College of Veterinary Medicine, Cornell University, 925 Warren Drive, Ithaca, NY 14850.

The qualitative usefulness of LC/MS, if it really is a routine analytical technique, is not a disputed point. However, the past seven or eight years of rather slow development or progress in LC/MS has not convinced many users that the technique is practical enough to implement in their laboratory. This reluctance to either join in and help develop LC/MS or explore the analytical utility of commercially available LC/MS interfaces comes in spite of considerable published work which demonstrates that the technique can be analytically useful (1,2). We have initiated quantitative analysis micro LC/MS experiments to demonstrate that this technique is reliable enough to carry out for these more demanding experiments.

Since quantitative analysis demands very stable, reliable performance from the equipment and the technique, we have conducted quantitative analysis experiments for determining the disappearance curves and excretion times of corticosteroids in equine urine and plasma. This work can be compared with results obtained from conventional HPLC and a realistic assessment of the relative merits can be made. The added specificity available from micro LC/MS minimizes the possibility of measuring additive, endogenous interference which may co-elute with the drugs or metabolites of interest. This paper attempts to demonstrate the ion current stability during the course of three or four hour micro LC/MS quantitative experiments and the value of the specificity available from a mass spectrometer as an HPLC detector.

EXPERIMENTAL

All quantitative micro LC/MS analysis experiments were conducted using a modified Waters ALC 202 HPLC as described previously (2). The eluent was 70/30 methanol/water maintained at a flow rate of 40 μ L/min through a Whatman ODS 3 Microbore B reversed phase column (1mm id. x 25 cm). The micro LC/MS interface was the same as described previously (2) and was inserted into the standard direct inlet of a Hewlett Packard Model 5985B GC/MS fitted with the LC/MS option 01 which includes a liquid nitrogen cryopump. All experiments in this work were conducted in the negative ion chemical ionization (NCI) mode. The total effluent from the microbore column was directed into the micro LC/MS interface. The added extra column void resulting from a UV detector precluded its use prior to the mass spectrometer. All administration plasma and urine samples were collected post administration of therapeutic levels of betamethasone, spiked with the internal standard, 6 α -methylprednisolone, and extracted by liquid-liquid extraction. The extracts were concentrated subsequent to filtration through 0.2 micron fluoropore filters.

RESULTS AND DISCUSSION

This work utilized an internal standard, and verified that chromatographic separation was achieved for all components of interest. Three hour continuous micro LC/MS experiments began by obtaining a full scan (m/z 150-450) micro LC/MS analysis of an extract of control equine urine spiked with the standard and authentic betamethasone.

This experiment verified that endogenous hydrocortisone was resolved from the parent steroid. Triplicate injections of post administration urines collected through 48 hours were analyzed over the course of three hour periods of continuous micro LC/MS operation. These data represented concentrations down to 50 ng/mL urine and were readily detected by this technique.

Triplicate injections of plasma extracts through eight hours post administration were made. These experiments usually required up to four hours of continuous micro LC/MS operation without interruption of data acquisition. The data showed the resolution of the components of interest, the stability of the system over several hours of continuous operation, and the feasibility of utilizing micro LC/MS for quantitative analysis.

SUMMARY AND CONCLUSIONS

With the exception of some of the later hour urines this work could have been conducted utilizing HPLC with conventional detection. However, as the level of the parent drug or its metabolites decreased, interference from endogenous components became significant. In this case the selectivity of the MS is a substantial benefit and provides the analyst with the knowledge that there is interference when conventional HPLC detectors might not reveal this information. This work also demonstrates that instrumental stability and reliability is suitable for the more demanding requirements of quantitative analysis. Thus, if the technique is reliable enough for this type of application, then it certainly should be suitable for routine qualitative analysis.

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METASTABLE ION MONITORING FOR THE MEASUREMENT OF DELTA-9-TETRAHYDROCANNABINOL AND TWO OF ITS MAJOR METABOLITES

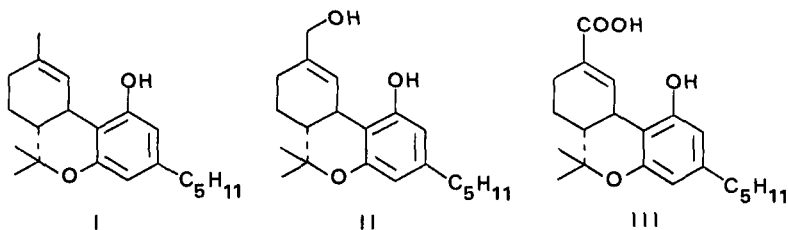
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Delta-9-tetrahydrocannabinol (delta-9-THC, I), the major psychoactive constituent of marihuana, is present in the blood at the low nanogram/ml range during intoxication and is thus difficult to quantify. In addition, its high lipophilicity results in extensive uptake into tissues and a rapid fall in the blood level so that few analytical methods can detect its presence for more than a few hours after use. Pharmacokinetic data on the drug are thus scarce and it is difficult to devise a reliable forensic test to determine its presence in the body. The most sensitive methods for measuring delta-9-THC in physiological fluids depend on radioimmunoassay, GLC or GC/MS and have been reviewed (1-3). Positive ion MS is capable of detecting delta-9-THC to the low picogram range from a clean sample, but when the drug is extracted from physiological media, the presence of co-extracted lipids limits the sensitivity of most assays to around 1 ng/ml and necessitates extensive clean-up of the sample, usually by column chromatography.

In our previously published method for quantification of delta-9-THC in plasma (4) and tissues (5) a double focussing mass spectrometer was used in the metastable ion mode to exclude most of the interfering ions produced by the endogenous lipids and enabled the drug to be measured to 5 pg/ml of plasma with no clean-up stage. [$1',1',2',2'-^4_4$]-cannabinol ($[^4_4]$ CBN) was used as the internal standard, the drug and standard were extracted with hexane, methylated with diazomethane to convert interfering fatty acids into methyl esters, and measured as TMS derivatives by means of the $M^+ \rightarrow [M-CH_3]^+$ (m/z 386 $\rightarrow$ 371) metastable fragmentation. This was focussed by tuning the magnet to m/z 371 and raising the accelerating voltage from 4.0 to 4.16 kV.

The major route of metabolism of delta-9-THC in all species so far studied is allylic hydroxylation at C-11 (II) followed by oxidation to delta-9-THC-11-oic acid (III).



11-Hydroxy-delta-9-THC (II) has an equivalent psychoactivity to delta-9-THC itself. The acid metabolite, although inactive, is frequently present in higher concentrations than the parent drug and is thus often preferred for forensic purposes.

We have now extended our metastable detection method to include measurement of these compounds. Quantification was again achieved with a VG Micromass 70/70F mass spectrometer interfaced to a Varian 2440 gas chromatograph containing a $2m \times 2$ mm glass column packed with 3% SE-30 on 100-120 mesh Gas Chrom Q. Focussing was achieved by decoupling the ESA voltage from the accelerating voltage which was maintained at 4 kV. The ESA reference was set by means of the peak selector unit to the value determined by M_2/M_1 , where M_2 is the mass of the daughter ion and M_1 the mass of the parent (the molecular ion, in each case) and the magnet was set to the mass of the metastable ion given by M_2^2/M_1 . [4_4]CBN was

again used as the internal standard and the fragmentation m/z 386 $\rightarrow$ 371 was used to monitor this compound and delta-9-THC as their TMS derivatives. The fragmentations m/z 474 $\rightarrow$ 371 and m/z 430 $\rightarrow$ 415 were used for the 11-hydroxy and 11-carboxylic acid metabolites as their TMS and Me/TMS derivatives respectively. Retention times of the four compounds at 250°C were: 1.2 min (delta-9-THC); 1.6 min ($[^3H]$ JCBN); 2.4 min (11-OH-delta-9-THC) and 2.8 min (delta-9-THC-11-oic acid). Immediately after the elution of the first two compounds, the ESA voltage was switched to the value for the hydroxy metabolite and the magnetic field adjusted manually by means of the mass marker. The conditions for focussing the acid metabolite were then set up after elution of 11-hydroxy-delta-9-THC. Detection limits, precision and accuracy for measurement of delta-9-THC were comparable to those of our previous assay. The detection limit for 11-hydroxy-delta-9-THC was about half that for delta-9-THC using peak height measurements and the limit for the acid metabolite was comparable with that for delta-9-THC.

Using the original extraction method (hexane), 11-hydroxy-delta-9-THC could be recovered to the extent of about 35% and could be measured to 100 pg/ml. The acid metabolite was not extracted under these conditions. The method has been used to measure the level of the hydroxy metabolite in mouse plasma one hour after an intravenous injection of 10 mg/kg of the drug. This was found to be 1.57 ± 0.28 ng/ml ($n = 4$) corresponding to a delta-9-THC level of 322.5 ± 33.0 ng/ml.

For simultaneous measurement of both metabolites, the following scheme was used. The internal standard in ethanol (1-5 μ l) was added to 1 ml of the plasma and allowed to equilibrate with plasma proteins for about 1 hour. The four compounds were then extracted with 3 volumes (2 ml) of ethyl acetate with the aid of centrifugation to split the resulting emulsion. The combined extracts were then chromatographed on a 100 mm x 5 mm column of Sephadex LH-20 in chloroform. The first 1.5 ml was discarded and a 6 ml fraction was then eluted with 10% methanol in chloroform. This was methylated and converted into TMS derivatives as before. 10% of the resulting sample was then analysed by GC/MS. Detection limits using this method were somewhat less than those achieved by the simpler hexane extraction for delta-9-THC and the hydroxy metabolite, these being around 100 pg/ml for both compounds. At present, therefore, the original technique would appear to be the best for these two compounds. The acid metabolite could be measured after extraction with ethyl acetate to 50 pg/ml in the equivalent of 0.1 of plasma. Work is currently under way to improve the extraction and clean-up stages to enable all three compounds to be measured to lower levels using only one extraction.

The method has been used to measure the levels of the drug and its metabolites in rabbit plasma following an intravenous injection of delta-9-THC at a dose of 1 mg/kg. Levels of the acid metabolite were very similar to those of delta-9-THC for at least the first 8 hours after drug treatment, whereas levels of 11-hydroxy-delta-9-THC were about one tenth of the delta-9-THC level. Typical values after 4.5 hours were: delta-9-THC, 3.0 ng/ml; 11-hydroxy-delta-9-THC, 180 pg/ml; and delta-9-THC-11-oic acid, 2.7 ng/ml.

Acknowledgements. We thank the Wellcome Trust and the Medical Research Council for financial support, and Miss J. Hughes for expert technical assistance.

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FD AND FAB MASS SPECTRAL IDENTIFICATION OF METHOTREXATE-POLYGLUTAMYL METABOLITES IN PATIENTS RECEIVING HIGH-DOSE METHOTREXATE TREATMENT

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The metabolism of the anticancer drug methotrexate (MTX) is less significant at conventional therapeutic doses (≤ 50 mg/kg) but becomes increasingly complex and pharmacologically important in the high-dose therapy which has been successfully introduced for the treatment of several human neoplasms. Among other metabolic pathways leading to relatively nontoxic excretion products, the conjugation of MTX with additional γ -glutamyl residues has found particular interest because of evidence for the high cytotoxicity of these metabolites. Although polyglutamyl derivatives have been known for the natural MTX analog, folic acid, the occurrence and pharmacologic role of MTX-polyglutamates in clinical chemotherapy have not yet been elucidated. In combination with reversed-phase HPLC methods, FD and more recently FAB mass spectrometry have been successfully applied to the unequivocal identification of several MTX- γ -polyglutamyl metabolites in erythrocytes of patients after single and multiple high-dose MTX therapy. As found in previous studies of MTX metabolites (1) and synthetic folate derivatives (2), FD mass spectrometry has been particularly suited for the direct structural characterization of MTX-glutamyl conjugates.

Erythrocytes obtained from patients 3-7 days after receiving MTX doses of 8-15 g/m² as adjuvant treatment for osteogenic sarcoma were analyzed following the presumed formation of intracellular metabolites from studies using biological assays. Isolated erythrocyte supernatant was concentrated and subjected to HPLC on a C-18 Bondapak column using a 50 mM NH₄-HCO₃/methanol gradient eluent optimized to high resolution for MTX- and folate-polyglutamates (3). Metabolite fractions of increasing polarity relative to MTX were isolated (313 nm UV) and lyophilized. Further purification for some samples was obtained by cation exchange chromatography with dilute pyridine or pyridinium acetate (4). FD and FAB mass spectra were obtained with a Finnigan MAT 312 spectrometer (EI/FD/FAB source) using a precisely adjustable insertion probe with interchangeable tips for FD emitter and FAB target. Emitter current programming and spectra accumulation techniques employed for FD analysis were as previously described (1,4). FAB spectra were obtained with a 7 keV Xe neutral beam (saddle-field gun).

MTX-polyglutamates eluted at regularly decreasing retention times prior to MTX with increasing chain length, at the HPLC conditions used (3). These metabolites were not present in serum but were detected in erythrocytes in significant amounts with time after therapy as shown in Fig. 1 for a sample obtained after 72 hrs (MTX- γ -mono- and -diglutamate; MTX-G1, -G2). MTX-G5 has been the highest conjugate detected and identified thus far. FD spectra of all metabolites in aqueous pyridinium or ammonium buffer solution yielded abundant MH⁺ ions, associated by intense M2H²⁺ ions for the larger conjugates (Fig. 2). Detailed structural information is provided by the series of fragmentations at the NH-alkyl bonds along the peptide chain, and at the N(10)-amino group, leading to complementary fragment ions (e.g., m/z 539, 177 for MTX-G2). Abundant MH⁺ ions were also obtained by FAB spectra using acetic acid/glycerol solutions. Major fragmentation, in contrast to FD, occurred at the peptide and pteroyl-amide bonds yielding structural data complementing that obtained by FD (e.g., m/z 406, 308 for MTX-G2; Fig. 2,b). However, the strong acidification of the matrix required for FAB mass spectral analysis presented problems in some cases because of the high lability of MTX-polyglutamates upon acid treatment.

These results and first quantitative studies by HPLC indicate polyglutamyl conjugates as the major intracellular forms upon systemic MTX administration at high doses and for prolonged periods of time. Their intracellular accumulation, and absence of any detectable levels in serum and urine are consistent with the previously suggested role of MTX-polyglutamates to cause delayed erythropoietic and hepatic toxicity after high-dose MTX. Further studies are presently carried out to characterize in detail the formation of these metabolites in the clinical high-dose MTX therapy.

This work has been supported by the Bundesministerium für Forschung und Technologie, Bonn, West Germany (PTB 8460).

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Fig. 1 (a), HPLC separation of MTX and MTX- γ -glutamyl conjugates ($n = 1-6$); (b), detection of MTX-G1 and MTX-G2 (arrows) in erythrocytes from a patient 72 hrs after high-dose MTX treatment (8 g/m^2).

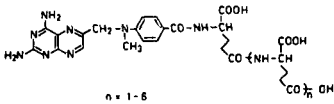
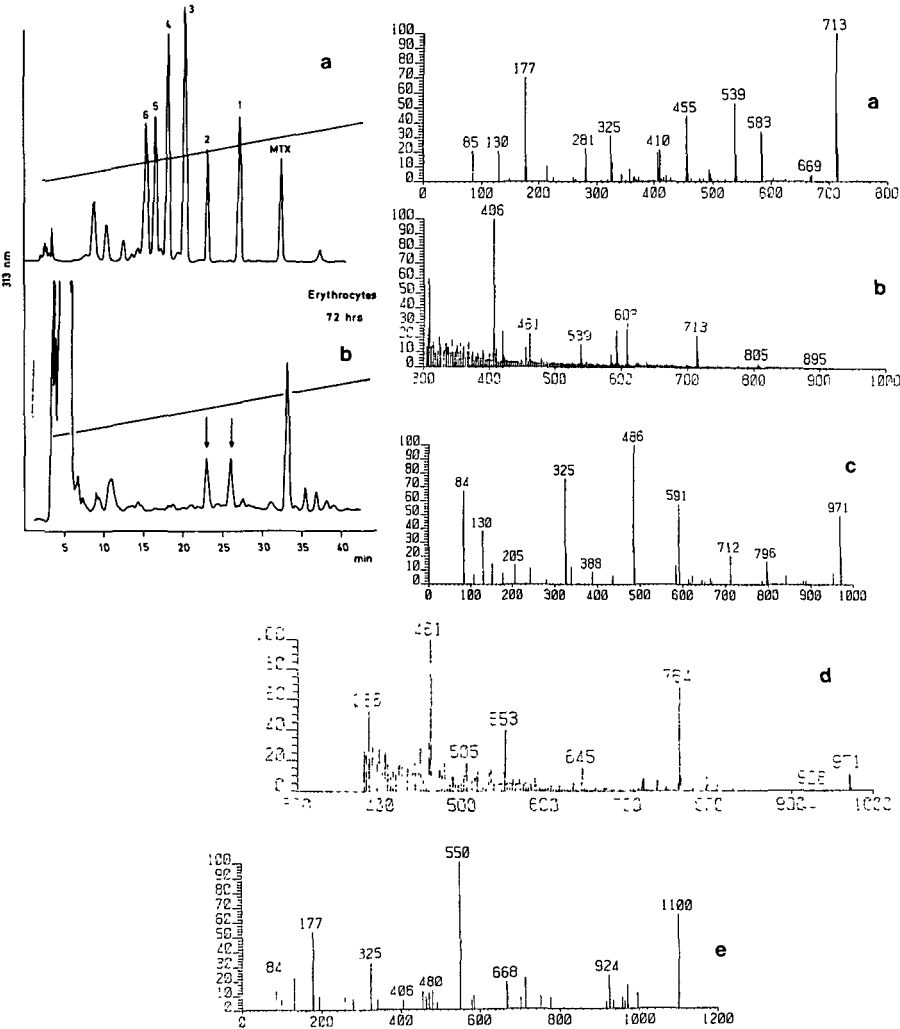


Fig. 2 FD and FAB mass spectral identification of MTX- γ -glutamyl conjugates from erythrocytes; (a), MTX-G2, FD; (b), MTX-G2, FAB; (c), MTX-G4, FD; (d), MTX-G4, FAB; (e), MTX-G5, FD.



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CONTINUUM FLOW ION EXTRACTION FROM AN ICP

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The use of an atmospheric pressure plasma as an atomic ion source for a mass spectrometer has attracted increasing attention as a potentially important analytical technique since it was first described (1). Very low detection limits were achieved, even in the early work, and the potential for ultra-trace analysis was seen. The introduction of an ICP as the plasma (2) was an important step towards overcoming the low plasma temperatures of the original work. Once continuum sampling with large apertures was achieved (3), the full performance expected from the ICP could be approached (4). Detection limits were initially poorer but much more uniform over the required mass and ionization energy range. Other papers at this meeting (5,6,7,8) and elsewhere (9,10) describe both systems now available and the performance obtained with them.

Rapid isotope ratio measurements to precisions $<0.5\%$ (11,8) together with very simple sample preparation and introduction are major advantages of the technique. However detection limits in solution for many elements of between 0.1 and 1 ng.ml^{-1} , first reported in 1982 (4,10) and again at this meeting, are still poorer than really required for much ultra-trace analysis.

Recent studies of the factors limiting the ultra-trace performance have enabled these values to be lowered considerably. The result of this latest work and the performance now achieved are discussed. The background signal is considered in relation to the total system ion transmission for the wanted species. This is defined as the count rate detected per unit solution concentration in the sample presented to the instrument. It has typical values of between 5 and $30 \times 10^3 \text{ counts.sec}^{-1} \text{ per } \mu\text{g.ml}^{-1}$. Random background from all sources is roughly constant across the mass range, but particularly below $100u$, small molecular peaks from ion molecule reactions between the plasma support gas and the vaporized solvent also contribute to background levels at certain discrete masses. Once detection limits much below 1 ng.ml^{-1} are achieved contamination in solvents and reagents can also contribute significantly to the blank level. These components of spectral background then become much more significant than the random level.

Random background from excited ion decay and from stray ions passing the analyzer increases with transmission but it has been found possible to limit the total random level to about $30 \text{ counts.sec}^{-1}$ while increasing the transmission to about $5 \times 10^5 \text{ counts.sec}^{-1} \text{ per } \mu\text{g.ml}^{-1}$. Detection limits for some important elements resulting from this improvement are shown in Table 1. Only for Hg was the blank free of minor peaks from contamination or reaction products. However the level for Hg of 0.01 ng.ml^{-1} does suggest what might ultimately be achieved in clean laboratory conditions. Certainly values below this may reasonably be expected in the future.

Table 1. Solution Detection Limits ng.ml^{-1} (2σ)

Li	B	Be	Co	Zn	Ge	As	Se	Ag	Cd	Sn	Au	Hg	Pb	Th	U
0.06	0.2	0.04	0.03	0.7	0.1	0.3	7	0.02	0.04	0.05	0.04	0.01	0.03	0.06	0.06

These detection limits were measured in the single ion mode, using 10 second integrations of blank and line at 100 ng.ml^{-1} . Such an integration for Hg at 1 ng.ml^{-1} is shown in Fig.1.

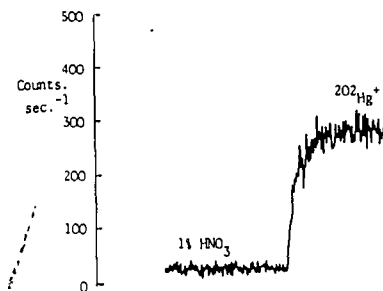


Fig.1. Single Ion Plot of Blank and 1 ng.ml^{-1} Hg.

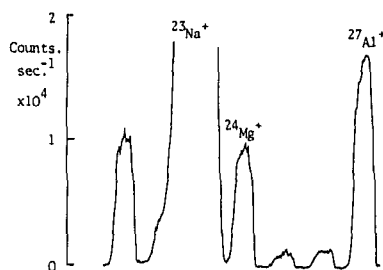


Fig.2. Spectrum of Mg at 10 ng.ml^{-1} in 1000 µg.ml^{-1} Na.

To use the full matrix tolerance of the ICP good abundance sensitivity of the mass analyzer is important. Fig.2 shows the response to Mg in the presence of a high concentration of Na in the adjacent mass channel.

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Acknowledgements.

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NEGATIVE ION GLOW DISCHARGE MASS SPECTROMETRY OF THE LANTHANIDE ELEMENTS, R.B. Keefe, P.J. Savickas, and W.W. Harrison, Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901.

The d.c. glow discharge has previously been applied in our laboratory to trace element mass spectrometric analysis of the lanthanide elements in the positive ion mode. A system has been constructed which also allows the detection of negative ions using pulse counting circuitry. The primary interest is in the negative ions of the sputtered species. A comparison is made between the types of positive and negative ion species observed.

Experimental

The source has been designed to hold a solid or pressed powder sample in a shielded vice grip parallel to the quadrupole axis (Figure 1). In another configuration, solution residue samples can also be examined from a vitreous carbon cup held perpendicular to the quadrupole axis.

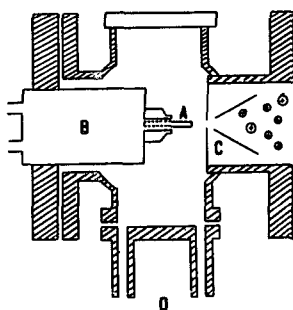


Figure 1. Solids Ion Source (A) Single pin cathode, (B) Water-cooled pin holder, (C) Ion exit orifice leading to high vacuum chamber for quadrupole mass filter, (D) Gas inlet and pumping port.

Results and Discussion

(1) Reactive Oxygen Sputtering

Figure 2 shows the negative ion spectrum obtained from a pressed Ce/Ag pin in a pure O_2 discharge. This sample shows a trace amount of CeO_2^- , the major peaks in the spectrum corresponding to CeO_3^- , CeO_4^- , $CeO_5H_2^-$, and $CeO_6H_2^-$. The major mode of negative ion formation appears to be attachment of O^- and O_2^- background species to the neutral sputtered oxides. Figure 3 shows the corresponding spectrum for a pure Sm pin, with a series of peaks extending from the SmO_2^- to SmO_6^- region. No Ln^- species are observed, as the electron affinities for these atomic negative ions are estimated to be <0.5 eV.

This contrasts for the positive ion situation, where the major lanthanide species in an O_2 discharge are LnO^+ and LnO_2^+ .

(2) Sputtering with Ar/ O_2 Mixtures

For a Ce/Ag sample, as the percentage of O_2 in the discharge is reduced, the total number of negative ion species formed in the discharge is lessened due to the reduced population of O^- and O_2^- background species available for attachment, but the relative percentage of CeO_x^- species do not vary. This contrasts with the positive ion mode, where the total ion yield increases with increasing percentage of argon due to the more efficient sputtering of the sample as the oxide layer is reduced. Also, the

relative percentages of LnO_2^+ , LnO^+ , and Ln^+ change drastically when the O_2 is decreased, until in a pure Ar. discharge the major Sm species from an Sm_2O_3 (low oxide bond strength) pressed pin is Sn^+ , and from a CeO_2 (high oxide bond strength) pin is CeO^+ .

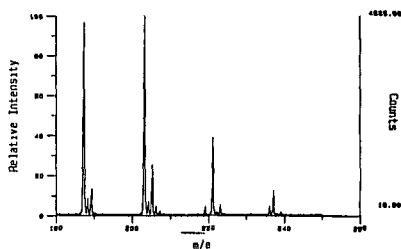


Figure 2. Ag pin with 40% Ce as CeO_2 . 10 scans at 800 usec/channel, 40 points/amu; O_2 discharge.

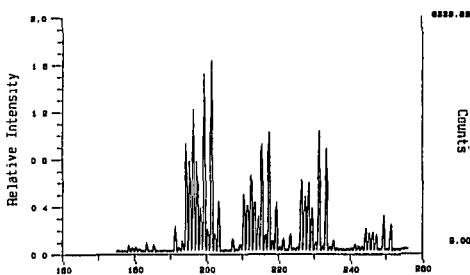


Figure 3. Pure Sm pin, O_2 discharge.

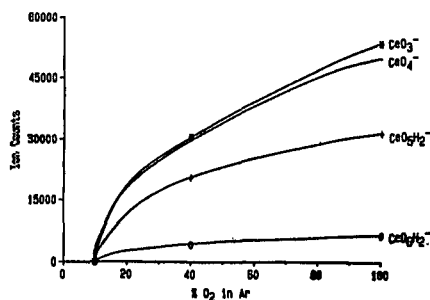


Figure 4. % O_2 in Ar discharge vrs. Peak intensity of a Ag pin with 40% Ce as CeO_2 .

MASS SPECTROMETRY OF NANOGRAM QUANTITIES OF Th AND U FROM (Th,U)O₂ FUELS

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Measurements of Th and U isotopes in irradiated (Th,U)O₂ fuels are required for fuel burnup determination and for verification of reactor physics calculations. The major actinide isotopes in an irradiated (Th,U)O₂ fuel are the following: ²³²Th, ²³³U, ²³⁴U, ²³⁵U, ²³⁶U and ²³⁸U. ²²⁸Th and ²³²U are produced in trace quantities, but, because of their relatively high specific activities, they cause the total Th and U to have relatively high radioactivities. The high radioactivities limit the quantities of U and Th used for mass spectrometry to the nanogram level to avoid radiation hazards and mass spectrometer contamination.

With the conventional solution loading technique used with thermal ionization mass spectrometry, sufficient sensitivity was not obtained for analysis at the nanogram level. To obtain higher sensitivity, the following alternative sample loading techniques were investigated: the Dowex resin bead technique (1), a similar technique that used a 1 mm diameter disc of Acropor anion-exchange membrane instead of the Dowex bead, and a technique that involved carburization of a solution deposit and filament in a benzene atmosphere. To compare the sensitivities obtained with each technique, their sample utilization efficiencies were determined. The U and Th ion currents were integrated over the lifetime of the sample and divided by the number of atoms of each deposited in the source; the results are shown in Table I.

Table I. Utilization Efficiencies

Sample Loading Form (Centre Filament)	Element	
	U	Th
Solution	1/100,000	
Dowex bead	1/2,000	
Acropor disc	1/2,000	1/100,000
Benzene carburized	1/2,000	1/40,000
Starch-Rhenium covered Acropor disc	1/500	1/10,000

The differences in the utilization efficiencies obtained with the various forms were likely due to differences in their ionization efficiencies. The higher ionization efficiencies obtained for the reduced forms compared with the solution forms may be explained as follows. First, the reducing environment likely reduced U oxides to the atomic state, and thus increased the abundance of the atomic state, which was the state that was measured. Second, other workers (2) have reported that carburized Re has a higher work function than the pure metal; the filament in the region of the sample would have been carburized by the burning bead or disc.

The increase in utilization efficiency obtained when the starch-rhenium mixture was deposited over the disc was possibly due to an increase in the fraction of actinide atoms that made close contact with the surface. In the absence of the starch-rhenium deposit, a significant portion of the U or Th was likely volatilized with the decomposition products of the bead or disc. The starch-rhenium deposit likely held the U and Th atoms in the surface region longer, allowing a larger proportion of them to diffuse to the filament surface to become ionized; the atoms must approach the surface within a few atomic radii for ionization to be possible (3). The starch-rhenium covered disc was the optimal filament loading form studied, because it yielded steady ion beams and the highest utilization efficiencies. Furthermore, the discs were more convenient to work with than were the other forms. The efficiencies for Th and U with the starch-rhenium covered disc were high enough for precise analysis of 5-20 ng of actinide, which was suitable.

The differences in the efficiencies for U and Th were mostly due to the .8 eV difference in ionization potential between the two; from this the efficiency for Th was expected to be ~ 50 times less than that of U.

Distribution coefficients were determined for U(VI) and Th(IV) in HNO_3 solutions by the radiotracer technique, and the results showed that the Acropor discs had a much higher affinity for Th(IV) than for U(VI). Because of this and the high Th to U ratio (~ 40) in the fuel, the discs were unsuitable for direct sampling of a $(\text{Th,U})\text{O}_2$ fuel solution; an excessive amount of Th would have been present for U analysis. Other extractants were investigated and tributylphosphate (TBP) was the most suitable. To adapt the procedure to a micro-scale 0.6 mm diameter XAD-2 resin beads were impregnated with tributylphosphate and equilibrated with the fuel dissolved in 0.08 M $\text{HF}/6$ M HNO_3 ; this achieved the desired separations and extracted Th and U from the fuel in approximately equal proportions. However, these beads were not suitable for mass spectrometry; the ion signals for U and Th were very erratic and noisy.

To transfer the U and Th to a suitable sample loading form, they were back-extracted in a nitric-hydrofluoric acid solution and nanogram quantities were adsorbed onto an Acropor disc, which was then transferred to the centre filament. The two elements were analyzed sequentially from the same disc; U first at 1750°C then Th at 1950°C . The peaks were well defined and interference free (Figure 1); Pu appeared during the initial heating period but it evaporated quickly and was not a problem. There was an interference at mass 238 due to trace U contamination in the filaments, but this was avoided by using zone refined Re filaments. Relative standard deviations of isotope ratios were typically between 0.1 and 0.3%.

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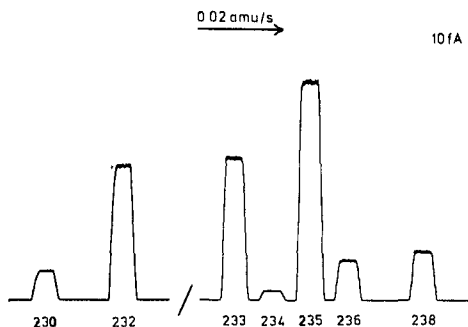


Figure 1. Mass spectrum of Th and U from irradiated $(\text{Th,U})\text{O}_2$ fuel. 40 ng Th, 14 ng U.

THE DEVELOPMENT OF AN ISOTOPIC DILUTION
TECHNIQUE TO DETERMINE FEMTOGRAM AMOUNTS OF ^{97}Tc , ^{98}Tc ,
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Alamos National Laboratory, Box 1663, Los Alamos, N.M.
87545

A method to chemically purify and isotopically analyze femto-gram amounts of technetium 97, 98, and 99 is being developed. The method will be utilized to measure technetium in secular equilibrium with uranium in various ores, technetium as a 14 MeV neutron detector in weapons diagnostics, and technetium-97 and -98 as reaction products of solar neutrinos on molybdenum-97 and -98.

There are several complicating factors in both the chemistry and the mass spectrometry. Molybdenum, at this level, is a ubiquitous contaminant. The elimination of Mo both chemically and as a background impurity in the instrument itself is the major obstacle. Since technetium is a relatively inefficient ionizer compared to molybdenum, the problem becomes compounded.

The major thrust has been concentrated on devising an ionization enhancement method for technetium while reducing the instrumental isobaric molybdenum background. Nominal one nanogram (10^{-9} gm) loads of technetium are being used to evaluate various methods of ionization. Ionization efficiencies from 10^{-5} to 2×10^{-4} have been observed. Molybdenum-98 signals have been reduced to <200 cps at 1900°C using a canoe filament.

A PRACTICAL CHARGE-INTEGRATION TECHNIQUE FOR SMALL SIGNAL MEASUREMENTS IN THERMAL IONIZATION MASS SPECTROMETRY. E. L. Callis; Argonne National Laboratory, Argonne, Illinois, 60439

Charge integration has long been recognized as a very sensitive method for the measurement of ion currents as small as 10^{-17} amps. While several dual collector systems have been described¹ for measurement of relatively large currents, $\geq 10^{-11}$ amps, and the advantages of the method for smaller currents was reiterated by Herzog² several years ago, application of the method to single collector measurements has apparently not been very successful. This may be due to the problems associated with computerization of the method, a virtual necessity for routine measurements.

In this work, techniques were developed and modifications made to an existing data acquisition system which permit routine utilization of the charge integration method. The mass spectrometer employed is of NBS design, single stage, equipped with a Faraday cup collector and a vibrating-reed electrometer, Cary Model 401MR. The signal is digitized via voltage-to-frequency conversion followed by a precision counter. The counter is internally triggered at one-second intervals and serves also as the system clock. A Hewlett-Packard 9830B desktop computer controls the data acquisition. Only a minor hardware modification was made to the standard system to permit operation of the electrometer input-shortening relay under computer control.

Setup of the system, including peak-centering and focusing, is performed manually with the electrometer in the high-resistance leak mode, which greatly facilitates these operations. At the start of the measurement sequence, the electrometer is switched to the charge mode. Ion currents are measured by integrating charge for periods of three to ten seconds for each peak, while measuring the electrometer output voltage at one-second intervals. A linear least-square regression then gives dV/dt , which is proportional to the charging current. Baseline measurements, which consist of the sum of the input current drift plus any actual signal appearing at baseline positions, are made prior to each block of ratio measurements.

The primary obstacle in automation of charge integration measurements is the necessity of detecting spurious "steps" in the integrated signal. These steps are primarily due to α -decay of naturally occurring radioactive elements in the electrometer construction materials. These discontinuities occur at a rate of 20 to 30 per hour in a typical electrometer, producing a charge, on average, of 5×10^{-15} coulomb per event. The least-square regression offers an excellent method of detecting these events by testing for goodness of fit. Measurements in which the residuals exceed an experimentally determined value are rejected and a second integration is performed. For ion currents greater than 10^{-13} amps, this test becomes less effective since ion beam fluctuations become important. Accordingly, shorter integration times are employed for signals greater than 10^{-13} amps to reduce the probability of encountering a spurious event during the measurement.

In general, up to five isotopes are measured per cycle. Since discharging of the capacitor and subsequent opening of the input can produce strain currents, these operations are minimized by only discharging the capacitor before and after integration of the major peak. That is, the signals from two or more smaller peaks are allowed to accumulate in turn, generally on a more sensitive range than that used for the major peak.

This technique has been in use for about two years. The average noise level observed is 2 to 3×10^{-17} amps, which is at least a factor of 5 lower than that obtained in the high-resistance leak method. This has permitted analyses of uranium and plutonium samples as small as 10 ng as well as improved measurements on ratios $< 10^{-2}$ with relatively large (1 μ g) samples.

¹Willey, John F. and James W. Taylor, Anal. Chem. 50, 1930 (1978).

²Herzog, L. F., Proceedings of 20th Annual Conference on Mass Spectrometry and Allied Topics, p. 295, June, 1972, Dallas, Texas.

KNUDSEN EFFUSION AND TRANSPIRATION MASS SPECTROMETRY STUDIES OF HALOGEN-ALKALI SILICATE INTERACTIONS

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Complex alkali silicate systems, such as glass and coal slags, exhibit inherently low alkali activity and hence partial pressure. However, in the presence of chloride-containing species, the rate of alkali vapor transport can be greatly enhanced. The results of Knudsen effusion or Transpiration Mass Spectrometric experiments are discussed for the systems:

- (a) NaCl (s, ℓ , g) + K₂O-containing coal slag
- (b) HCl + Na₂O, K₂O-containing glass
- (c) HCl + dolomite (a K₂O, Na₂O-containing mineral)

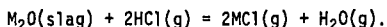
An example of system (a) using excess slag containing 19.4 wt% K₂O was obtained by transpiration mass spectrometry. The exchange process is due to the reaction,



This result is demonstrated in figure 1 where the observed partial pressures of NaCl and KCl are expressed in thermodynamic activity form. A marked decrease in NaCl activity and concomitant increase in KCl activity take place just above the melting point of NaCl. By the end of the experiment 90 percent of the initial NaCl was converted to Na₂O(slag) with stoichiometric release of KCl. During the isothermal, constant activity period of the exchange process the effective K vapor concentration was 10⁴ times greater than in the absence of NaCl.

The exchange process was also observed in Knudsen effusion experiments using a higher salt/slag ratio with a slag containing 8.7 wt% K₂O. If complete exchange of NaCl with K₂O took place, the maximum concentration of KCl in the salt phase would be 20 mol%. The phase diagram of NaCl-KCl indicates that at this concentration, a solid solution would exist over the entire temperature range studied. The activity of KCl in the salt phase as a function of temperature based on the KCl(g) and K₂Cl₂(g) pressures is shown in figure 2 compared with literature data. At 1000 K the effect, in K concentration in the vapor, is 10⁷ times that in absence of NaCl. Observation of monomers, dimers and mixed dimers were also made during the exchange reaction. Calculation of equilibrium constants for gas phase reactions and comparison with previous effusion data were made.

Data for system (b) were obtained using a gas inlet Knudsen effusion system. The chemical reaction taking place is,



Initial measurements were made at HCl flow rates which later proved to be too high to obtain steady state. During this period Na and K transport were as much as 10² times greater than in the absence of HCl. At lower flow rates it was possible to obtain steady state conditions over a limited HCl pressure range.

In the steady state region the ratio of theoretical to observed $A_{\text{K}_2\text{O} \cdot \text{K}}$ is 10⁶ showing that the process is far from equilibrium. However, this ratio is an upper limit because the K₂O activity decreased during the measurements. Representative data are shown in figure 3 as the equilibrium constant times the activity of K₂O (determined in a previous experiment) plotted against the pressure of HCl. At pressures above 10⁻⁶ atm, the value of $A_{\text{K}_2\text{O} \cdot \text{K}}$ falls below the steady state value. In the steady state region the observed value is six orders of magnitude below the theoretical value showing that the transport process is far from equilibrium. However, this is a lower limit because the K₂O activity decreased in the slag during the measurements.

The reaction of HCl(g) with calcined dolomite represents system (c). The dolomite initially contained 100 PPM K_2O and 10 PPM Na_2O . Because of the low concentrations of alkali and reaction of CaO and MgO with the HCl(g) it was not possible to determine if a steady state could be established. A comparison of $A_{\text{K}_2\text{O}} \cdot K(\text{obs})$ vs $10^4/T$ and the theoretical line is shown in figure 4. Although the theoretical and observed curves approach each other they differ by 10^{14} at 1100 K and it is clear that no significant approach toward equilibrium will be achieved at physically attainable temperatures. Yet, the loss of K_2O and Na_2O could be detected at a temperature 200 K below the temperature where measurable vaporization of Na and K were detected in the absence of HCl . In addition to the formation of KCl(g) and NaCl(g) , the dolomite reacted with HCl to form a solution of $\text{CaCl}_2(\text{s})$ and $\text{MgCl}_2(\text{s})$ which could be observed vaporizing at higher temperatures.

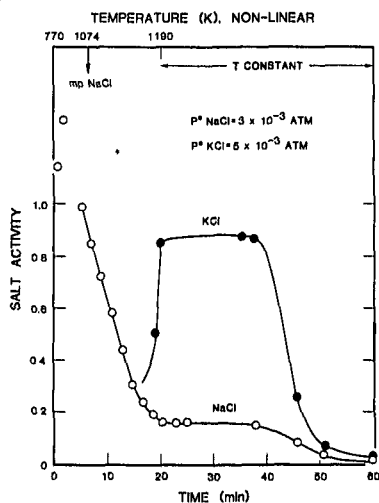


Figure 1.

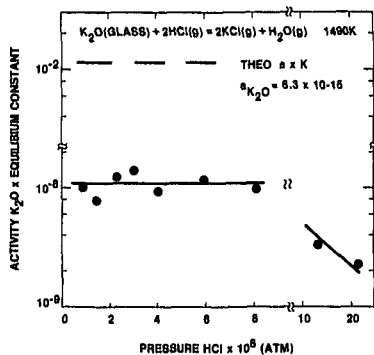


Figure 3.

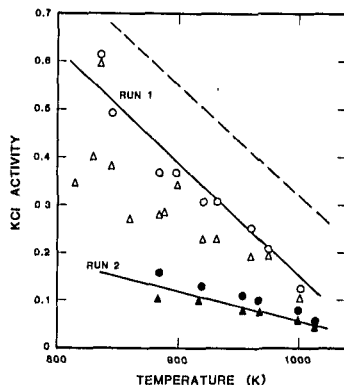


Figure 2.

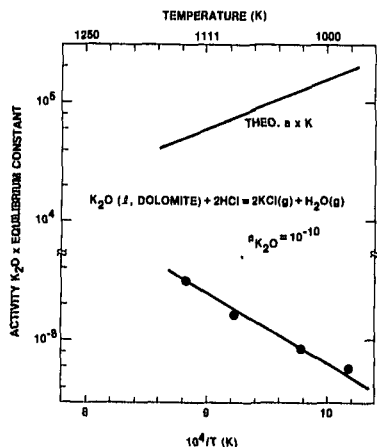


Figure 4.

RESPONSE OF SOME CARBIDES AND OXIDES TO PULSED-LASER VAPORIZATION

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INTRODUCTION

Vaporization characteristics of several carbides and oxides have been studied by heating them with a pulsed laser operating in the burst mode and analyzing, in situ, the vapors induced by each pulse of the laser with a time-of-flight mass spectrometer (TOFMS) having a nude ion source configuration. This technique has provided information on the amount and composition of vapors (including transient species) in terms of laser irradiance and material parameters.

EXPERIMENTAL

The laser parameters for the heating conditions in this work were as follows:

Wavelength	1.06 μm
Operating mode	Normal (burst)
Pulse duration	400-800 μsec
Energy density on target	25-600 J/cm^2
Beam diameter on target	1-6 mm
Peak irradiance (in time)	50-1000 kW/cm^2
Beam cross section	Uniform (non-Gaussian)

Efforts were made throughout to maintain uniform irradiance over the targeted spot on the planar surfaces of the sample materials; earlier work [1,2] has shown that laser-induced vaporization from a circular spot on a planar surface in a low ambient pressure produces a quasi-steady, supersonic free-jet expansion wherein the irradiated spot acts as if it were a sonic orifice between a high-pressure reservoir of the vapors and the low-pressure environment. Thus, the pressure at the irradiated surface can be quite high (even in a vacuum), but the rapid gasdynamic expansion quickly "freezes" the vapor species in a collisionless regime. For this reason we are reasonably confident that the species detected by the mass spectrometer several centimeters away are the same as those generated at the surface of the sample material. In this work the distance between the heated sample and the ionizing electron beam of the TOFMS was 14 cm; relative positions are shown in the vacuum system diagram of Fig. 1. The baffle serves as a partial vacuum barrier for the vapor pulses but chiefly as a protection of the nude ion source structures from the condensables in the pulses. The TOFMS was operated at a rate which produced 40 spectra per millisecond, and the data acquisition system has been described previously [3].

RESULTS

One example of oxides investigated was the reusable surface insulation (RSI) materials employed as the thermal protection system of the Space Shuttle. These are pure, low-density, rigidized, fibrous silica (LI-900) and a similar material containing about 3% by weight 1200-grit silicon carbide powder (LI-2200). This small amount of SiC makes a significant difference in the response of the material to 1.06- μm radiation. The pure silica (LI-900) is very reflective at this wavelength, and only after several laser "shots" (540 kW/cm^2) on the same area does the surface begin to darken and "densify," i.e., melt and fill adjacent pores. Subsequent laser shots produce O_2 and a lesser amount of atomic oxygen as the major vapor components. By contrast the presence of the small amount of SiC in the silica increases the quantity of vapor produced per laser pulse (200-330 kW/cm^2) and changes the major vapor component to CO plus small amounts of O_2 and CO_2 . Post-test examination of the irradiated target spot revealed that the vaporization of the silica had been nonuniform and occurred primarily around the SiC particles which act as light absorbers. This, then, accounts for the preponderance of CO in the vapor products despite the low concentration of carbides in the bulk.

Another group studied was pure carbides and metal-impregnated carbon materials to compare their high-temperature behavior to that of graphite in an attempt to understand the role of the metals during the laser heating of the metal-impregnated carbons. One of the first objectives was to determine the amount of heavy metals in the vapor phase. Carbonized tungsten-bearing resin (TBR) at 380 kW/cm^2 peak irradiance yielded the same ratios of carbon species typical of a graphite spectrum, but no measurable W. However, these spectra show very prominent peaks of B, Al, Ca, and Ti and small ones for Na, Fe, Ba, Sr, and hydrocarbons, which indicates that the instrumentation is sensitive to even the minor metal impurities. Significantly, after several laser shots the irradiated area of the solid material became covered with agglomerates of metallic material which appears to be a cooled melt.

At approximately 75 kW/cm² peak, 38% TaC in graphite produced only typical graphite spectra and no perceptible tantalum. The vapor products from WC containing 4% cobalt were C₃ and C₂ (and possibly C) with the predominant peak in the spectra being Co. Also prominent in the spectra were the peaks from the metal impurities Ca, Ti, Fe, Al, Cr, and Ba, but no W. Similarly, 99.9% pure W₂C at 1 MW/cm² peak irradiance produced vapors of (in descending order) CO, C, C₃, C₂H₂, and CO₂, and again no W. Note that in this case the amount of C exceeds C₃. The large amounts of hydrogen and oxygen compounds are presumably due to oxides on the surface and/or water absorbed in the pores. As with the TBR, a layer of metallic agglomerates was left behind on the irradiated surface of the solid samples; scanning electron micrographs clearly portray a cooled melt which apparently wetted the W₂C surface. The most consequential effects of this metallic layer are the increased reflection of the incident laser beam and a depletion of carbon at the surface so that as more of the metal is formed on successive laser pulses striking the same area, less vaporization occurs. This is shown in Fig. 2.

This observed absence of the heavy metals in the vapor phase agrees with vapor pressure data [4] which show that the vapor pressure of carbon in the 3000-4000 K range is three orders of magnitude greater than W or Ta. It also tends to support the conclusion of Storms [5], who pointed out that the tungsten carbide systems lose carbon preferentially at all compositions and temperatures.

ACKNOWLEDGMENT

Appreciation is extended to Bruce A. Raby for his assistance in obtaining the data and providing mass calibrations.

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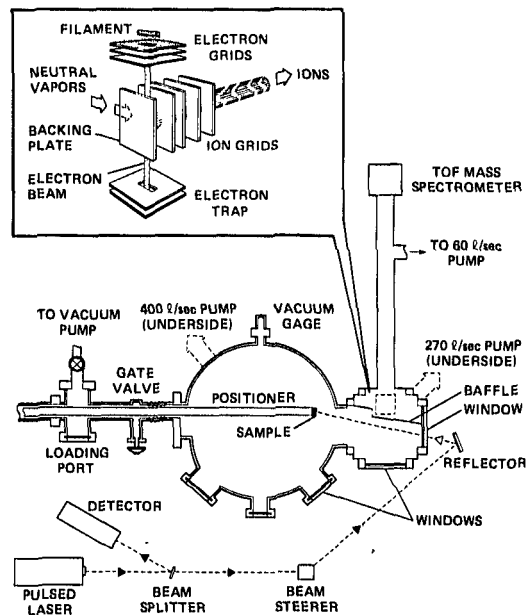


Fig. 1. Advanced laser/mass spectrometer system.

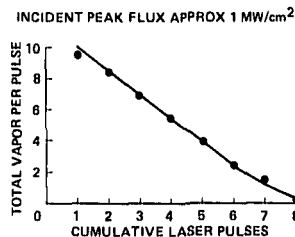


Fig. 2. Vapor produced from sintered W₂C (99.9%).

ANALYSIS BY PLASMA SOURCE MASS SPECTROMETRY

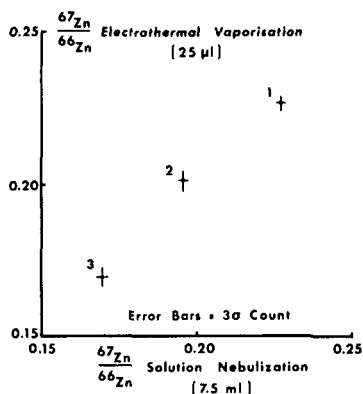
Alan R. Date, Institute of Geological Sciences, 64/78 Gray's Inn Road, London WC1X 8NG, UK.
 Alan L. Gray, Department of Chemistry, University of Surrey, Guildford GU2 5XH, UK.

The development of continuum flow ion extraction from an inductively coupled plasma (ICP) is described in a companion paper (Gray and Date, FOC2), with a complete reference list. The characteristics of the ICP as an emission source in atomic spectroscopy are now available for its use as an ion source in atomic mass spectrometry. The advantages of this successful development are:

1. high sensitivity,
2. wide element coverage,
3. simple spectra,
4. wide linear dynamic range,
5. tolerance to complex matrices,
6. isotope ratio capability,
7. speed of analysis,
8. ease of automation.

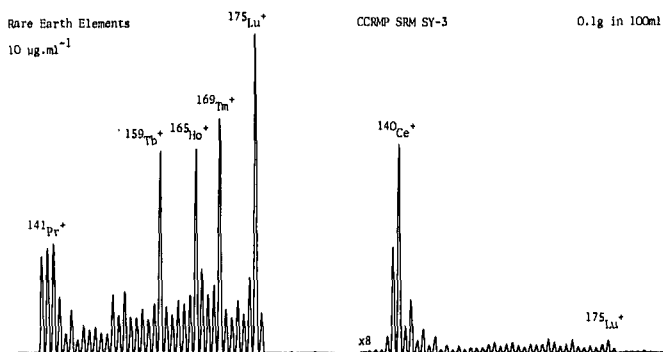
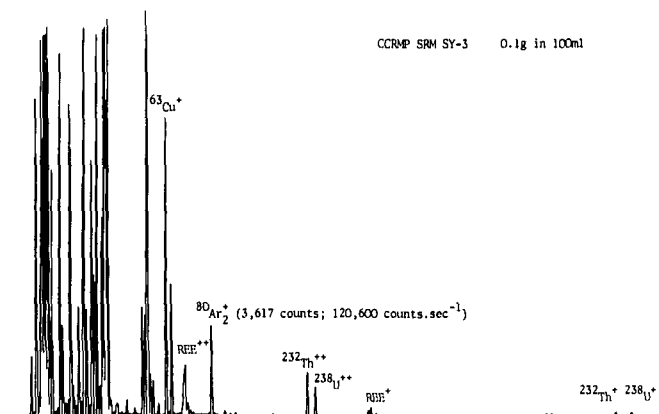
The main disadvantage, with true plasma sampling from the ICP, is the presence of doubly charged species for those elements having second ionization energies below the first ionization energy of the plasma support gas, in this case argon (15.76eV).

The Institute of Geological Sciences in the United Kingdom has a two-fold interest in this development, rapid isotope ratio determination for geochemical prospecting and multi-element trace analysis, particularly for the traditionally 'difficult' elements, e.g. the rare earth group, the hydride group, and the platinum metals. The method has already been applied to the rapid determination of lead isotope ratios in minerals^{1,2} and is expected to find wide application for isotope ratio determinations where error in the range 0.1 to 1% is acceptable, e.g. stable tracer studies in biochemistry. During the course of the work on lead isotope ratios, the authors were given some ⁶⁷Zn-enriched extracts from blood plasma and faeces. Data obtained for three blood plasma extracts with sample introduction by conventional solution nebulization, previously published², are compared below with electro-thermal vaporization values (concentration 2µg.ml⁻¹ Zn, aliquot 25µl). Agreement is excellent.



The work on lead isotope ratios is now extended to multi-element analysis of a wide range of

geological materials. Data are presented below for the Canadian Certified Reference Materials Project SRM SY-3, a silicate rock spiked with a mixture of rare earth elements. Concentrations calculated using one blank (1% v/v HNO₃) and one standard solution are compared with previously published values. The method clearly has great potential.



Element	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Concentration Found (µg/g)	1365	2435	255	821	126	16	191	23	151	30	96	13	75	10
Published Data (3)	1350	2200	1207	800	1007	14	55	117	807	207	507	87	65	87

ACKNOWLEDGEMENT

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A THEORETICAL ANALYSIS OF TEMPERATURE DEPENDENT ELECTRON IMPACT FRAGMENTATION

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Fragmentation of ions produced in electron impact is a ubiquitous phenomenon in all aspects of mass spectrometry, whether to be avoided in, e. g. Knudsen Effusion Mass Spectrometry (KMS), or desired as an analysis tool in structural mass spectrometry. In all of these cases, the process is normally assumed to be temperature independent, both because $kT \ll eV$, and because of lack of evidence for the existence of the effect. One known exception is the report by Akishin et. al. [1] of a 20% variation in the ratio $Cs^+ / CsCl^+$ in the temperature region from 800-900 K. The explanation is a simple Franck-Condon argument, asserting poor vertical overlap of the ground state of $CsCl$ with the ion state. Models for that system have been proposed by Dronin and Gorokov [2], and Nikolaev [3]. They have simply evaluated the overlap of a harmonic oscillator ground state model of $CsCl$ with a hypothetical ion state located by requiring that the experimental R be given by

$$R = \frac{M^+}{MC l^+} = 1 \div \left(\sum_{v=0}^{\infty} \left[\int_{r_c}^{\infty} \left(Y_v^* Y_v dr \right) \cdot f_v(T) \right] - 1 \right) \quad (1)$$

where Y_v is the vibrational eigenfunction for vibrational state v , $f_v(T)$ is the population of the v th state at temperature T , and r_c is the vertical from the intersection of the repulsive wall of the ion state with the dissociation energy of that state. The requirement of eqn (1) is equivalent to arguing that transitions from the ground state to the right of r_c lead to ion formation, and to the left of r_c produces dissociation to fragments (see figure 1). Although Nikolaev [3] presented calculations of r_c based on a model of the ion state, he found it necessary to compute r_c for $CsCl$ empirically from the experimental fragmentation ratio, obtaining $r_c = 0.342$ nm, compared to his calculated value of 0.323 nm.

Recent development of the Transpiration Mass Spectrometer (TMS) system [4] has provided new insight into this process. The isentropic expansion used to form a representative non-interacting beam from a high temperature region provides extreme rotational and vibrational cooling of the molecules. This has displayed itself by producing anomalously high ratios of M^+ / MX^+ in alkali halide studies. The above model provides a mechanism for interpreting these experimental data since the proper temperature describing molecules in the beam is given by the isentropic temperature of the expansion rather than the source temperature. For suitable orifice dimensions, the temperature can be altered over a wide range by varying the system pressure. At high temperatures, the vibrational levels are occupied substantially up to $v = 10$ or more. In the $NaCl$ case, considered in detail here, less than half of the molecules at 800 K are in $v = 0$ (harmonic oscillator assumption), and 1% or more occupancy is found up to level $v = 8$. This makes empirical interpretation based on the observed high temperature ratio so insensitive to small changes in the value of r_c that almost no information can be obtained. The TMS data plotted in figure 2 for $NaCl$ show a low temperature limit near $R = 27$. The harmonic oscillator fit used $\omega_e = 363.62$ cm^{-1} and $r_e = 0.23606$ nm. Equation 1 was evaluated by numerical integration of Y_v and direct summation over v from 0 to 12. The evaluation was iterated on r_c with $T = 10$ K until $R = 27 \pm 0.5$ was obtained. The value of r_c determined was 0.24648 ± 0.0001 nm. The extreme sensitivity of this process is evidenced by the high precision of r_c obtained. The temperature dependence curve based on this fit is plotted in figure 2 as the HARMONIC OSCILLATOR MODEL. It is clear that the qualitative shape of the data (including effusive measurements taken from the literature [4]) is modeled by the harmonic oscillator, but even with a possible error of a factor of two in beam temperature (which has been applied to the data), the model obviously does not correctly fit either the TMS or KMS effusive data. The obvious explanation is that the ground state well is anharmonic, and the harmonic oscillator eigenvalues of the levels underestimate upper level participation. An anharmonic model has now been developed as follows. The potential well has been computed by the Rydberg-Klein-Rees method from the spectroscopic data selected by JANAF [5], $\omega_e = 363.62$, $\omega_e x_e = 1.72$, $B_e = 0.21691$, all in cm^{-1} . These values imply $r_e = 0.23671$ nm. $f_v(T)$ was summed directly from the anharmonic eigenvalues $E_v = \omega_e(v+1/2) - \omega_e x_e(v-1/2)^2$ normalized by summing to $v = 15$, where the

population even at 1200 K was insignificant. The anharmonic eigenfunctions were approximated by mapping harmonic oscillator eigenfunctions onto the actual potential well. The result is plotted in figure 2 as the ANHARMONIC OSC. curve ($r_c = 0.2445 \pm 0.0001$ nm). The agreement is now excellent for the TMS data, however it still fails to predict the effusive KMS data. Possible explanations are: 1) Harmonic oscillator eigenfunctions are not a good model at high temperature - the "true" eigenfunctions do exhibit higher amplitude toward the right wall of an anharmonic potential than toward the left; 2) Centrifugal broadening of the potential and high rotational level occupation may be important in the calculation - at $T > 800$, $J(\max)$ is of the order of 60. The effect on $f_v(T)$ and Y_v may be significant, the effect on the potential is less likely a problem; 3) Avoided crossing distortions of the potential well may be important at high temperatures although Oppenheimer and Berry [6] calculate that point for NaCl to be at $r = 0.94$ nm, where no significant thermal occupation would exist; 4) The actual form of the potential may not be accurate enough above $v = 2-3$; 5) The separation of the two states of the ion expected to exist [7] may cause the simple overlap model to fail at higher T . An ab initio calculation for this problem is now possible [7], and probably warranted. However, many important systems are not yet calculable, and fragmentation at a single bond is a very important case. A simple diatomic model such as has been described would be very valuable in modeling such effects. The sensitivity shown by this model also points to the possibility of directly using this effect to test existing ab initio calculations at the higher energy levels.

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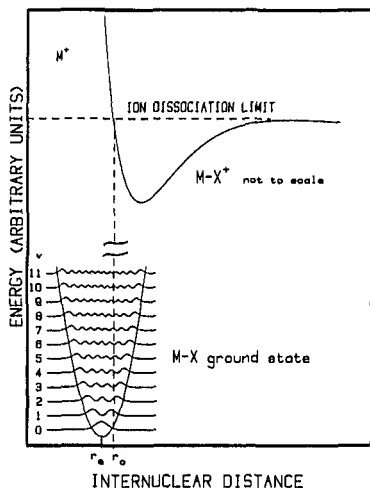


Fig. 1. Model for M^+/MX^+ fragmentation, showing details of the square of the oscillator eigenfunctions.

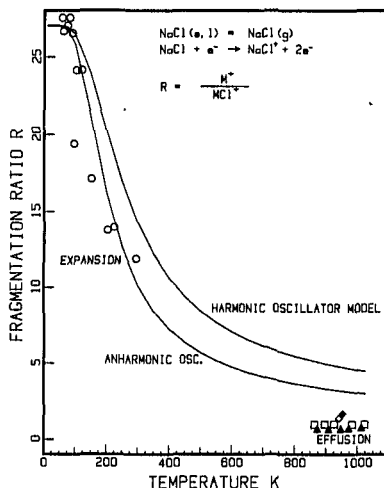


Fig. 2. Experimental and literature KMS/TMS fragmentation ratios, and 2 model fits. TMS points are plotted at $2 \times T$ calculated from expansion parameters.

THE CURRENT STATUS WITH MULTIPLE COLLECTORS IN THERMAL IONIZATION MASS SPECTROMETRY

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The recent introduction of the concept of multiple collectors for the simultaneous detection of thermally produced ion beams has not only improved the quality of the results, but has also led to a new class of problems in collector technology.

The internal precision of ratio determinations and the measuring time to achieve a certain level of precision can be significantly improved as compared to a single collector peak jumping experiment. A measuring experience of approximately one year shows that a relative internal precision (σ_R/R) of 10 - 30 ppm can be achieved routinely within 10 - 20 minutes total data acquisition time for isotopic systems like Sr, Nd, Pb or U. Two modes of data acquisition have been used:

- a) The simultaneous detection of all relevant ion beams (5 collectors in the case of Pb and U, and 3 collectors for Nd and Sr) together with a separate calibration of the amplifier gain factors, using a precise current source.
- b) The combination of simultaneous double or triple collector acquisition with peak jumping, to cancel the influence of the gain factor of the amplifiers in the case of Nd. This is a direct transfer of the well-known Sr double collector technique from Sr to Nd.

Experience shows that the simultaneous detection method results in a 3 to 6 times shorter measuring time to obtain a certain level of precision as compared to the multiple collector jumping method, as long as the gain ratio of the amplifiers can be held at a relative stability of approx. 5 ppm during the run.

However, concerning external (i.e. sample to sample) precision, both methods show approximately the same error limits. The explanation for this observation is found in small sample to sample variations of the ion optical transmission, which slightly depends on magnetic field (mass) and on the path an ion beam takes through the system.

Such variations, finally, are also the limiting parameter for the accuracy which can be expected from a multiple collector isotope ratio measurement. An assessment of these limits can be obtained by measuring the ion optical transmission by jumping with one ion beam to all collectors. This requires, in addition, a precise calibration of the amplifiers and, furthermore, the proof that the efficiency of each collector approaches 100 %. Hence, the overall quality of a multiple collector measurement cannot be better than the precision of an isotope ratio obtained by a single peak jump procedure (including amplifier calibration).

FOD 2 | A LASER MASS SPECTROMETRY LABORATORY FOR STABLE ISOTOPE MEASUREMENTS AND VAPORIZATION DIAGNOSTICS; J. C. Travis, J. D. Fassett, L. J. Moore; National Bureau of Standards, Center for Analytical Chemistry, Inorganic Analytical Research Division, Washington, DC 20234.

A modular laser mass spectrometry laboratory has been established at NBS to develop highly sensitive and selective isotope measurement technology and to investigate the vaporization, isotopic discrimination and ionization behavior of inorganic species in thermal ion sources. Laser and mass spectrometry systems were selected to provide maximum flexibility to access all but the most difficult portion of atomic ionization potentials in the periodic table. A 700 m Joule pulsed Nd-YAG laser, which pumps a tunable dye laser coupled to a frequency doubler, generates highly coherent wavelengths down to the near ultraviolet (260 nm). The output of the pulsed laser system can be directed to the ionization region of a time-of-flight (TOF) or a magnetic sector mass spectrometer. A second laser system consists of a continuous wave (CW) argon ion laser used to pump a scanning single mode tunable dye laser. The output of the CW system also can be routed to the ionization region of either mass spectrometer or to the detector region of the magnetic sector instrument for laser-induced ion fluorescence studies. Using this system, resonance ionization feasibility has been demonstrated for eighteen chemically disparate elements from ground state and metastable vapor phase atom reservoirs, including elementally selective ionization from binary mixtures and isotopically selective ionization of uranium. Other potential applications include highly sensitive and selective abundance sensitivity measurements, multielement trace determinations, and as a general purpose, diagnostic tool for elementally and isotopically selective probing of high temperature vapors.

FOD 3 | RIMS--RESONANCE IONIZATION MASS SPECTROMETRY
OF 18 ELEMENTS FROM THERMAL VAPOR ATOM RESERVOIRS;
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Resonance ionization mass spectrometry (RIMS) has been employed to ionize 18 elements from thermal vapor atom reservoirs. Laser wavelength scans over the range 292-302 nm while focusing on the major isotope of the element have demonstrated ion formation due to the two-photon resonant absorption of energy by both ground-state and metastable excited-state neutral atoms. Atomic ion mass spectra were recorded at major spectral lines for most of the elements. Included in the list of elements investigated are several that are difficult to ionize using conventional thermal ionization mass spectrometry, such as Fe, Ni, Hf, Ti, and Zr. Sample sizes for most measurements were 0.5-1.0 μg . Reduction of the element on a rhenium substrate in a hydrogen atmosphere from a solution of the appropriate salt of the metal has been used for most of the measurements. In situ graphite reduction was used for a number of elements. The elements investigated are: Co, Dy, Er, Fe, Hf, In, Lu, Mo, Ni, Re, Sr, Ta, Th, Ti, U, V, Y, and Zr.

The relatively low temperature of the thermal vapor sources, together with the collision-free high vacuum environment and mass selectivity of RIMS results in the observation of spectral features that do not appear in atomic transition tables for several of the elements. Experimental evidence indicates that significant populations of atoms exist in low-lying energy states at thermal vapor temperatures and can exhibit large photon absorption cross-sections.

RESONANCE IONIZATION MASS SPECTROMETRY*

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A powerful new method for isotope ratio mass spectrometry is described, in which a high-power tunable laser is used to selectively ionize the element of interest, followed by mass analysis. It is based on the technique of resonance ionization spectroscopy (RIS) which has demonstrated the ability to detect single atoms in a complex matrix.¹ In RIS, the sample is vaporized and the resulting cloud of atoms is illuminated by a focused beam of monochromatic light, usually from a dye laser. If the energy of the photons matches an allowed electronic transition in the element of interest, resonant absorption will occur. Ionization of the atoms can be achieved by using the proper combination of photon energies for that element. At sufficiently high photon flux, normally obtained with pulsed dye lasers, this ionization will be saturated, i.e., all atoms of that element within the laser volume will be ionized.

Our studies thus far have been directed toward understanding the processes and experimental parameters which affect the analytical application of resonance ionization mass spectrometry (RIMS) to a number of important elements. The initial demonstration of elemental selectivity was carried out using the elements neodymium and samarium.² By properly choosing the energy of laser excitation, either element could be measured free from isobaric (same mass) interference in a mixture of the two. This capability is a marked improvement over the thermal ionization method, which has virtually no elemental selectivity.

From studies of Nd and Sm, we obtained optical ionization spectra showing the wavelengths of laser excitation which produced an ion signal for each element.³ This led to a better understanding of the thermal vaporization process, and opened up new analytical wavelengths to use for measuring these two, as well as other, rare earth elements. In fact, we have measured the element europium using a single color, two-photon process in contradiction to the previously held theories of RIS. It appears that this ionization of Eu proceeds through dissociation of a dimer species into highly excited Eu atoms, from which it is easier to reach ionization.

Our most recent thrust has been the measurement of plutonium isotope ratios, free from isobaric overlap by uranium (mass 238) and americium (mass 241).⁴ Initial experiments successfully demonstrated that plutonium is accessible to a single color, two-photon RIMS process. Further work will be presented in which a flashlamp-pumped dye laser has been coupled to a two-stage magnetic sector mass spectrometer system. This combination offers the best situation for obtaining high quality analytical measurements of Pu in the presence of U and Am.

*Research sponsored by the U. S. Department of Energy, Division of Basic Energy Sciences, under Contract W-7405-eng-26 with the Union Carbide Corporation.

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EOD 5 | RAPID DETERMINATION OF ISOTOPE RATIOS OF
ELEMENTS IN SOLUTION WITH AN INDUCTIVELY COUPLED PLASMA/
QUADRUPOLE MASS SPECTROMETER (ICP/MS) SYSTEM.

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We have previously described the use of a plasma mass spectrometer system for isotope ratio determinations (1). The initial work was performed with a microwave induced plasma. An inductively coupled plasma is now in use in this lab. Solutions are sprayed into the plasma and the resulting elemental ions are extracted into a quadrupole mass spectrometer for analysis. All sample (solution) handling is at atmospheric pressure. Almost entirely singly charged ions are formed and oxide ions are present only at low levels. Spectral overlap is rare so that in many cases extensive sample work-up or clean-up is not required. Isotope ratios can be determined with a precision of 0.1-1% with a measurement time of ≈ 5 min per sample if the elemental concentration is $\approx 10 \mu\text{g ml}^{-1}$. With a conventional pneumatic nebulizer, total solution volumes of ≈ 10 ml are required. Almost the entire periodic table is accessible for analysis. An improved signal averaging procedure and a lower noise, more stable nebulizer have been incorporated. The improvement in accuracy and precision will be reported and the capability of the SCIE[®]X ELAN[®] 250 ICP/MS for rapid isotope ratio determinations will be described.

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IMPROVED PRECISION AND SPEED OF THERMAL IONISATION MASS SPECTROMETRY WITH A MULTICollectOR

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We have designed a multiple Faraday collector configuration which permits simultaneous collection of up to 8 ion beams. Of the 7 spacings between the collectors 4 are adjustable from outside the vacuum. This facility means that the analyst retains maximum flexibility and does not have to restrict his analysis to a limited number of mass numbers and separations. We have so far measured isotopes of lead, strontium, uranium, neodymium, and hafnium using this multicollector.

Simultaneous collection of the ion beams makes far better use of the signal available. It allows continuous integration of all the ion beams during the measuring period and no settling time is needed for the amplifiers and magnet. Because all the beams are collected and integrated simultaneously the system is very insensitive to fluctuation in ion beam intensity. Thus difficult samples producing unstable, rapidly growing or rapidly decaying beams give analysis precisions similar to standards. With peak jumping systems, even with the best interpolation methods a rapidly changing beam intensity causes significant losses in precision.

To get good data from a multiple collector using simultaneous collection the relative gains of the amplifiers must be very accurately calibrated using a high stability current source. Further the high coefficient of resistance with temperature of the high value measuring resistors - several hundreds of ppm per degree C - require the resistors and amplifiers to be inside an enclosure which is evacuated and maintained to better than .01°C.

The multiple collector has been used to measure strontium and neodymium isotope ratios in standard materials, using a combination of static and peak jumping techniques. Typical data are summarised in the tables below.

STRONTIUM ANALYSIS TRIPLE COLLECTOR

Bead No	Ratio 87/86	Standard Error per mil	Time in Minutes
01	0.710182	0.005	182
02	0.710199	0.005	177
03	0.710210	0.005	176
04	0.710208	0.005	187
05	0.710206	0.004	187
06	0.710209	0.005	185
07	0.710197	0.005	185
08	0.710199	0.006	176
09	0.710214	0.005	177
10	0.710213	0.005	188

Mean_i = 0.7102037

Standard Deviation = 0.001% 1 Sigma

NEODYMIUM ANALYSIS MULTI COLLECTOR

Bead No	Ratio 143/144	Standard Error per mil	Time in Minutes
07	0.511124	0.006	122
08	0.511122	0.004	137
09	0.511127	0.005	147
10	0.511127	0.004	132
11	0.511131	0.004	131

Mean = 0.511126

Standard Deviation = 0.000003 1 Sigma

In addition we have recently analysed minor isotopes of uranium using a method which measures the U-234 or U-236 minor ion beam using a Daly photomultiplier whilst the U-238 major ion beam is being measured on a Faraday collector simultaneously. This technique will be extremely useful in the measurement of a series of isotopes where one or more is significantly lower than the remainder.

Summary of minor isotope ratios on UO10 using simultaneous Daly/Faraday measurement

Bead No.	234/238 ppm	236/238 ppm
1	55	72
2	55.3	71.5
3	55.3	71.2
4	55.3	71.1
5	55.2	70.6

DETERMINATION OF IODINE-129 IN THE ENVIRONMENT
USING A TWO-STAGE MASS SPECTROMETER

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In the nuclear industry, the monitoring of low levels of ^{129}I is of current interest. Because of the long half life (1.57×10^7 years), the Idaho National Engineering Laboratory has long been concerned with the measurement of small amounts of ^{129}I released to the environment from reactor operations and fuel reprocessing activities.

Released iodine combines with natural ^{127}I , already present in the environment, to produce a background with I ratios ($^{127}/^{129}$) in the range of 10^4 to 10^8 . At the present time, neutron activation analysis is the most commonly used method for the determination of these wide-ratios.

This presentation outlines the demonstration of a negative thermal ionization method that was applied to air samples with iodine 127 to 129 ratios in the range of interest. A two-stage Nuclide mass spectrometer, equipped with dual detectors, was used to perform the analyses. The results show precision of about 1% between the detector systems, and, when applied to samples as small as 1 nanogram total iodine, showed precisions in the lower part of the range as low as 4%. The end result is a cost effective method for the analysis of iodine in the environment at ratios up to 10^8 .

The Status of the Production and Certification
of an Equal Atom Plutonium Isotopic Standard

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The New Brunswick Laboratory (NBL), in cooperation with the National Bureau of Standards (NBS), is producing and certifying an equal atom $^{239}\text{Pu}/^{242}\text{Pu}$ isotopic reference material, NBS Standard Reference Material (SRM) 957.

The SRM 957 starting materials, consisting of isotopically pure separated isotopes of ^{239}Pu and ^{242}Pu have been procured from Oak Ridge National Laboratory (ORNL), dissolved in NBS double-distilled acids and stored in Teflon bottles. Elemental impurity analysis of these solutions has been performed at ORNL using spark source mass spectrometry. Various ion exchange techniques are now being evaluated to effect removal of certain undesirable impurities in the master solutions and insure the production of a chemically pure isotopic reference material. Following the ion exchange purification, portions of the ^{239}Pu and ^{242}Pu solution will be assayed for total Pu content, then blended by mass to form the equal atom mixture. Interim certification will be based on the mass measurement blending data and isotopic measurements performed relative to uranium. The final certification of absolute isotopic ratios will then be made, using the NBS calibration mixture technique employed for atomic weight determinations.

The calibration mixtures representing various $^{239}\text{Pu}/^{242}\text{Pu}$ isotopic ratios will be prepared from the chemically and isotopically pure separated isotopes. Mass spectrometric bias, determined from the isotopic analysis of these mixtures, can then be applied to the isotopic measurements of the equal atom mixture to yield an absolute isotopic ratio from which the final certification is made.

High precision wet chemical assay and mass spectrometric techniques have been developed at NBL for use in the characterization of these materials. Precision of the wet chemical assay by controlled potential coulometry was demonstrated by analyses of aliquots of SRM 949f plutonium metal. Assay results showed a relative uncertainty of less than 0.01% at the 95% confidence interval for 49 measurements. Mass spectrometric analyses on 100- nanogram filament loadings of an experimental equal atom blend of ^{239}Pu and ^{242}Pu demonstrated a sample-to-sample reproducibility of better than 0.02% around the mean of the major isotope ratio for 11 measurements.

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EVALUATION OF INTERNAL STANDARDS IN URANIUM AND PLUTONIUM ISOTOPIC RATIO MEASUREMENTS

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Since its first proposal by Dietz, et al.,¹ the use of internal standards (or double isotopic) spikes has been widespread. In this technique, a spike is synthesized by mixing two highly enriched isotopes of the element in question. The isotopic composition of the resulting mixture is measured as accurately as possible; the mixture is then used to spike the samples. By comparing the measured ratio of the two high isotopes in the spike with the known value, a bias correction applicable to the specific analysis in question can be calculated. Isotopes of low abundance in the sample are desirable for the spike; ²³³U and ²³⁶U are the obvious choices for that element. The technique does not appear to have been put into routine application for uranium analyses as applied to the nuclear fuel cycle. In safeguards and other accountability applications, the technique will yield extremely high rewards.

We have synthesized and characterized one plutonium and two uranium internal standards; isotopic information is presented in Table 1. Because of the small amount of high purity ²³⁶U available, a "low" purity spike was synthesized for isotope dilution applications, which require about one mg per sample. A "high" purity spike is used to obtain refined ²³⁵U/²³⁸U ratios of high precision and accuracy.

To evaluate the potential of the technique in our laboratory, where pulse counting is used exclusively, a series of NBS isotopic uranium standards was analyzed with the high purity spike. The results are given in Table 1a. The "low" purity spike was used to analyze a SALE sample; results are shown in Table 1b. Information concerning plutonium is in Table 2.

The results of Tables 1-2 show an improvement of about a factor of 5 over those routinely obtained by conventional pulse-counting analyses. The power of the technique is exemplified by the results given in Table 3, where the results of a single loading were analyzed to exhaustion; each ratio represents the average of ten individual determinations, each representing 15-20 minutes of data collection; only a few of the data points are listed. Even though fractionation was pronounced during this extreme test, the average of all 11 analyses give a precision of better than 0.1%, while the average determined by application of an "average" bias correction yielded a precision higher than 1%. That the concept of "average" bias correction is fallacious is shown by the fact that the standard deviation of the bias correction for a typical analysis is +30% or so.

We now routinely obtain external precisions (standard deviations) of 0.1% or better on isotope dilution analyses of both uranium and plutonium. We suggest a concerted effort to initiate enrichment of enough ²³⁶U of high purity (>99%) for its widespread use in safeguards applications.

Table 1a. Isotopic Composition of Internal Standards

Isotope	233	234	235	236	238	233/236
High Purity Spike						
233 Component	99.925	0.0317	0.0032	0.0014	0.038	
236 Component	0.0108	0.0008	0.201	99.674	0.113	
Mixture	46.935	0.0155	0.1084	52.864	0.0771	0.88785
Low Purity Spike						
233 Component	99.528	0.185	0.062	0.015	0.209	
236 Component	0.000	0.119	9.25	89.27	1.32	
Mixture	47.899	0.153	4.903	46.250	0.795	1.0357

Table 1b. Typical Results

Sample	Analyses	235U/238U
NBS U010	6	0.012192 ± 0.08%
SALE H-66	6	0.025702 ± 0.1%

Table 2. Pu Internal Standard

	238	239	240	241	242	244
High 242	0.0004	0.0048	0.0518	0.0246	99.853	0.0648
High 244	0.0015	0.0409	0.0863	0.0008	0.911	98.960
Mixture	0.0007	0.0226	0.0690	0.0097	50.0349	49.8631

Typical Results, NBS 947

	238/239	240/239	241/239
Certified*	0.00358	0.24139	0.03469
Measured	0.00359	0.24133	0.03468
%RSD	0.6	0.08	0.12

*Corrected to February 15, 1983.

Table 3. Results from a Single Resin Bead Run to Exhaustion, SALE H-66

Run	Uncorrected 238/233	Corrected 238/233	Uncorrected Wt. %	Corrected Wt. %
2	0.6403	0.6341	88.66	87.80
4	0.6417	0.6338	88.86	87.77
7	0.6386	0.6340	88.43	87.79
9	0.6388	0.6347	88.46	87.89
11	0.6350	0.6348	87.93	87.90
Avg. (N=11)	0.6370	0.6343	88.21	87.84
S.D.	0.0067	0.0043	0.92	0.06
%RSD	1.05	0.07	1.05	0.07

Acknowledgment

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FOD 10 | COMPUTER CONTROL OF PADUCAH ISOTOPE

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Six 30-year-old mass spectrometers have been uprated and automated by the Paducah Isotope Measurements Laboratory. Two H-P 9825 desk-top computers are employed, each operating under programmable interrupt to control a bank of three mass spectrometers. A send/receive teleprinter at each spectrometer permits local control and also provides hard copies of analytical results. Circuits utilizing solid state design have replaced all obsolete tube circuits. Automatic ion beam centering and sample gas flow control is featured. Amplified ion current signals from the dual collector are input into a ratimeter. Through various interfaces, the computers automatically sequence digitally controlled manifolds, time programmed delays, read ratimeters, record analyzer pressures, calculate isotopic results, and check some raw data parameters for each spectrometer.

Performance and economy have improved since systems were assembled and programmed by Paducah Laboratory personnel in the 1980-82 period. System description and performance will be discussed.

SOFT IONISATION LC-MS

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The effective combination of LC with MS using a continuous moving-belt interface has traditionally been limited by available ionising methods. The use of this type of interface with Fast Atom Bombardment, arguably the most versatile of ionising techniques, will considerably extend the applicability of this approach to LC-MS.

Investigation by EI or CI of involatile or thermally labile chromatographic components, requiring their thermally induced volatilisation, can result in their decomposition. In extreme cases, temperatures at the practical limits of heater supplies and belt materials can fail to evaporate compounds of interest. Fast Atom Bombardment ionisation affords analysable spectra from the greatest range of compounds without the need for sample heating.

In general the reliability of the moving belt interface is dependent on the heat sources to the belt, in particular on the heat requirement for evaporation. Modifications to the belt interface which allow its use with F.A.B., in which all heat sources to the belt can be eliminated are discussed. They include the replacement of the traditionally employed clean-up heater and infrared solvent evaporator by a solvent wash-system and a spray deposition-device. The removal of said heat sources to the belt can be expected to increase the reliability of the interface, whilst reducing its mechanical and electrical complexity.

The incorporation of these and other modifications to a commercially available moving-belt interface, allow its use as a FAB-LCMS interface.

Preliminary investigations employing the modified system as exemplified by the reconstructed mass chromatograms from two components in a simple on-line separation, and the spectrum of one of them, demonstrate the applicability of the interface in this mode of operation.

The constraints imposed on the investigator by this interface configuration will be discussed and improvements, currently under investigation which can be expected to diminish those constraints, described.

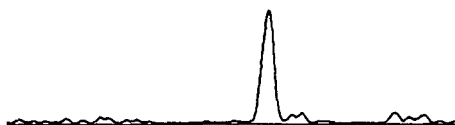
A direct liquid introduction system for LC-MS studies will be briefly discussed.



FAB LC-MS



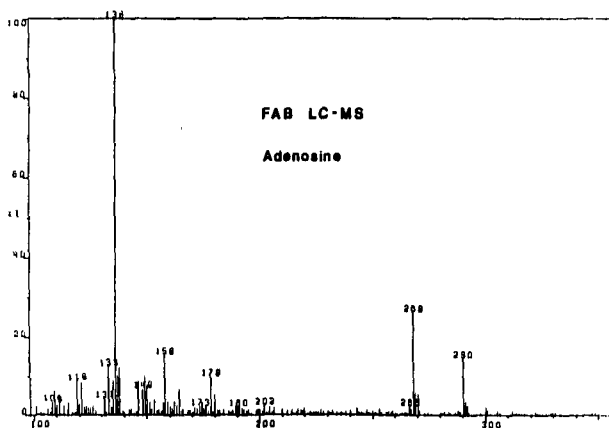
URIDINE (M+Na)⁺ m/z 267



ADENOSINE (M+1)⁺ m/z=268



Column Waters C₁₈ Radpak
Solvent H₂O:CH₃OH:C₃H₈O₃
(59.6:39.7:10.7)
Flow 0.25 ml min⁻¹
Sample load 10 µg



DESIGN AND PERFORMANCE OF A NEW TOTAL LIQUID INTRODUCTION LC/MS INTERFACE

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Introduction

Liquid chromatography is rapidly becoming the method of choice for the separation of compounds which cannot be analyzed by gas chromatography because of their low volatility. As the technology of liquid chromatography improves, there is a growing desire to couple liquid chromatography and mass spectrometry to provide a system suitable for the analysis of involatile compounds in a wide variety of biochemical, industrial and environmental applications. The requirements of an LC/MS system are: a) reliability; b) reproducibility; c) compatibility with standard LC column technology; d) acceptance of aqueous mobile phases and buffers commonly used by chromatographers; e) sensitivity; f) specificity. The technique of LC/MS/MS with the new SCIEX® LC/MS interface coupled to a TAGA® 6000 Triple Quadrupole MS/MS system offers these features by providing the capability for obtaining full scan LC/MS data under gentle Atmospheric Pressure Chemical Ionization (APCI) conditions, together with the selectivity of LC/MS/MS to provide structural information. The eluent from a wide bore LC column (at flowrates up to 1 ml/min) is introduced without splitting into the APCI source, where the liquid is sprayed into a heated vaporizer. The rapid desolvation and vaporization of the droplets and entrained analyte minimizes thermal decomposition and preserves molecular identity for ionization by proton transfer in the APCI source. The introduction of the entire sample from the column results in full scan detection limits in the low nanogram range. Buffers are readily tolerated by the atmospheric pressure source without significant contamination problems, and the flash vaporization of the sprayed eluent allows up to 100% water to be used without problem.

LC/MS/MS Interface

The LC/MS interface schematically shown in Figure 1, consists of a sprayer coupled to a heated desolvation/vaporization chamber and transfer line, in a configuration which interfaces to the SCIEX® Atmospheric Pressure Chemical Ionization source. The eluent from the LC flows through narrow bore stainless steel tubing directly into the nebulizer, where it is converted into a fine mist by a high velocity jet of air or nitrogen. The droplets are swept by the nebulizing gas through a quartz tube heater which vaporizes the droplets and analyte in the gas stream. The heated gas and sample mixture flows from the quartz tube into the reaction region of the APCI source, where the sample is chemically ionized by charge transfer and proton transfer from the reagent ions.

The vaporizer is temperature controlled (either manually or from the keyboard), resulting in reproducible and stable vaporization conditions independent of flowrate and mobile phase composition. Up to 100% water can, therefore, be used without encountering the problems often associated with aqueous phases.

The minimum bore of all connecting tubing and passages in the nebulizer is 0.009 inches, which eliminates potential problems of clogging by particles from the column. The continual use of nonvolatile buffers requires thus only an occasional (every one or two days) five minute source cleaning step to maintain performance.

The process of converting relatively involatile molecules, solvated in a liquid, into a molecular gas without inducing excessive decomposition which is used in this interface is one of dispersing the flowing liquid stream in the form of small droplets suspended in a hot carrier gas. At a sufficiently high temperature the droplets are vaporized at a rate which allows intact organic molecules to be desorbed from the droplets or particles with minimal thermal decomposition.

Results

Figure 2 shows the signal from a repetitive injection of 10 ng caffeine. The maximum variation in the peak intensity is $\pm 5\%$. Due to a low dead volume past the UV detector the chromatographic integrity is practically undisturbed as can be seen from results shown in Figure 3. Only a small tailing is observed in the lower 5% portion of the signal.

With a fast vaporization and a gentle ionization process thermally labile compounds such as dexamethasone, cholesterol or aflatoxin show very little thermal breakdown. With the gentle vaporization and ionization processes, the linear dynamic range extends over three orders of magnitude. Figure 4 shows a calibration curve for the $MH^+ = 421$ from 750 pg to 750 ng of a sulfone analogue of the drug sulfinpyrazone.

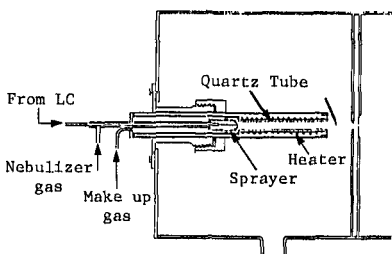


Figure 1. Schematic diagram of the LC/MS interface.

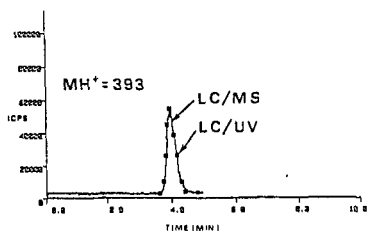


Figure 3. Comparison of the LC/MS response with the LC/UV response.

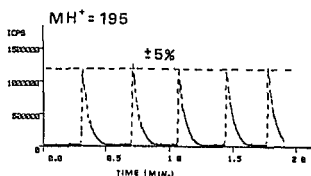


Figure 2. Reproducibility of 10 ng caffeine (without column).

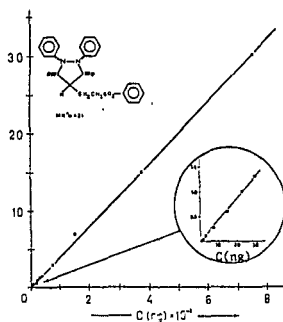


Figure 4. Calibration curve from 750 pg to 750 ng.

A direct liquid introduction LCMS interface for use on sector mass spectrometers.
J.R. Chapman, S. Evans and K.R. Compson. Kratos Analytical Instruments, Manchester, UK.

In the design of an LCMS interface for existing Kratos mass spectrometers, three criteria were considered to be of particular importance (i) the interface should be able to directly sample relatively high flow rates (including aqueous solvent systems used in reverse phase chromatography) to achieve compatibility with smaller bore LC columns without stream splitting, (ii) vaporization of labile and involatile samples should be possible without decomposition or loss of chromatographic resolution, (iii) electrical breakdown from the high potential ion source should be prevented.

A survey of the literature data and user experience led us to the conclusion that a method based on the direct liquid introduction (DLI) principle was to be preferred to a mechanical transport system. In order for such a system to be compatible with existing mass spectrometer instrumentation and particularly with existing pumping systems without stream splitting it was also decided to make use of the jet separator principle where part of the solvent flow is preferentially removed as vapour.

In the present configuration, liquid flow from the LC column passes through a narrow bore (0.1 mm) capillary chosen so as to minimise dispersion effects and any consequent loss of chromatographic resolution. The tip of this capillary is electrically heated in a heater assembly whose construction allows the ready replacement of the capillary if necessary. The design of the heated area also ensures a rapid vaporization of the majority of the flow resulting in the formation of a well collimated, fast moving beam of solvated sample molecules (droplets) and solvent vapour.

A large percentage of the vapour flow is removed by the auxiliary rotary pump in the separator stage and the sample beam then passes into the source housing region via the counter-orifice. A narrow gap interposed between the counter-orifice assembly and the ion source provides a region of lower pressure in which electrical breakdown does not occur so that the ion source is effectively isolated from the inlet system. The beam formed is, however, able to transfer the solvated sample molecules across this gap to enter the ion source.

Modifications to the ion source inlet port have been made to provide a path length in which further desolvation of the droplet stream may occur. The solvent vapour is then used as a chemical ionization reagent gas to promote ionization of the sample. These modifications are also consistent with the maintenance of a source pressure that is suitable for chemical ionization operation (normally 0.1 - 0.2 torr). The introduction of additional reagent gas via a separate inlet system can be used either to supplement the source pressure or to change the nature of the reagent gas mixture.

Preliminary tests of the interface showed that it was capable of handling samples with a satisfactory transfer efficiency and could operate at ion source potentials up to 4000 volts without electrical breakdown. Tests with acetonitrile/water and methanol/water mixtures containing up to 50% water showed that satisfactory operating pressures were possible with total flow rates in excess of 100 $\mu\text{l minute}^{-1}$. The complete interface can be retrofitted on any Kratos mass spectrometer equipped for CI operation without any modification of the existing pumping system.

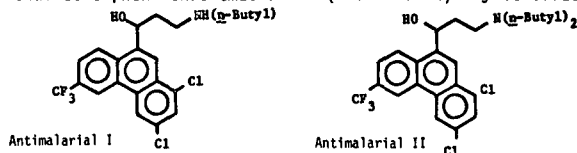
The interface has been fitted to a standard Kratos MS25 mass spectrometer for further evaluation. A standard reverse phase column and a test mixture containing benzhydrol and benzophenone was used to assess the effect of the interface on chromatographic efficiency. A comparison of plate numbers calculated from selected ion monitoring traces with plate numbers obtained using a conventional UV detector under these conditions indicated a loss of resolution that varied from zero to a maximum value of 10%. Most separations have used directly interfaced 2 mm columns but the use of lower flow 1 mm columns is also possible.

Samples of adenosine and underivatized leucyl alanine were used to assess the performance of the interface with less volatile samples. The spectra of both compounds showed strong quasi-molecular ions and there was no evidence for sample memory following their elution into the ion source. Other compounds such as chloramphenicol, ampicillin, tomatidine and rubijervine can be introduced via the interface and give quasi-molecular ions. Improvements in the performance of the interface are expected to result from a better control of the desolvation process and from a better collimation of the beam formed at the capillary tip. The design and implementation of appropriate modifications is in progress.

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS OF CANDIDATE ANTIMALARIALS

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The emergence of drug resistant malarial strains and the restricted use of pesticides raise the possibility of the resurgence of malaria. Efforts to develop new antimalarial drugs have shown that some phenanthrenemethanols (shown below) may be effective agents.



Little is known about the metabolism or kinetics of the candidate drugs due to the absence of specific and sensitive analytical methods. Recently ion-paired reverse phase liquid chromatography (LC) became the method of choice for analytical information on these drugs in biological fluids. At best, however, detection limits (absolute on-column injection) for LC are about 2 ng with UV detection and about 10 ng with fluorescence detection. Liquid chromatography/mass spectrometry (LC/MS) may offer significant improvement in analytical methodology as a result of increased specificity and decreased detection limit for these antimalarials compared to conventional LC detection modes.

The LC/MS methods evaluated include the thermospray and direct liquid introduction (DLI) interfaces. In the thermospray technique, ions are formed through reactions of cluster ions in solution and the analyte (1). The DLI technique uses the solvent as the chemical ionization reagent gas to ionize the analyte (2). We have compared results obtained from the two different LC/MS interfaces in the analysis of candidate antimalarials.

The separation of the antimalarials was performed on a C₁₈ (150 x 4.6 mm) column using a mobile phase of 80% acetonitrile / 20% water buffered with ammonium acetate (0.1 M) and acetic acid to a pH of 4. A flow rate of 2 mL/minute produced retention times of 3.3 and 4.9 minutes for antimalarials I and II, respectively. A UV detector (254 nm) was incorporated between the interface and the column. The DLI probe was operated with a 1/100 split of the total LC flow and sensitivity was optimized by positioning the probe and adjusting the split to give the maximum solvent cluster ion intensity. The Finnigan 4500 quadrupole mass spectrometer was operated in CI mode (100 eV electron energy, 0.3 mA emission current, and a source temperature of 180°C) when using the DLI probe. On the other hand, the LC conditions for the thermospray interface were identical as those mentioned above except methanol was used instead of acetonitrile and the flow rate was 1.5 mL/minute. The interface was operated without a split of the total LC flow with a vaporizer temperature of 200°C, a jet temperature of 220°C, and a source temperature of 160°C. No external ionization source was used in the thermospray interface which was also mounted on a Finnigan 4500.

The spectra for the two antimalarial candidates obtained by the thermospray technique using positive ion detection consist of only the protonated molecular ion and the characteristic isotopic pattern for a two chlorine-containing compound (Figure 1). When the thermospray interface was used, selected ion monitoring (observing m/z 444, 446, 500, and 502 each for 0.4 seconds) detection limits of 800 pg injected on column with less than a 15% deviation for duplicate injections were obtained (Figure 2).

The DLI spectra, however, are not as simple as those obtained with the thermospray interface (Figure 3). Using negative ion detection, both antimalarials showed signals at m/z 308, 342, and 368 for the loss of -Cl and the alkyl chain at the alpha carbon, the loss of the alkyl chain, and the loss of -OH and one or two butyl groups, respectively. Antimalarial I exhibited a loss of HCl and H₂O (m/z 407 and 425, respectively) and has a molecular anion which is the base peak at m/z 443, with the proper isotopic pattern. Antimalarial II showed a loss of HCl (m/z 463) and a molecular anion at m/z 499 along with the expected isotopic cluster. Both antimalarials also underwent losses of hydrogen to form [M-1]⁻ and [M-2]⁻ ions. It appears that resonance electron capture is the main method of negative ion formation for these compounds. The NCI spectra are qualitatively similar to the methane NCI spectra obtained from direct probe analysis. Positive ion detection for these drugs produced no signal at the 500 ng level.

The detection limits for the antimalarials using the DLI probe and negative ion detection were 160 ng for full scan spectra and 5 ng for SIM (monitoring m/z 342, 443, 445, 499, and 501 each for 0.4 seconds; Figure 4). The detection limit for the analysis could be

reduced if micro-LC techniques were used to avoid the 1/100 split of LC effluent.

Either LC/MS technique appears to offer better sensitivity and specificity for the antimalarials than can be obtained by UV or fluorescence detection. While the thermospray technique seems to have better detection limits, the DLI method provides more specificity due to the greater number of fragment ions formed.

Acknowledgment

We thank W. McFadden and Finnigan for analyzing the antimalarials using the thermospray interface.

- (1) Blakley, C.R.; Vestal, M.L. *Anal. Chem.* 1983, 55, 750.
- (2) Voyksner, R.D.; Hass, J.R.; Bursey, M.M. *Anal. Chem.* 1982, 54, 2465.

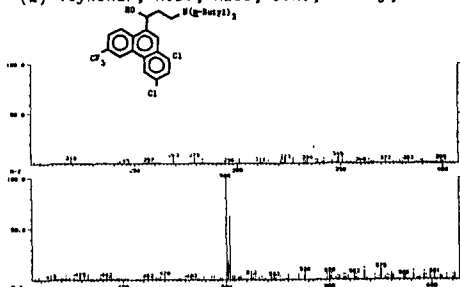


Figure 1: The full scan spectra of 5 ng of antimalarial II using the thermospray interface.

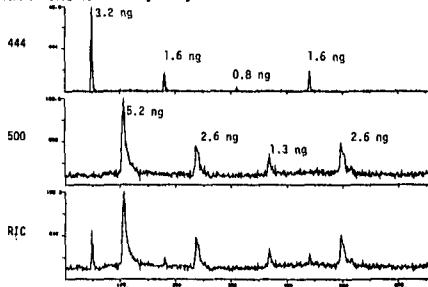


Figure 2: Selected ion monitoring traces for different amounts of antimalarial injected on column using the thermospray interface.

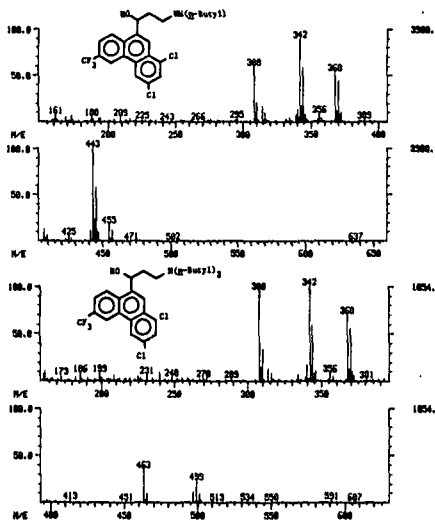


Figure 3: The full scan spectra of 300 ng of antimalarials I and II using the DLI interface and negative ion detection.

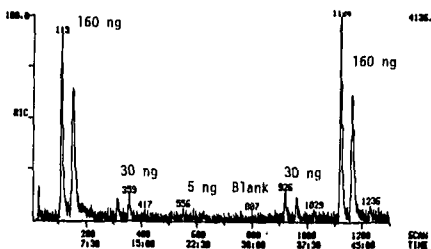


Figure 4: SIM trace for various amounts of antimalarial injected on column using the DLI interface.

PROCESS DEVELOPMENT AND ANALYSIS USING DIRECT SUPERCRITICAL FLUID EXTRACTION-MASS SPECTROMETRY

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Supercritical fluids are attracting increased interest as media for new separation techniques and in new analytical techniques for the separation and characterization of complex materials products. The solvating power of supercritical fluids under controlled conditions can often provide for the extraction of specific compounds (e.g., caffeine from coffee) as well as efficient extraction of a wide range of compounds under more severe conditions. A major deficiency in the development of supercritical fluid extraction (SFE) processes is related to the difficulty in direct and continuous analysis of high pressure and, sometimes, high temperature fluids. In particular, the desired analysis method should be selective, sensitive, applicable to all extracted compounds and should not disrupt the process. New methods developed in our laboratory now allow the efficient and detailed characterization of supercritical fluid processes for the purpose of process development.

Two similar instruments have been utilized in our supercritical fluid studies to date. The first is a micro-scale supercritical fluid extraction/reaction facility designed to allow the rapid study and characterization of the fluid phase under a wide range of conditions. The second instrument is utilized for analytical studies which include direct fluid injection-mass spectrometry⁽²⁾ and capillary column supercritical fluid chromatography-mass spectrometry⁽³⁾. The instrumentation has been described previously⁽¹⁻³⁾. Instrumentation for direct supercritical fluid extraction involves replacement of the injector by an extraction cell containing the material to be studied, while SFC-MS involves addition of a suitable open tubular capillary column between the injector and DFI probe⁽³⁾.

The direct fluid injection (DFI) method allows any compound soluble in a supercritical fluid to be transferred to the gas phase for ionization using conventional methods⁽²⁾. The DFI method has the following advantages: quantitation is straightforward; the process is applicable to essentially any compound; various CI reagents may be selected to vary the degree of spectral complexity (fragmentation); and the technique is inherently sensitive.

The DFI probes have been designed to couple with the direct probe inlet of a modified "simultaneous" chemical ionization-electron impact (CI-EI) ion source⁽¹⁻³⁾. Chemical ionization provides both excellent sensitivity and flexibility due to the potential for the addition of different or mixed CI reagent gases while the electron impact source allows monitoring of the major fluid components. For cases where additional structural information is necessary for identification one can use either more energetic CI reagents or the collision-induced-dissociation (CID) capability of the tandem quadrupole analyzer. The use of CI analysis is particularly attractive since the gentle ionization mechanism primarily produces protonated molecular ions, which allows information on the molecular weight distribution to be obtained directly from the mass spectra.

The last few years have seen a dramatic growth in the application of supercritical fluid extraction methods to a variety of extraction, separation, and process areas. The strong and often selective solvating power of supercritical fluids under controlled conditions often allows extraction of specific compounds from complex matrices, as well as efficient extraction of a wide range of compounds under more severe conditions. Under more extreme conditions the "extraction" process for materials such as coal is undoubtedly a combination of chemical reactions resulting in breakdown of the complex matrix combined with the extraction-distillation process of the supercritical fluid.

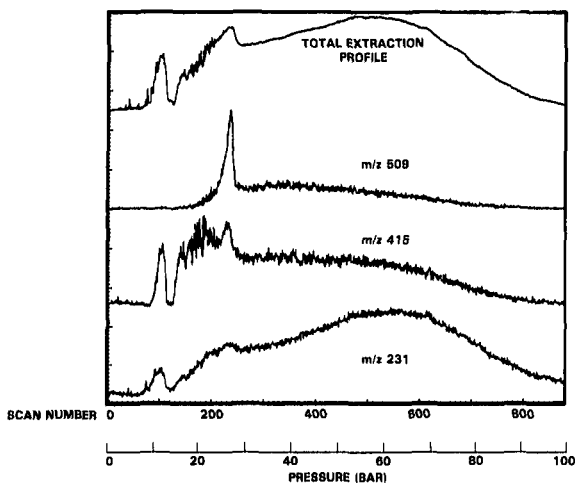
An example of such a process is the extraction of coal. The present methods for direct analysis of coal supercritical fluid extraction (SFE) processes involve the continuous removal of a small sample from an extraction cell for direct mass spectrometric analysis. An example of a nonisobaric study is given in Figure 1. A 40 mg sample of a bituminous coal, sized to approximately 80 μm average particle size and previously washed with pentane, was packed into a 75 μL cell for these experiments. The extraction temperature was 280°C and the solvent was a 95% n-pentane-5% 2-propanol mixture; thus the temperature was main-

tained well above the estimated critical temperature of the solvent mixture. The "gentle" CI conditions result in little molecular fragmentation and a dominant protonated molecular ion, greatly simplifying mass spectral interpretation. The experiment shown in Figure 1 involved an initial 2 hr period at 10 atm after which the pressure was increased at a rate of 0.4 atm/min between 10 atm and 100 atm. The figure gives reconstructed single ion profiles for several typical ions in the mass spectra and the total ion current (TIC) or extraction profile, which serve to illustrate the solubility of extractable components as a function of pressure. Large groups of compounds are clearly extracted in the 10-13 atm and 25-28 atm ranges and similar results showing greater structure are obtained with slower pressure ramps. Between 40 and 80 atm a large amount of complex higher molecular weight material, is extracted. Mass spectra for these conditions show that species having molecular weights extending to over 1400 are extracted(1). Initial experiments show significant structure in the extraction profiles, suggesting that the potential exists to readily alter the extracted product slate and to obtain significant product fractionation. The information obtained from such studies can be directly applied to the development of larger scale supercritical fluid extraction processes, allowing more rapid and efficient process development.

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SUPERCRITICAL COAL EXTRACTION PROFILES AT 280°C



DIRECT ANALYSIS OF INTRACTABLE ENDOGENEOUS MOLECULES BY THERMOSPRAY LC/MS

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The thermospray LC/MS interface permits direct analysis of molecules that, by reason of charge state, involatility or lability, are difficult to analyze by conventional mass spectrometry. The methodology has been described recently in some detail (1). In summary, it permits the use of reverse phase LC using buffered solvents at conventional flow rates (.5-1.5 ml/min). The entire solvent stream is input through the ion source, but the manner in which the solvent is heated and the expands into the source to form a highly directed stream allows the entire solvent flow to be removed by a mechanical pump. For convenience a standard cold trap is placed in front of this pump to eliminate daily oil changes. Ions are desorbed directly from charged droplets in the flowing vapor stream, and a portion of them pass into the source ion optics through a small grounded sampling orifice. The relative intensity of fragment ions varies with temperatures in the interface. At higher vaporizer and transfer line temperatures (Sensors 1 and 3 in Fig. 1) the base/nucleotide ratio of adenosine changes to 10/1 but the m/z 146 of acetylcholine becomes predominant. As these temperatures are reduced from 250°C to 230°C, the adenosine base/nucleotide ratio becomes 3/1, but the m/z 132 peak of acetylcholine becomes dominant. Derivatization for volatility or to produce chromophores is unnecessary.

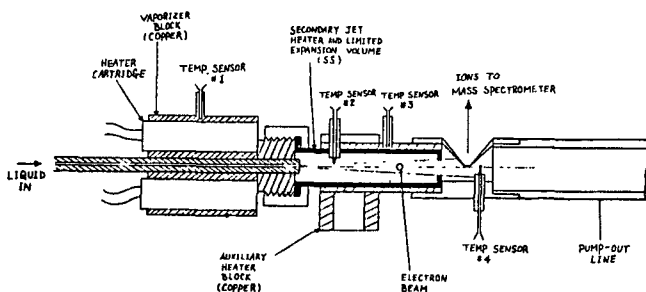


Figure 1. Schematic diagram of thermospray system showing cross sectional view.

Spectra of a wide variety of materials of physiological or biochemical interest have been obtained using this technique. The spectra tend to be simple, but some do contain information of structural significance. Spectra are summarized in Table I.

Table I

Material	Compound	Positive Ion Spectra		Spectrum
		M.W.	base peak	ion
peptides	valinomycin	1111	1112	(M+18)>(M+H)
	(glycine) ₂	132	133	(M+H)
phosphatidylcholines	18:0, 18:1-Pch	788	M-183	(M-183)>(M-183-FA)>
	12:0, 12:0-Pch		M-183	(M)
steroids	Δ4-androstenedione	286	287	(M+H)
	11-deoxycortisol	346	347	(M+H)>(M+H-18)
	α-ecdysterone	448	(M+18)	(M+18)>M>
	β-ecdysterone	464	(M+18)	sequential water loss
arachadonic acid derivatives	arachadonic acid	304	305	(M+H)>(M+H-18)
	12-HETE	320	321	(M+H)
	12-HPETE	336	354	(M+18)>(M)>(M-16)
	glucose	180	194	(M+18)>(M)> sequential water loss
	adenosine	267	135	(M-base+H)2x>(M+H)
	acetylcholine	146	146	(M)>(M-15+H)
	propionylcholine	160	160	(M)>(M-15+H)

The most commonly seen ions are protonated or ammoniated molecular ions for uncharged species. The presence of one or the other (or both) of these depends upon the relative basicity of the molecule and solvent ions as one would expect. Precharged ions typically show the molecular cation as the highest mass ion. Fragmentation occurs typically at bonds to functional groups so that the base/sugar pair of adenosine is cleaved and sequential water losses occur from the heavily hydroxylated ecdysones or from glucose. In these last three cases the ion at the molecular weight is probably (M+NH₄-H₂O). No structurally significant fragmentations are seen for the arachadonic acid derivatives, so one would need to depend on chromatographic separation to distinguish isomers in mixture. Acetylcholine and propionylcholine show an ion that appears to correspond to the pyrolytic loss of methyl followed by protonation. The phosphatidylcholines show ions corresponding to loss of phosphatidyl choline as well as fragments associated with loss of either fatty acid residue. These fatty acid loss ions could be used to characterize the lipids.

Sequential dilution sensitivity studies of materials in Table I show that in general, for materials with molecular weight lower than 500 daltons, response can be obtained at a particular ion from a full spectrum scan of 1-10 pmoles of the material. By scanning only specific ions the sensitivity of those materials could be reduced to 1-10 pg. Sensitivity was not nearly so great for the three heavier materials of Table I, and was in the 1-10 nmole region. Negative ion sensitivity was generally about 10-fold lower than positive ion sensitivity. The absolute response of adenosine, scanning the molecular ion ± 5 m, was about 10⁻¹¹ caol/μg, and the overall instrumental efficiency (including fragment ions) was 10⁻⁷ ions/molecule.

(1) C.R. Blakley and M.L. Vestal, Annal. Chem. **55**, 750 (1983).

THERMOSPRAY LC/MS PROGRESS WITH A COMMERCIALLY AVAILABLE GC/MS, Jack Henion, NYS College of Veterinary Medicine, Cornell University, 925 Warren Drive, Ithaca, NY 14850.

We have used the concepts pioneered by Vestal (1) to construct a thermospray probe which differs only in that it can be readily removed from the solid probe inlet of a commercially available GC/MS. We have disconnected the GC from the mass spectrometer so an auxiliary 300 L/min rough pump can evacuate the chemical ionization source from the GC side. In addition, the mass spectrometer is equipped with a liquid nitrogen cryo-pump over the ion source region to further complement the pumping capacity of the system. The HPLC columns used were 2.1 mm id. Brownlee microbore columns operated in the range of 100-500 μ L/min wherein the entire flow rate is introduced into the mass spectrometer. In these experiments all ion current and mass spectra were generated without any external source of electrons, eg. by thermospray.

EXPERIMENTAL

The commercial GC/MS instrument utilized in this work was a Hewlett-Packard Model 5985B. The Model 5840 GC which is interfaced to this mass spectrometer was disconnected from the MS and moved approximately 12 in away from the MS on its track. A half inch od. stainless steel pump out line (Figure 1) was inserted into the GC side of the CI source and connected to a 300 L/min Alcatel rough pump with an in-line isolation valve. This thermospray pump out line was equipped with a one-eighth in. stainless steel thermocouple tube which reached through the pumpout line to the ion source chamber to facilitate monitoring the source pressure.

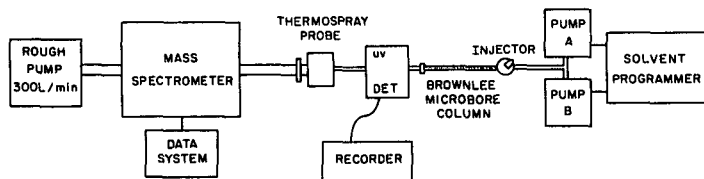


Figure 1

The thermospray LC/MS interface developed for this work was very similar to that reported by Vestal. It consists of a half in. od. x 3/8 in. id. stainless tube threaded to a heated, copper vaporizer through which a 0.004 in. id. x 0.062 in. od. microbore stainless steel tube is silver soldered. The vaporizer was equipped with two 50 watt 1/8 in. x 1 in. cartridge heaters and a 1/16 in diameter iron-constantine thermocouple. The microbore throughput tube of the thermospray probe was connected to the HPLC column via an in-line Valco 0.2 micron filter union. The MS filament was set at zero microamps emission current and 10 eV ionizing voltage to effectively "turn off" the filament of the mass spectrometer. A schematic of the system is shown in Figure 2

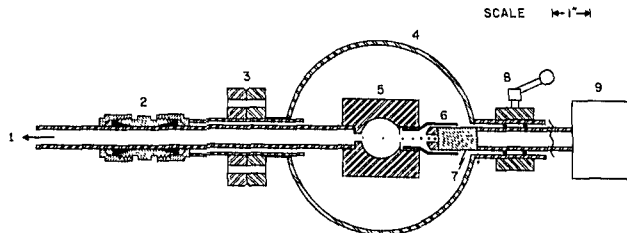


Figure 2

RESULTS

All thermospray LC/MS experiments reported in this work were conducted using a micro LC eluent flow rate of 150 μ L/min and a vaporizer temperature of 225 degrees. These conditions were chosen as a reasonable compromise for the Brownlee microbore columns. However, the system described herein easily could handle one-half mL/min and as much as one mL/min eluent flow rates.

A tuning solution of 10 ppm adenosine in 50/50 MeOH/ 0.05 M NH_4OAc was used to tune for thermospray operation. When this condition had been reached, injections of various xanthine drugs and steroids were injected on-column to obtain thermospray LC/MS mass spectra. The ion current and thermospray operation were very stable and mass spectra were extremely simple consisting essentially of only $M+1$ ions as has been reported (1). The ion current profiles and plotted mass spectra of a mixture of theophylline (MW=180), theobromine (MW=180), and caffeine (MW=194) were obtained. Although the detection limits of these experiments were not determined, sensitivity appeared to be comparable to micro LC/MS results reported earlier (2). The chief difficulty encountered in this work was an unusually high amount of electron multiplier noise that could not be suppressed. It is not known as yet whether this is due to x-rays and/or neutrals entering the multiplier or whether there is excessive pressure in this region of the MS. When the system is operated with the filament on as is done in DLI experiments, conventional DLI type CI mass spectra are obtained without the excessive detector noise.

CONCLUSIONS

This home made thermospray probe provides for its removal from the MS, implementation on a commercially available GC/MS, and further demonstration of the thermospray technique. We are confident that this technique is a valuable complement to well established DLI LC/MS methods.

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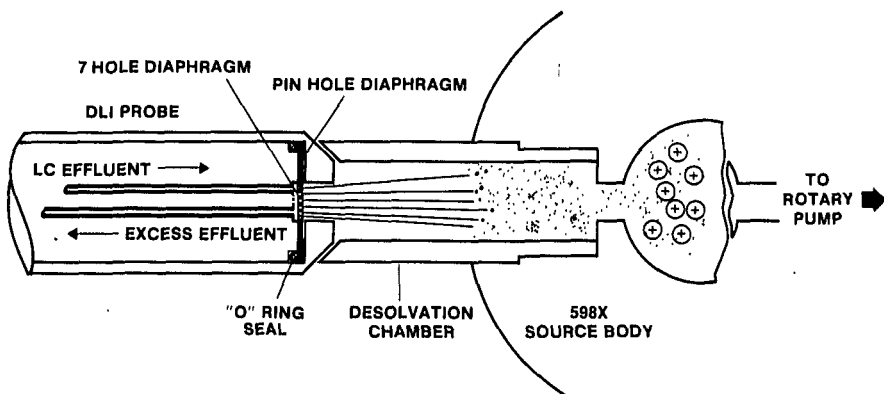
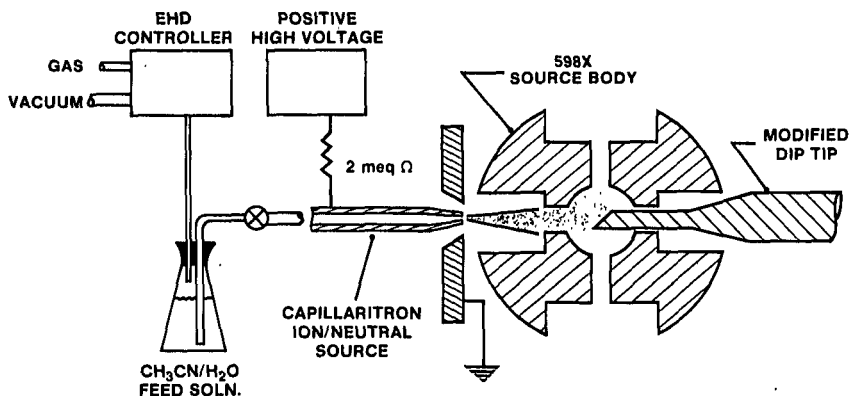
EXPERIMENTAL APPROACHES TO LC/MS

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Two experimental approaches for introducing liquids from a high pressure liquid-chromatograph (HPLC) into a quadrupole mass spectrometer were examined for feasibility. The first approach examined the feasibility of electrospray ionization (ESI) for generating the charged species from a liquid and mass analyzing these ions as they exit a small nozzle held at a high voltage (1-3). A modified Capillary Ion Bombardment (CIB) (4) source was installed as shown in Figure 1 to the gas chromatographic inlet port of a Hewlett-Packard 5985B quadrupole mass spectrometer. A special stainless steel DIP probe tip cut at a 45° angle was designed to locate 4 cm from the Capillary Ion Bombardment nozzle and deflect the ions toward the axis of the mass spectrometer source lens and analyzer. Two liquid combinations were examined: glycerol/NaI and acetonitrile/H₂O. The liquid flow to the 100µm tungsten needle was controlled by a differential pressure sensing transducer which used the primary ion beam current as a feed back loop to supply 25 µL/minute to the MS ion source. Ion beam currents from the Capillary Ion Bombardment were controlled at 20 uA and 6 kV of energy. The positive ion mode gave ion clusters of [(glycerol)_nNa-glycerol]⁺ where n=8. In the negative ion mode, the major ions were I⁻ and NaI₂⁻. No other major ions were observed. Water clusters were observed up to n=30 for the acetonitrile/water solution when detecting positive ions. No sample molecules of Rhodanile Blue were observed during this experiment. The analytical potential for ESI use for LC/MS must still be explored.

A second approach used to introduce a liquid into the mass spectrometer source was to use a standard direct liquid inlet (DLI) probe (5-7) which had a 5 µmeter stainless steel pinhole type diaphragm as the liquid exit nozzle. Typically, the single pinhole diaphragm allows 20 to 40 µl of the HPLC column effluent to enter the mass spectrometer ion source and exit into the high vacuum system. For conventional HPLC columns having two through four mm i.d., one to three percent of the total HPLC effluent is allowed to enter the mass spectrometer ion source. Large diffusion pumps and cryopumping assist in maintaining the source and analyzer pressure in the 10⁻⁵ and 10⁻⁷ Torr ranges, respectively, on the standard commercial system. Ultimate sensitivity could be increased by allowing more liquid sample (solvent/solute) to reach the MS source provided larger high vacuum pumping capacity were available. For this experiment, the high capacity pumping requirement was circumvented by using a rotary vacuum pump to capture the excess gas load created by the excess liquid before it enters the high vacuum region outside of the ion source body. Also a special diaphragm containing six laser drilled 5 µmeter pinholes spaced regularly on a base circle of 0.2 mm radius with one hole in the center was designed to allow additional liquid eluent into the ion source. The purpose of the experiment was to examine the effects of the excess liquid droplets on the vacuum system and mass spectrometer sensitivity. The diaphragm was constructed of 302 stainless steel to fit the standard HP DLI probe. A 200 liter/minute rotary pump was coupled through a custom heated pump-out adapter using a polyimide seal to the GC entry port of an HP 5985B mass spectrometer as shown in Figure 2. The rotary pump-out line was connected directly to the opposite side of the CI source chamber and a pressure of 700 mTorr could be maintained while the DLI was jetting 200 µL/min of liquid/droplets. No significant pressure increases were observed in the high vacuum manifold region provided close tolerances were held so that the sealing of the source body to the repeller plunger and pump-out line were maintained. Preliminary results using Benzanilide as a test compound showed a factor of five increase in the ion intensity of m/z 198 when the seven hole diaphragm was tested under the same source pressure conditions and multiplier voltage as was used for the single hole diaphragm used on the standard HP DLI LC/MS interface.



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CALCULATIONS ON CAPILLARY INTERFACES FOR LC/MS

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The method of introducing a liquid into a mass spectrometer via a capillary tube, as an alternative for a heated batch inlet system, has been pioneered by Talroze and coworkers. Various research groups have employed a narrow capillary tube as an LC/MS interface. One conclusion that can be drawn from the experimental data is that the capillary has to be heated in order to achieve a stable ion source pressure. A cooled capillary is not suitable as an LC/MS interface.

The essential part of the interface built in our laboratory is a 70 cm long piece of 50 μm i.d. fused Silica tubing. The tip of the capillary is heated by thermal contact with the source via a copper block (1,2).

Talroze and coworkers have presented a discussion about the situation at the end of the capillary: does the liquid evaporate inside the capillary or, alternatively, does the liquid flow into the vacuum system? Their calculations and experiments have shown that either case is possible.

The rate of evaporation of a liquid at the liquid-vacuum interface at temperature T can be calculated from the kinetic gas theory (2, and references cited therein). In SI units:

$$G = P_0 A \sqrt{\frac{10^{-3} M}{2 \pi RT}} \quad \text{kg.s}^{-1}$$

The area A (m^2) is assumed to be a hemisphere with radius r (m, radius of the capillary). P_0 (Pa) is the saturated vapour pressure of the liquid at temperature T (K). M is the molecular weight, R is the gas constant ($\text{Joule.K}^{-1}.\text{Mol}^{-1}$). Figure-1 shows the calculated evaporation rate if $r = 25 \mu\text{m}$. The 10 $\mu\text{l/min}$ liquid flow from a micro LC can be evaporated at

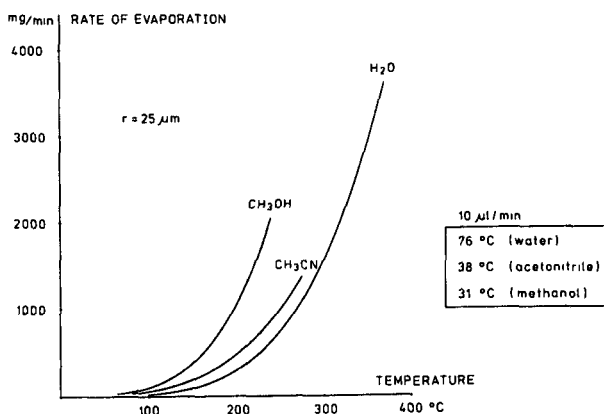


Figure 1

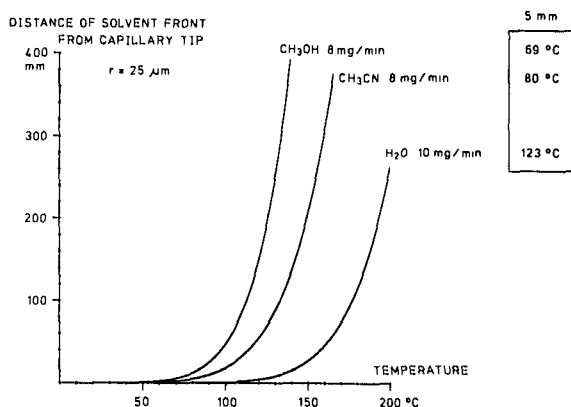


Figure 2

a fairly low temperature. A higher temperature of the capillary will make the solvent front retract into the capillary. One can calculate the pressure build up of solvent vapours as they flow through the capillary and the distance of the solvent front from the capillary tip. See figure 2 (2).

The data presented thus far indicate that a micro LC flow rate, combined with a capillary radius of $25 \mu\text{m}$ results in complete evaporation of solvent and solute inside the capillary. On the other hand, if complete evaporation has to be avoided, the same formulae can be used to calculate upper temperature limits. The thermospray interface, as used by Vestal et al. for thermospray ionization and by Junclaus et al. for combined LC/²⁵² Cf MS, and the nebulizer interface designed by Tsuge et al. are based on droplet spray formation.

In the case of a nebulizer in which $30 \mu\text{l/min}$ of mobile phase is transported through a $100 \mu\text{m}$ i.d. capillary, the temperature of the liquid has to be kept below 69°C , 80°C , and 123°C for water, acetonitrile and methanol respectively, to prevent complete evaporation. Higher temperatures are permissible if a narrower capillary is used.

CONCLUSIONS

Theoretical evaporation rates are remarkably high.

But if the liquid is not pure, and if the liquid surface is contaminated with involatile solutes, the real evaporation rate can be much lower (3) so that salts and sample components may reduce the rate of evaporation.

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PRACTICAL DLI LC/MS WITH IMPROVED DETECTION LIMITS, Jack Henion, New York State College of Veterinary Medicine, Cornell University, 925 Warren Drive, Ithaca, NY 14850

Combined LC/MS has received slow acceptance in research and service laboratories for a variety of reasons. Although both transport and direct liquid introduction (DLI) type LC/MS interfaces have been commercially available for several years, they are routinely used by only a few laboratories. This is in part due to the belief that neither approach to LC/MS is really routine or adequately sensitive. Since most mass spectrometry laboratories are familiar with the sensitivity performance of GC/MS, comparable performance is expected from LC/MS. This is both reasonable and possible.

We have investigated the practical limitations of the DLI approach to LC/MS, and have focussed on making it routine with detection limits comparable or better than GC/MS (1). At the present time optimum sensitivity requirements for DLI LC/MS appear to be met by implementing micro LC/MS techniques. The utilization of micro high performance liquid chromatography (micro LC) improves the detection limits of both the transport and the DLI LC/MS interface because the concentration of the eluting chromatographic peaks is significantly higher. In addition, approximately 20 times less LC eluent is introduced into the vacuum system because micro LC utilizes liquid flow rates in the neighborhood of 20-50 microliters per minute rather than one to two milliliters per minute. However, micro LC equipment is not readily available commercially and many laboratories are reluctant to implement this technique. Alteration of conventional LC equipment is possible but can cause experimental difficulties. We have recently focussed on a means of combining the advantages of conventional HPLC equipment with the improved detection limits of micro HPLC technology.

EXPERIMENTAL:

This work utilized a Hewlett-Packard 5985B GC/MS mass spectrometer and its associated options for DLI LC/MS. The LC/MS interface is available commercially from Hewlett-Packard. This DLI interface is equipped with a variable split valve which allows the operator to vary the amount of the LC effluent that enters the MS ion source. When this interface is used with conventional LC flow rates of 1 mL/min. only about 1% of the total LC effluent enters the ion source.

We have optimized the use of 2mm id x 25cm microbore RP 18 HPLC columns (Brownlee Labs, Inc.) at eluent flow rates of 100 μ L/min for use with the above described DLI LC/MS interface (Figure 1). The split ratio with these columns is increased from 1% to 50% so this approach offers a 50 fold increase in LC/MS sensitivity without any modification or alteration of equipment. A conventional LC detector may be used with the LC/MS interface which is not practical in micro LC/MS experiments. This feature is very useful since it provides the operator an indication of overall performance of the LC system and a check on the LC/MS performance. In addition, extra column band broadening is much less of a problem at these flow rates. The Brownlee Labs 2mm id microbore columns have the added desirable feature that they are housed in a convenient cartridge holder which also accommodates a guard column butted firmly against the analytical column. The guard column protects the analytical column and allows injection of rather dirty samples without harm to either the latter or the LC/MS system. A final advantage is that the 2mm id microbore columns allow larger injection volumes and accommodate higher levels of endogenous biological substances without becoming chromatographically overloaded.

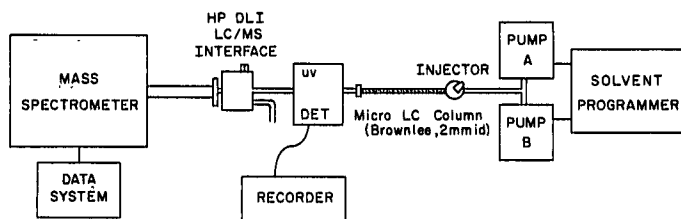


Figure 1

RESULTS AND DISCUSSION:

Experimentally this system is much easier and faster to set up for operation. Method development on the 2mm id columns is faster because flow rates ranging up to 500 $\mu\text{L}/\text{min}$ may be used and column equilibration times are relatively short. Usually an off line separation is established at about 200 $\mu\text{L}/\text{min}$ because run times are short and the flow rate can then be halved for the LC/MS experiment or used as is with 25% LC effluent introduction into the MS. The variable split ratio of the HP DLI LC/MS interface is also convenient for adjusting the optimal jet length into the ion source if an extended desolvation chamber is not used. (2) The entire method development can be accomplished with the LC/MS interface installed in the plumbing system (not in the mass spectrometer) so the LC/MS experiment may commence as soon as satisfactory separation has been achieved. When the DLI LC/MS operator has developed sufficient confidence, method development may be undertaken using the mass spectrometer (the better detector) for detection purposes.

We have investigated a variety of biologically important substances including antibiotics, steroids and drugs using this system. The detection limits of this system in the full scan mode is approximately 50 ng injected on column and is satisfactory for many applications. Certain metabolism studies require better detection limits than this wherein we utilize micro LC/MS. However, for many problems the system described here is very satisfactory and routine.

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Mass Spectral Identification of Chemical Residues
Encountered in the Total Diet Program

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The Total Diet Program of the Food and Drug Administration consists, in part, of the surveillance of residue levels of pesticides and industrial chemicals in table-ready foods. Each sample, or market basket, is comprised of 276 grocery items prepared to form 234 table-ready foods. Analyses are performed using procedures found in the Pesticide Analytical Manual of the Food and Drug Administration.¹

Routinely, these foods are examined for the presence of several hundred chemical contaminants. The determinative step for the analysis of organic contaminants is usually gas-liquid chromatography. During the course of examination, a number of chemicals of unknown identity have been encountered.

Chlorpropham or CIPC (isopropyl 3-chlorophenylcarbamate) is an organic carbamate compound used as a herbicide with some plant growth regulation properties. In the latter use, CIPC is applied to potatoes to prevent sprouting during storage. One method of application involves the use of a 5% concentration dip with an application rate of one pound/60,000 pounds of potatoes; or nearly 17 ppm.²

Residues of CIPC are occasionally encountered in the Total Diet Survey. Associated with the higher residue levels is a second gas chromatographic peak. The compound represented by this peak elutes from an OV-101 column at a retention time relative to chlorpyrifos (Rc) of 0.75, and was thought to be a metabolite of CIPC. The parent compound, CIPC, elutes at a relative retention time of 0.32 relative to chlorpyrifos under identical conditions.

To be sure that this UAR was really a metabolite and not an impurity in the CIPC formulation, a sample of Sprout-Nip^R was obtained and analyzed by GLC. The sample was simply diluted to a concentration consistent with HECD detection and compared to a standard CIPC solution. No impurities were found in Sprout-Nip^R.

To substantiate the existence of a CIPC metabolite, several home-grown potatoes were dipped in a 5% solution of Sprout-Nip^R and analyzed by the previously described procedures. At 70 hours after being dipped, the potatoes showed no evidence of the metabolite at Rc 0.75. After 458 hours (19 days) a metabolite at Rc 0.75 was seen. Both CIPC and its metabolite were examined by GC/MS. The electron impact mass spectrum of CIPC (Figure 1) exhibits the loss of 42 amu (171 m/z) representing the rearrangement loss of isopropyl function; the loss of 59 amu (154 m/z) representing the propoxy group; and 86 amu for the rearrangement loss of the ester function. The resulting chloroaniline ion (127 m/z) appears as a relatively stable ion.

The mass spectrum of the CIPC metabolite (Figure 2) showed the same fragmentation losses as the CIPC itself. An increase in molecular weight of 30 amu was assumed to be a substitution on the aromatic portion of the molecule--most likely a methoxy group. Positive ion, methane chemical ionization was used to confirm the molecular weight of 243 amu. From the ion resulting from the loss of the ester function (m/z 157) an additional loss of 15 amu, generally a methyl group, is observed. This lends support for the fairly stable ion at 142 amu to be a quinoid structure. The very small ion cluster at 114-116 amu may, then, be the chloropyridinium cation resulting from the subsequent loss of carbon monoxide.³

Reaction of isopropylchloroformate with chloroanisidines as described by Strain⁴ resulted in the formation of isopropyl 2-methoxy-5-chlorophenylcarbamate and isopropyl 3-chloro-4-methoxyphenylcarbamate. This latter compound matched the mass spectrum and retention data of the metabolite of CIPC found in potatoes.

In conclusion, a previously unreported metabolite of CIPC (chlorpropham) found in potatoes of the Total Diet Program of the Food and Drug Administration has been identified by mass spectrometry as isopropyl 3-chloro-4-methoxyphenylcarbamate.

The author thanks PPG Industries for both Sprout-Nip^R and isopropylchloroformate.

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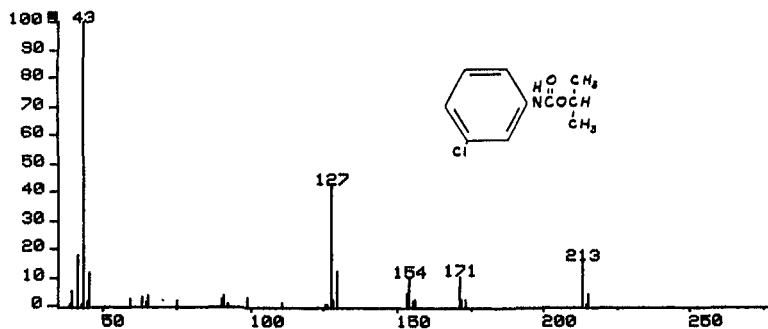


Figure 1 - Electron impact mass spectrum of chlorpropham (CIPC).

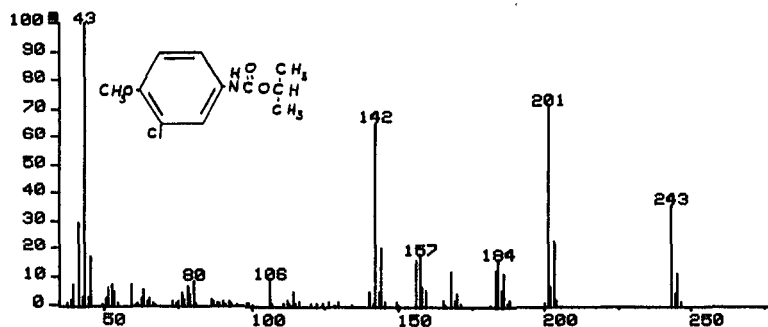


Figure 2 - Electron impact mass spectrum of isopropyl 3-chloro-4-methoxyphenylcarbamate.

PROPOSED STRUCTURE OF ANTIOXIDATIVE ARGININE-XYLOSE MAILLARD PRODUCTS

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An antioxidative Maillard reaction product (MRP) can be formed from arginine and xylose in equimolar ratio. The antioxidant produced may possess considerable utility for the food industry depending on its cost of production, and its effectiveness in preventing rancidity. The possible structures of antioxidative melanoidins from MRP's such as the arginine-xylose combination are here reported for the first time. The MRP was synthesized by refluxing mixed aqueous solutions of arginine HCl and xylose for 20 hrs. A 1:1 molar ratio gave products of highest antioxidative activity. Removal of low-molecular-weight materials by dialysis through tubing of 1000 MW cutoff (nominal) for 24 hr yielded about 30% retained compounds with an antioxidative activity of 9.7 units as measured by an oxygen electrode with linoleic acid as reference.

These compounds have been examined by NMR, IR, UV and mass spectrometry. It was not possible to analyze them by electron impact because of their extreme lability, but analysis by FAB was found to be fairly satisfactory; it worked for a portion of the partially purified mixture which was soluble in glycerol. The MS-50 spectrometer modified into an MS/MS mass spectrometer equipped with a FAB source was used to obtain the spectra. Fig. 1 shows the MS/MS spectra and from that one is able to determine molecular weight ($M^+ - 421$) and some fragment ions [m/z , 403 ($M^+ - 18$) and m/z 289 ($M^+ - 1$ mole of xylose)]. High resolution mass spectra gave the following exact masses: $M^+ 421$ ($C_{16}H_{29}N_4O_9$); 421.1035, dev. 0.1 ppm; m/z 403; $C_{16}H_{27}N_4O_8$; 403.1808, dev. -5.2 ppm; m/z 289 ($C_{11}H_{21}N_4O_5$), 289.1523 dev. 3.9 ppm. This leads to the proposed structure shown in Fig. 2a. The structure shown has exactly the number of carbons predicted without a loss of CO_2 , and the rest of the molecule can be formed from the 3 successive dehydrations to form the substituted partly dehydrated imidazole lactone compound. This substituted imidazole may be a repeating unit of a polymeric substance that shows antioxidative activity or it may be the molecule that exhibits such activity. Since the material is only partly purified (retentate of 1000 MW cutoff tubing) we are unable to say. The CA MIKES spectrum showed several interesting features: the carboxylic acid group was found to be unesterified based on the loss of 45 u, and the sugars were both linear and showed losses of 30, 31, 32 u. Also seen from the CA MIKES spectrum of m/z 421 (Figure 2) is m/z 310, which may be from arginine fragmenting to lose 103 u: $CH_2CH_2CH_2NH_2CO_2H$. The loss of arginine is not frequently encountered.

Partial hydrolysis of the dialyzed compounds followed by lyophilization with 0.1 N NaOH refluxed for 1 h yielded on the FAB source products of m/z 436, 490 and 528, and these are shown in Fig. 4. Since we have found that CO_2 liberation follows the antioxidative pattern we were led to think of the decarboxylation product of arginine which might be involved. Fig. 2 indicates that possibility; in addition it shows that the imidazole ring may be formed from the guanidine group of arginine by becoming attached to the 3 and 4 positions of xylose. By this arrangement the repeating unit of Fig. 2b becomes possible.

Hydrolysis with 0.1 N trifluoroacetic acid at reflux for 3 hrs yielded mainly one product with m/z 1249 and the structure is shown in Fig. 5. The compound(s) shown in Fig. 5 contained C---43.06; H---4.67; N---10.14; O---23.91; F---13.85 and Cl---8.38%. The chlorine was present because we started with arginine hydrochloride. It would have been predicted that the Cl^- would be lost by dialysis but we have found that these molecules associating-dissociating activity upon dialysis. Actually repeated dialysis up to 12 days results in the loss of about 50% antioxidative activity. The Cl^- would not be seen on the FAB source.

Research supported in part by Oklahoma Agricultural Experiment Station, by NSF Grant No. INT-8018220 to GRW and NSF Grant No. CHE-8211164 to MLG.

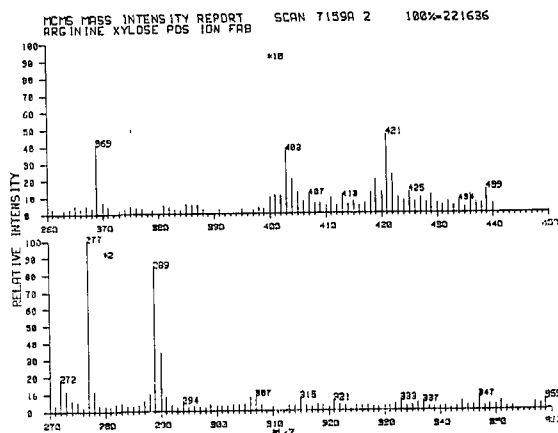


Figure 1 MS/MS Mass spectrum of a partially purified antioxidant arginine-xylose product (MS-60 modified)

Legend: Antioxidative activity = 1

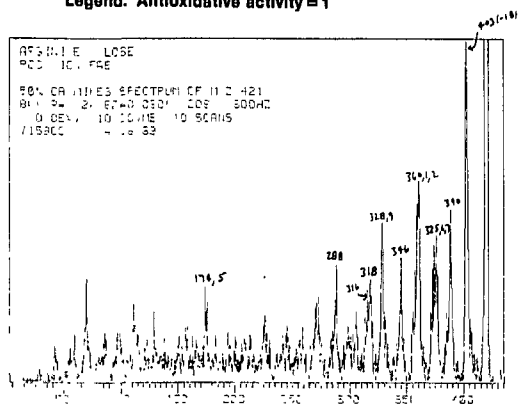


Figure 2 50% CAD spectrum of ion m/z 421 from Figure 1

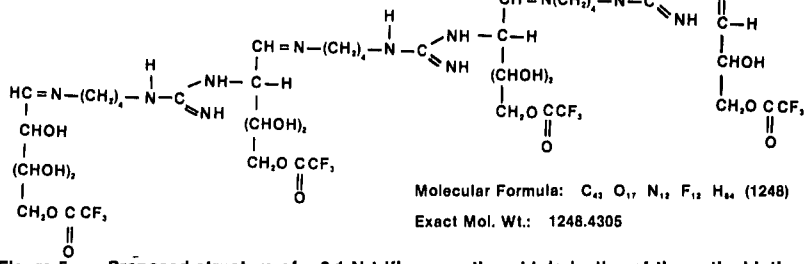


Figure 5 Proposed structure of a 0.1 N trifluoroacetic acid derivative of the antioxidant arginine-xylose product

Legend: Antioxidative activity = 0.65

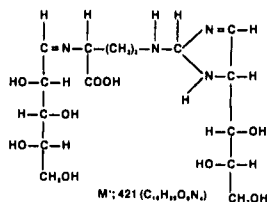


Figure 3 Proposed structure of a partially purified arginine-xylose product (a) modified imidazole

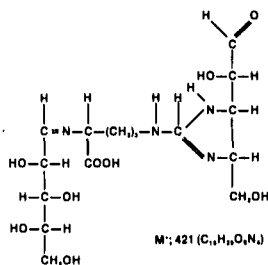


Figure 4 Proposed structure of a NaOH derivative of the antioxidant arginine-xylose product

Legend: Antioxidative activity is unknown

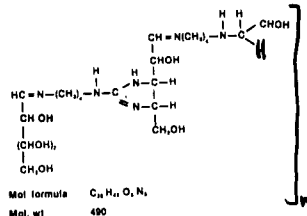


Figure 4 Proposed structure of a NaOH derivative of the antioxidant arginine-xylose product

Legend: Antioxidative activity = 0

Molecular Formula: $C_{41}O_{17}N_{13}F_{12}H_{44}$ (1248)

Exact Mol. Wt.: 1248.4305

The Analysis of Volatile N-Nitrosamines in Rubber Nipples by GC CID/MIKES.
D. Andrzejewski, D.C.Havery, P.Dreifuss, K.White, T.Fazio and J.Sphon

Volatile N-nitrosamines were reported in rubber nipples for babies' bottles by Preussmann et al.¹ These findings prompted the Food and Drug Administration to investigate the levels of N-nitrosamines in rubber nipples, as well as their migration into milk and infant formula during sterilization and storage.² Three nitrosamines, N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA) and N-nitrosopiperidine (NPIP) were detected by GC/MS in rubber nipple extracts.

An EI GC/MS confirmatory method was developed based upon full mass scan data from the injection of 100 ng of apparent nitrosamine as determined by GC/TEA quantitation. The confirmatory method required both a silica gel and an acid celite column clean up. These two column clean up steps, along with the final concentration step, result in a significant loss of the volatile nitrosamines present. To compensate for the loss, three to six cleaned up nipple extracts were pooled to provide the 100 ng of apparent nitrosamine needed for confirmation. In an attempt to improve the confirmatory method we investigated the usefulness of GC CID/MIKES in the analysis of rubber baby bottle nipples.

Using standards, EI GC CID/MIKE spectra were recorded on the molecular ion of NDMA, NDEA, and NPIP at the 50 ng level. MIKE spectra were obtained on a VG Analytical ZAB-2F mass spectrometer by scanning the electric sector with an Incos 2300 data system. The electric sector was scanned in 2 sec from 1600 to 8000 eV. Helium collision gas was introduced into the second field free region gas cell where the pressure was maintained at 3.0×10^{-7} mbar. Splitless injection of samples were made onto a 50 M Carbowax 20M fused silica capillary column, temperature programmed from 80°C to 170°C at 8°/min. The column exit was coupled directly to the ion source of the mass spectrometer.

In the sample clean up for CID/MIKES analysis, elution from an acid celite column was unnecessary. The nipple extract, following elution from a silica gel column was quantitated by GC/TEA and concentrated to a volume capable of transferring 50 ng of suspected nitrosamine in a 2 or 3 μ l injection. Sample extracts were sufficiently clean to confirm NDMA, NDEA, and NPIP by GC CID/MIKES. However, because of software constraints, only one nitrosamine could be confirmed from a single injection. Confirmation was based upon a comparison of the CID/MIKE spectra and relative retention times recorded for authentic standards against the same data acquired from nipple extracts.

The added specificity of the GC CID/MIKES method has reduced the need for extensive sample clean up, as well as increased the sensitivity of the confirmatory method when compared to the conventional EI GC/MS method. Confirmation of NDMA and NDEA in the nipple extracts examined by CID/MIKES was not possible by EI GC/MS from full mass scans.

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RAPID SCREENING OF T-2 TOXIN AND DIACETOXYSCIRPENOL IN GRAIN PRODUCTS BY
APCI/MS/MS AND GC/CI/MS/MS;

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Trichothecene mycotoxins are a class of naturally occurring toxins produced by *Fusarium* and some other species of molds. Little is known about the toxicological effects of these trichothecenes on humans. However, it is believed that they are, deoxynivalenol (vomitoxin or DON) in particular, responsible for vomiting and feed refusal in animals after contaminated food was eaten. There have been cases of natural contamination of grain crops and feed by several trichothecenes in both Canada and U.S.A. in recent years. Vomitoxin has the highest incidence. Concern is rapidly rising over this contamination problem at both government and industrial levels.

Present methodology for trichothecene analysis involves extraction, extensive sample clean-up and chemical derivatization to either TMS or heptafluorobutyrate derivative, prior to GC/EC or GC/MS/MID analysis. The whole procedure is quite laborious and time consuming. It is desirable to develop a simple and rapid method for the screening of trichothecenes in a large number of samples. One of these methods under our evaluation is the MS/MS technique. SCIEX TAGA 6000 MS/MS system is the instrument used in our extensive evaluation and validation program.

A Direct Insertion Probe (DIP)/APCI/MS/MS method has been applied to vomitoxin analysis in various grain products with some success. The results have been reported elsewhere. However, we encountered a severe matrix effect when we applied the same method to quantitative determination of diacetoxyscirpenol (DAS) and T-2 toxin in wheat. The response of the same amount of DAS and T-2 toxin in spiked samples was much lower than that of the standard alone. It appears that matrix components or residue of solvent(s) remaining in the sample deplete chemical ionization reagent ions, thereby inhibiting the ionization of the compound of interest. Two methods have been tried to circumvent this matrix problem:

- 1) Internal standard method: Same amount of an internal standard, T-2 triol, was added to both standards and samples spiked at various levels of DAS and T-2 toxin. Significant difference between the response ratio of DAS/T-2 triol in the spiked samples and that in the same amount of standards indicated that matrix components affected the internal standard and the compound of interest in a different manner. It appeared that a structurally similar internal standard was not able to solve the matrix problem.
- 2) Standard addition method: Using the same set of data in the wheat spiking studies, results obtained by standard addition method were consistently higher than the true values (around 40 ppb for DAS and 80 ppb for T-2 toxin). This background interfering level may vary from sample to sample and from matrix to matrix. If clean control sample is not available, results obtained by DIP/APCI/MS/MS method will be quite misleading.

GC/CI/MS/MS Method:

Underivatized trichothecenes (including vomitoxin, DAS and T-2 toxin) can be chromatographed on a short chemically bonded (DB-5, J & W) fused silica column. Coupled with a newly designed low pressure Townsend discharge chemical ionization source, this GC/CI/MS/MS combination proved to be the most effective way to overcome the matrix effect experienced with the APCI method. Underivatized DAS and T-2 toxin can be simultaneously determined within a 15 min. run. Figure 1a shows chromatograms of a clean Alberta wheat extract and Figure 1b clearly demonstrates that DAS and T-2 toxin can be detected in the same wheat extract spiked at 50 ppb level for both. The responses were the same as in the mixed standard alone.

As compared to the time required for DIP/APCI/MS/MS method (typically 2 minutes), the additional time required for GC/CI/MS/MS is worthwhile. Considering the time and complication saved for chemical derivatization, GC/CI/MS/MS is still considered as a rapid method and expected to be a sensitive,

selective and powerful technique in the screening of a variety of food contaminants. More detailed evaluation and validation studies in trichothecenes plus other pesticides are underway.

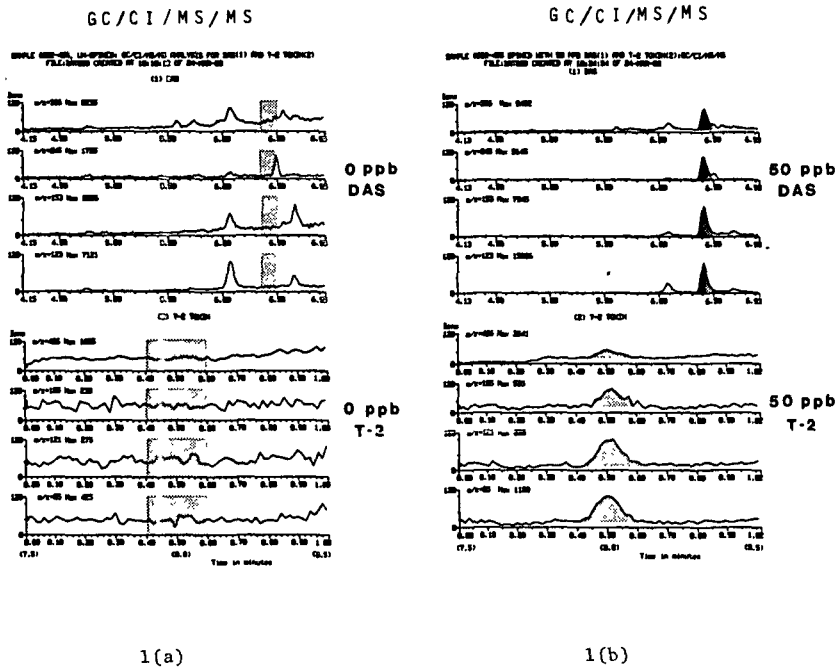


Figure 1. Simultaneous determination of underivatized diacetoxyscirpenol (DAS) and T-2 toxin (T-2) in an Alberta wheat sample (a) unspiked (b) spiked with 50 ppb of DAS and T-2.

APPLICATION OF THIN-LAYER AND LIQUID CHROMATOGRAPHIC SEPARATION WITH MASS SPECTRAL ANALYSIS

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Combinations of thin-layer and liquid chromatography with desorption ionization methods such as SIMS and FAB have been used to characterize a variety of pharmaceutical agents. Compounds such as the antibiotic aztreonam may be analyzed directly from thin-layer plates, however, detection limits are poor and the short-lived nature of the sample (ca. 20 seconds) precludes high resolution or ms/ms analyses. Under static ion flux conditions (SIMS), signals are long-lived but significantly reduced in intensity. The FAB results are generally similar to the direct examination of paper (1) and thin-layer (2) chromatograms as solids using SIMS.

Direct extraction using a vacuum-compatible solvent and subsequent FAB mass spectral analysis of aztreonam yields better results. Full positive and negative ion mass and ms/ms spectra have been obtained with separated components containing as little as five micrograms. High resolution FAB analysis is routinely used to characterize these separated materials, as static chromatography is well-suited to high resolution analysis.

Fluphenazine decanoate and its oxides have been examined using DCI and FAB (3). Thin-layer separation of mono-oxides of fluphenazine decanoate yields three components (Figure 1). Each species yields a representative mass spectrum at microgram levels. Ms/ms spectra are used to deconvolve complexities in the resulting spectra, to improve resolution on unresolved components, and for comparison with direct mixture analysis. For specific compounds, ms/ms may be applied to improve detection sensitivity to nanogram levels (Figure 2).

Compounds such as the ACE-inhibitor captopril, the antibiotic aztreonam, and the antipsychotic fluphenazine are directly extractable from a variety of thin-layer chromatograms, including cellulose, silica gel, and reverse-phase plates. Unlike high performance liquid chromatography where buffers and ion pairing agents often obscure the structure-spectrum relationship, little background is introduced into the spectrum. If additional separation is needed, often HPLC is the method of choice.

For the separation of arginine-aztreonam blends, sufficient secondary ion intensity is often obtainable on microbore effluents, eliminating concentration steps. The distinction of ion-molecule reaction products generated during sputtering from preformed aztreonam analogs is accomplished using ms/ms. Figure 3 demonstrates this difference for an HPLC fraction. The $(M+H+Arg)^+$ ion at m/z 610 shows SO_3 loss while the $(M+H+2Arg)^+$ ion regenerates the 610^+ ion exclusively under metastable conditions. The $784^+/610^+$ dissociation is expected for a proton-bound dimer generated during sputtering, while the preformed adduct shows covalent (SO_3) cleavages.

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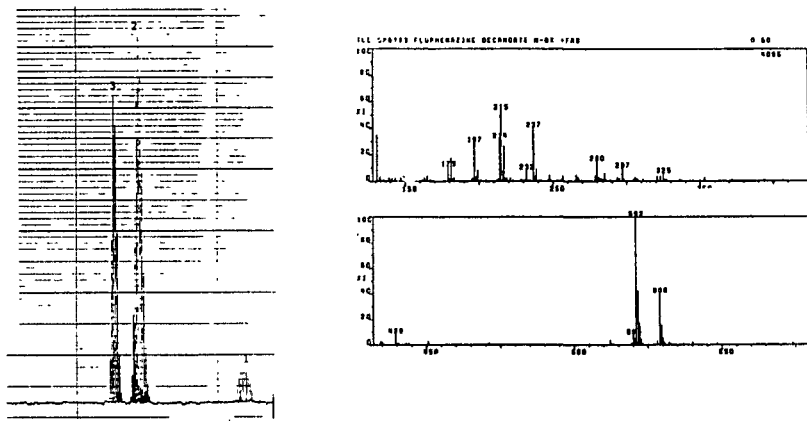


Figure 1

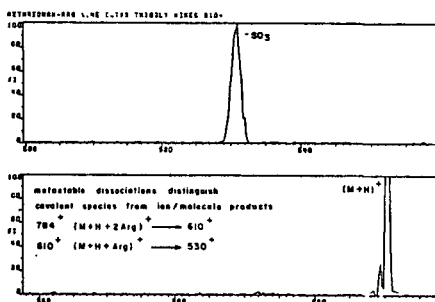
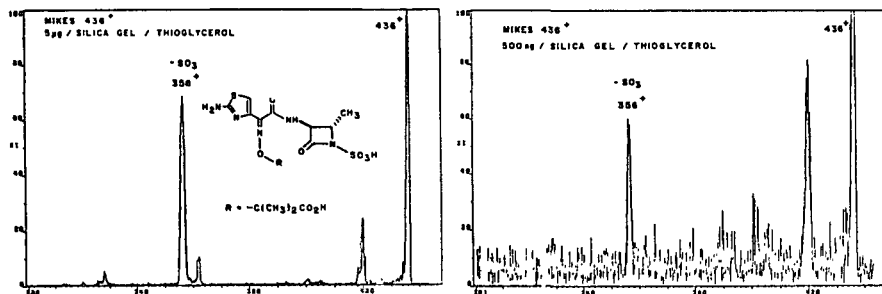


Figure 2

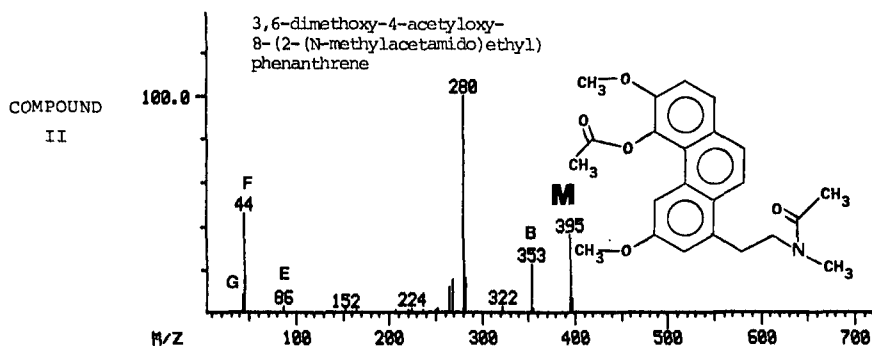
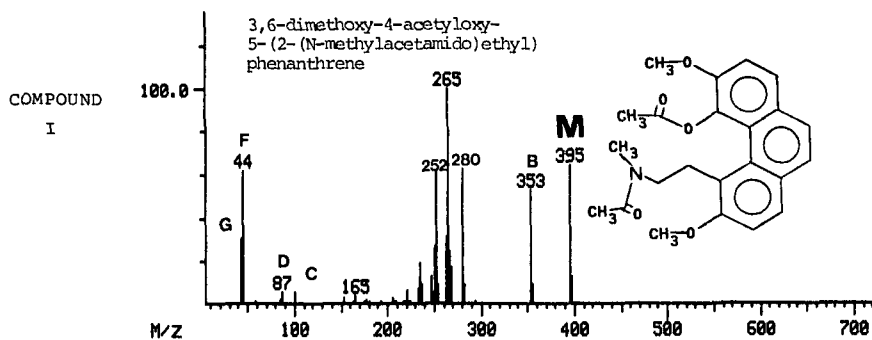
Figure 3

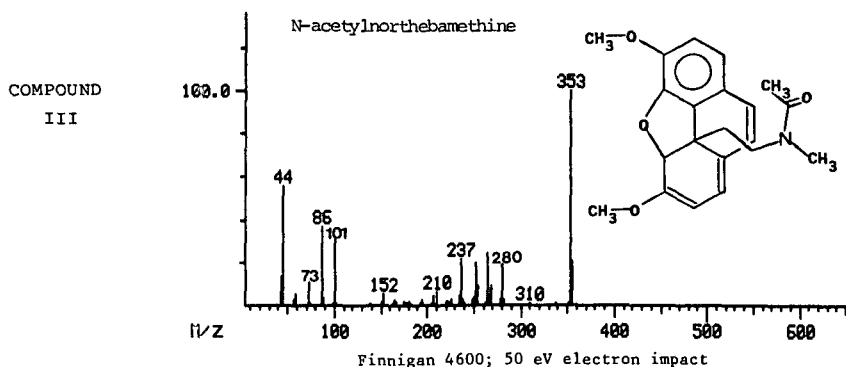


MASS SPECTRAL IDENTIFICATION OF NEUTRAL IMPURITIES FOUND IN HEROIN SAMPLES

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 Drug Enforcement Administration
 McLean, Va. 22102

Typical clandestine heroin samples contain a number of impurities which are unrelated to adulteration. These impurities are due to the presence of other opium alkaloids and synthetic by-products of the acetylation process. A considerable body of knowledge has been amassed concerning those impurities that are nitrogenous bases. However relatively little is known about those impurities that behave as neutrals. To our knowledge only five such compounds are presently known. Of these five, mass spectral data is available in the open literature for only two; that being for N,O⁶-triacetylnormorphine and meconin⁽²⁾. Along with morphine, the alkaloid thebaine is also found in opium and, therefore, invariably accompanies morphine through the acylation process which converts the morphine to heroin. It is the action of acetic anhydride upon thebaine, via a mechanism similar to that described by Bertgen et al.⁽³⁾, that results in the compounds reported here. Structural confirmations were obtained via accurate mass measurements (Kratos MS-30), C¹³ and H¹ NMR (Nicolet, 200 MHz), and synthesis.





Deutero-analogs were prepared by reacting thebaine with deutero-acetic anhydride. The mass spectra of the resulting deutero-analogs of I and II exhibited the mass shifts shown in Table I.

TABLE I

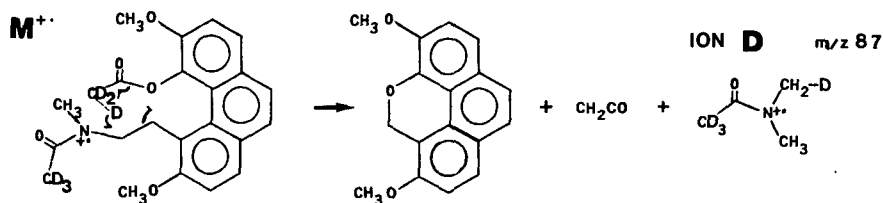
DEUTERATION RESULTS
for compounds I & II

ION	MASS*	EMPIRICAL COMPOSITION	DEUTERATION SHIFT*	
			I -compound-	II
M	395	C ₂₃ H ₂₅ N.O ₅	6	6
B	353	C ₂₁ H ₂₃ N.O ₄	4	4
C	100	C ₅ H ₁₀ N.O	3	---
D	87	C ₄ H ₉ N.O	4 (80%)	---
E	86	C ₄ H ₈ N.O	3	3
F	44	C ₂ H ₆ N	1	1
G	43	C ₂ H ₃ O	3	3

*nominal values in daltons

The deuteration shifts noted for ions M, B, C, E, and G are easily rationalized. However the shift observed for ion F is not quite so obvious and the shift noted for ion D (compound I) is of particular interest. A rationalization is available in the literature for ion F⁽⁴⁾ and scheme I below presents a mechanism for ion D.

SCHEME I



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SEX DETERMINATION FROM DRIED BLOODSTAINS USING GC/MS?

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A radioimmunoassay (RIA) technique was recently developed for the purpose of determining the sex of an individual by measuring the steroids testosterone, progesterone, and estradiol-17 β extracted from dried bloodstains (B. L. Brown, Journal of Forensic Sciences, Vol. 26, No. 4, Oct. 1981, pp 766-781). Although the method does allow sex determination from dried bloodstains, the necessary steroid measurements (involving sample extraction, a chromatographic cleanup/steroid separation step, and RIA quantitation of the steroids) are quite time consuming. Additionally, the RIA technique is best suited for analyzing a large number of specimens at one time. Thus, this method is not efficient in terms of time and reagents for the analysis of a single specimen. The purpose of the research project described in this report is to test the feasibility of determining the steroid concentrations by GC/MS.

Although it was found that standards of the free steroids could be separated and measured satisfactorily by splitless capillary column GC/MS, the sensitivity using either EI or CI selective ion monitoring was nowhere near that required to measure the steroids extracted from 0.1 ml dried bloodstains (about 2, 0.6, and 0.4 picograms for testosterone, estradiol-17 β , and progesterone, respectively). Reaction of the free steroids with heptafluorobutyric anhydride (HFBA) to form the HFB derivatives allowed the use of negative ion chemical ionization (NICI). Preliminary results show great promise for success using this technique. Although problems were encountered with background contamination in the GC/MS instrument, sufficient sensitivity appears to be available. In addition, a bloodstain extract appeared not to contain interfering materials.

THE CHARACTERIZATION OF SELECTED EXPLOSIVES BY COMBINED DIFFERENTIAL SCANNING CALORIMETRY/MASS SPECTROMETRY (DSC/MS), R. O. Yelton and D. M. Colman, Monsanto Research Corporation, Mound,* Miamisburg, Ohio 45342

Differential scanning calorimetry (DSC) is a widely used technique for the characterization of explosives and other energetic materials. Information relating to kinetics, sample purity and thermal stability is obtained by monitoring changes in heat flow as the sample is temperature programmed through decomposition. Gaseous by-products of decomposition are detected by the use of a thermal conductivity type effluent gas detector, however, the identity of these gases cannot be directly ascertained.

A system has been developed where in a DSC has been interfaced to a magnetic sector mass spectrometer in order that thermal data as well as mass spectrometric information on the gaseous decomposition products may be simultaneously obtained during the analysis of energetic materials. DSC/MS data have been obtained for a 2-[5-cyanotetrazolotopentamine] cobalt (III) perchlorate (CP), Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), 2-[3,5-dinitrophenyltetrazolotopentamine] cobalt (III) perchlorate (CP-DNP) and 2,4,6-trinitro-1,3-benzenediol, lead salt (lead styphnate).

INSTRUMENTAL

A Perkin Elmer DSC 1B was interfaced to the MS-25 mass spectrometer ion source via an all-glass, single-stage jet separator. Approximately 10 psi of helium was required to obtain a DSC purge flow of 30 cc/min. at the DSC outlet which necessitated the use of a 12" length of .010" I.D. x 1/16" O.D. tubing to serve as a pressure reduction device and was installed in series with the DSC and the mass spectrometer. Modification of the original DSC cell consisted of removing the glass windows and teflon purge plugs and TIG welding stainless steel closure disks in place to insure gas tightness and to minimize the potential explosion hazards. All samples were placed in open pans and were typically 1-2 milligrams in weight. The DSC was temperature programmed at a rate of 10° C/min., while the mass spectrometer was scanned repetitively at 10 s/decade in the electron impact mode. Thermograms were obtained directly from the DSC amplifier output, while changes in the M.S. total ion current were recorded via the spectrometer data system.

RESULTS AND DISCUSSION

The thermogram and total ion current profile obtained for CP is given in Figs. 1 and 2. CP exhibits a relatively good thermal stability to approx. 260°C at which point a step-wise decomposition is observed originating with the elimination of ammonia, followed by water and finally N₂, CO, CO₂ and NO as decomposition is accelerated. HMX, also considered to be a high temperature explosive, was found to be stable to approx. 250°C and results in the production of N₂, CO, CO₂, NO, H₂O, HCN and C₂N₂ gas as the decomposition exotherm proceeds.

CP-DNP, the dinitrophenyl analog of CP, also exhibits good thermal stability; however, mass spectrometric data have indicated a slow release of H₂O from room temperature to approx. 160°C. This is believed to be due to water of hygroscopicity rather than a water of hydration and exhibits similar behavior to silica gel (hygroscopic) when analyzed under similar conditions. The DSC/MS results obtained for lead styphnate (monohydrate) Figs. 3 and 4, show an endotherm from 145°C to 225°C and the corresponding mass spectrometric data verifies the loss of water of hydration during this event. The onset of decomposition of lead styphnate is observed beginning at approx. 260°C producing CO, CO₂ and N₂ as its gaseous by-products of decomposition. Thus, DSC/MS has been found to be a useful technique for the characterization of explosives and has provided a faster method of obtaining information pertaining to thermal stability and compatibility than by stand-alone analyses.

¹ House, Jr. J. E. and P. J. Zack (1977), J. Forensic Science, 22, 332.

* Mound is operated by Monsanto Research Corporation for the U. S. Department of Energy under Contract No. DE-AC04-76DP00053.

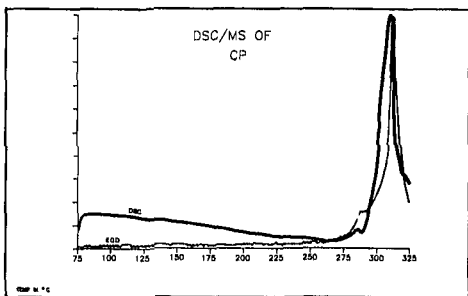


Figure 1. DSC Thermogram for CP

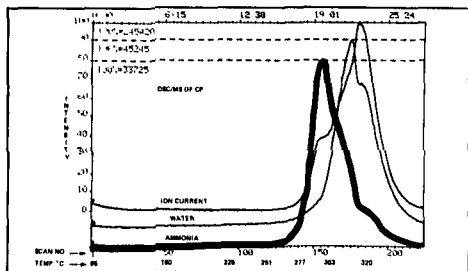


Figure 2. Total Ion Profile for CP

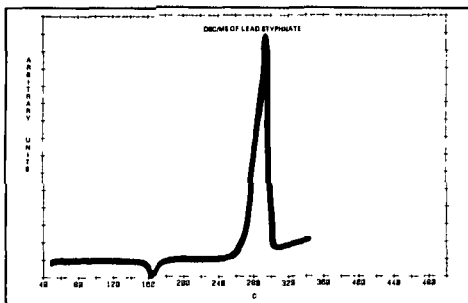


Figure 3. DSC Thermogram for Lead Styphnate

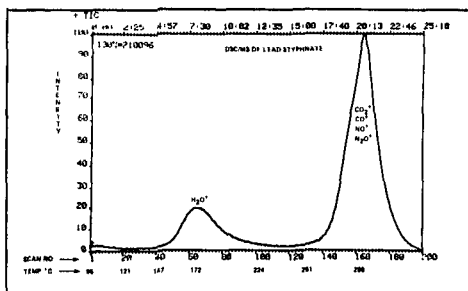


Figure 4. Total Ion Profile for Lead Styphnate

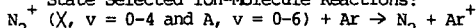
FPA 1 | METASTABLE DECAY OF Ar_2^+ ; K. Stephan, A. Stamatovic, T.D. Märk, Inst. f. Experimentalphysik, Leopold Franzens Universität Innsbruck, A 6020 Innsbruck, Austria

Of the rare gas dimer ions, only for He_2^+ metastable dissociations have been studied and compared with theoretical calculations. Recently, several studies have reported high quality ab initio CI calculations of Ar_2^+ including also spin-orbit coupling. The $I(1/2)_g$ state appears to have only a relative minimum and in addition seems to be crossed by the $I(3/2)_u$ state. This should give rise to the existence of metastable Ar_2^+ .

With the aim of obtaining more information on this property of Ar_2^+ , we have searched for a possible unimolecular dissociation process $\text{Ar}_2^+ \rightarrow \text{Ar}^+ + \text{Ar}$ using our molecular beam - electron impact ionization - double focussing mass spectrometer system. Employing the technique of decoupling the acceleration and analyzer fields we were able to confirm the existence of a metastable dissociation process with a measured metastable to stable ion ratio of $6 \cdot 10^{-4}$ in the time window between 3 and 8 μs . In addition collision induced dissociation with a cross section of $2 \cdot 10^{-16} \text{ cm}^2$ at 3 keV collision energy was observed. The occurrence of the metastable dissociation can be interpreted with help of the theoretical predictions of the potential energy curves as electronic predissociation and/or tunneling. Work supported by Österr. Forschungsfonds, S-18/05 and 08.

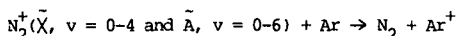
We have recently published (Can.J.Chem) an empirical scheme for calculating the heats of formation of homologous odd-electron organic cations. It reproduces very satisfactorily the thermodynamic stabilities of ions along eleven homologous series. Correction terms for chain branching, double-bond position and asymmetry effects are included. To extend the applicability of the scheme, particularly to even-electron ions, it is necessary to evaluate the effect on ionic stabilities of the substitution, singly and multiply, of functional groups such as CH_3 , OH , OCH_3 etc. at carbon atoms close to and remote from the principal locus of charge. Clearly, the substitution of a functional group near such a locus will alter the charge distribution; consequently the effect of multiple substitution will not follow simple additivity. When the point of substitution is sufficiently remote from the locus of charge, the effect thereof becomes equal to the normal substituent increment derived from additivity schemes for neutral molecules; that is, the ionization potential is not significantly altered by the substitution. The effects of functional groups on the rates of organic reactions have been extensively studied in solution; however the derivation of solely energetic parameters for functional group substitution in ions, derived from these rates, is obscured by the effects of solvation. Evaluation of the purely thermodynamic effects of functional groups from studies of gas-phase ion enthalpies is therefore being assiduously pursued in our laboratory. Results on multiple substitution effects, and relationships between the position of substitution and the distribution of charge in organic cations will be discussed.

State Selected Ion-Molecule Reactions:



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A time of flight mass spectrometer has been developed to study the effects of electronic and vibrational excitation on charge transfer reactions. Total absolute cross sections were measured for the reaction:



at 8, 14, 20 eV center of mass collision energies. The apparatus designed for the study of charge transfer reactions with state selected reactants is shown in Fig. 1. Pulsed (2ns wide at 13.5MHz) Synchrotron radiation from the storage ring was dispersed by a 1 meter normal incidence McPherson monochromator and focussed in the photoionization region I. The photoionization region was pressurized to approximately 10^{-4} torr. The photoelectrons were accelerated 0.7cm by a 2V/cm electric field and zero kinetic energy electrons were selected a combination of angular discrimination and electron time of flight. Thus, the corresponding photoion had an internal energy equal to the nominal photon energy. Upon detection of a threshold electron a 5μs wide, 12.8V/cm pulse was applied to the photonization region. The reactant ions were then accelerated and focussed on an effusive jet of Ar atoms oriented perpendicular to the ion beam. A 12V/cm electric field was applied to the reaction region II in order to extract the product Ar⁺. A sample time of flight spectrum is shown in Fig. 2. The peak at 10μs is the unreacted N₂⁺ and the peak at 14μs is due to the Ar⁺ product.

The derived cross sections for various N₂⁺ internal energies are shown in Fig. 3 for 8 eV center of mass translational energy. The fluorescence lifetime of the excited A state is between 10 and 15 μs. Hence some of the A state ions were converted to X state ions prior to the reactive collision, which occurred about 6μs after ion formation. The data of Fig. 3 were corrected for this fluorescence decay using the branching ratios of Maier and Holland.¹

The X v = 0 reactant state was found to be non reactive at all collision energies studied as also found by Kato et al.² No results for reactant N₂⁺ in the A electronic state have been published. Our results show a sharp drop in the cross section for N₂⁺(A) v = 4-6 at a relative translational energy of 8 eV. The reactivity of these vibrational levels increases substantially with increasing collision energy.

References

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*Department of Chemistry, University of North Carolina

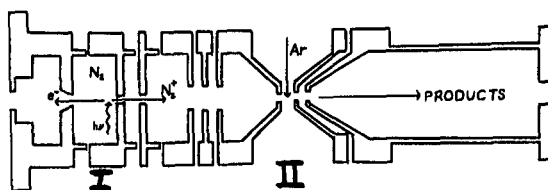


Figure 1.

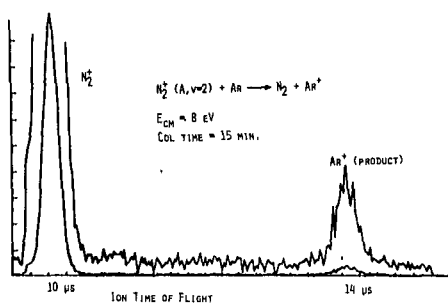


Figure 2.

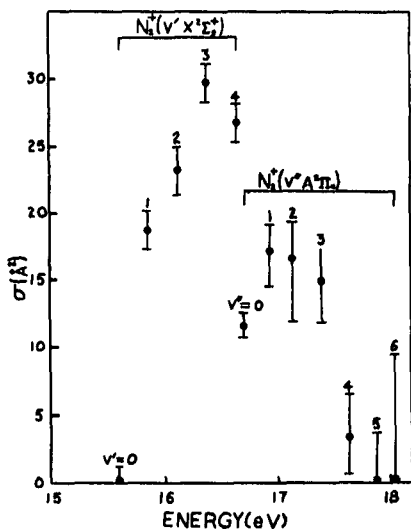


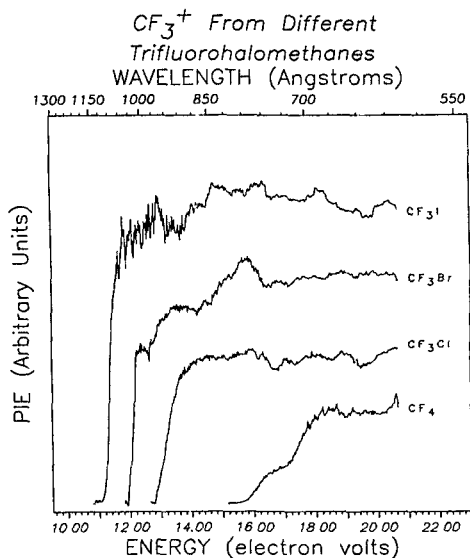
Figure 3.

PHOTOIONIZATION MASS SPECTROMETRIC STUDY OF THE TRIFLUOROHALOMETHANES
AND HEAT OF FORMATION OF THE TRIFLUOROMETHYL CATION (CF_3^+)*

Frank Cheng-Yu Wang and George E. Leroi

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A computer-controlled photoionization mass spectrometer¹ has been employed over the wavelength range 600-1200 Å (20.66-10.33 eV; conventional hydrogen and helium VUV sources) to measure photoionization efficiency (PIE) curves - corrected for detector background counts, stray light and sample pressure variations - of CF_3X (X=F, Cl, Br, I) parent and fragment ions. No detectable ion signal was found for CF_4^+ . However, values for the adiabatic ionization energies of the other parent ions were obtained from the inflection point of an Error Function - representing a Gaussian distribution of instrumental and equilibrium rotational contributions to the ion signal - fit to the threshold region of the appropriate PIE curve. The IP values in eV are: $\text{CF}_3\text{Cl}^+ = 12.60$, $\text{CF}_3\text{Br}^+ = 11.76$; $\text{CF}_3\text{I}^+ = 10.38$, each ± 0.02 eV. PIE curves for fragment ions were smoothed, extrapolated to the onset of ionization, and corrected for thermal contributions to obtain estimates of the "vortical" appearance potentials (APs). The corresponding enthalpies of formation for all observed parent and fragment ions have been calculated.²



Representative fragment PIEs, for CF_3^+ formed from each neutral precursor, are displayed in the Figure at the left. The APs extracted from the experimental data may be too high because of instrumental effects, kinetic shifts, competition with other fragmentation channels, etc. Thus the calculated fragment ion heats of formation must be considered upper limits to the desired adiabatic values. Clearly, the "best" ΔH_F^0 value for a given fragment ion will be the lowest of those obtained from the precursors studied. Table I (next page) lists the observed APs for CF_3^+ from CF_3X . The results are compared to previous determinations and used to calculate the $\Delta H_{F0}^0(\text{CF}_3^+)$ values given in the last column. The lowest upper limit is provided by CF_3I , $\Delta H_{F0}^0(\text{CF}_3^+) \leq 91.26$ kcal/mole. Other values reported for the enthalpy of formation of CF_3^+ are compared with this result in Table II; the best value appears to be that derived

*Work supported in part by the
National Science Foundation

in this work from the photoionization measurements.

Table I. Appearance Potential and Heat of Formation of CF_3^+ from Photoionization of CF_3X (X=F, Cl, Br, I)

Parent Neutral	CF_3^+ Appearance Potential (eV)		$\Delta H_{\text{FO}}^{\circ}(\text{CF}_3^+)$ (kcal/mole)
	This Work	Previous Work [Ref.]	
CF_4	15.44 ± 0.04	15.52 [3]	116.08
CF_3Cl	12.70 ± 0.04	12.55 [4]	95.94
CF_3Br	11.88 ± 0.04	11.71 [3]	95.01
CF_3I	11.11 ± 0.04	11.36 [5]	91.26

Table II. Comparison of $\Delta H_{\text{FO}}^{\circ}(\text{CF}_3^+)$ Values (kcal/mole) Obtained from Diverse Sources

Ion	This Work	Ref. 6	Ref. 7	Ref. 8
CF_3^+	91.3 ± 1.7	93.8	96.17	99.8

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MULTIPHOTON IONIZATION OF AMMONIA AND ITS CLUSTERS

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The resonance enhanced multiphoton ionization (REMPI) spectra of ammonia and ammonia clusters were obtained using a molecular beam-mass spectrometer-laser system (see Fig. 1). Ammonia was expanded through a pulsed nozzle with and without inert carrier gases up to total pressures of approximately 4 atmospheres. The resulting cooled beam was skimmed and crossed downstream with a pulsed YAG laser. The products of the multiphoton ionization were detected using a quadrupole mass spectrometer in early experiments and, more recently, a time of flight mass spectrometer. A dye laser was used to scan the relevant wavelengths of interest and data were obtained at 0.2 Å step intervals, with the bandwidth being approximately 0.5 cm^{-1} .

Well resolved vibrational bands showing rotational structure, were obtained in the 304-340 nm region via a 2+1 MPI process. The majority of the observed bands in this region coincide with the B state assignments for the $v=0-6$ vibronic bands, in agreement with single photon and 3+1 MPI studies reported in the literature [1]. Bands to the blue of the B(4) band exhibit structure corresponding to the C' state previously seen via a 3+1 process [2,3] (see Fig. 2 and Table I).

REMPI of ammonia clusters was also obtained at 355 nm. Data were acquired for clusters up to the octamer, leading to the protonated species containing one less ammonia molecule. The results of a power study for the formation of the protonated clusters suggest that ionization is taking place through a two photon resonance at 56338 cm^{-1} . This energy corresponds to a vibrational level in the A state of the monomer such that 11 quanta are excited in the ν_2 vibrational mode (reported as 56297 cm^{-1} ; see reference 4). Despite the fact that the A state is predissociated, it is evident that a multiphoton ionization process can compete with the rate of predissociation.

Data were also obtained for clusters in the 400-410 nm range. Spectra obtained for the tetramer (detected as the protonated trimer) displayed a sharp peak at 4009.8 Å. These preliminary observations are explained by the possible occurrence of a two photon absorption to either a perturbed A or D state in the cluster, followed by subsequent absorption of a third photon which ionizes the tetramer and also causes fragmentation to the protonated trimer that is ultimately detected.

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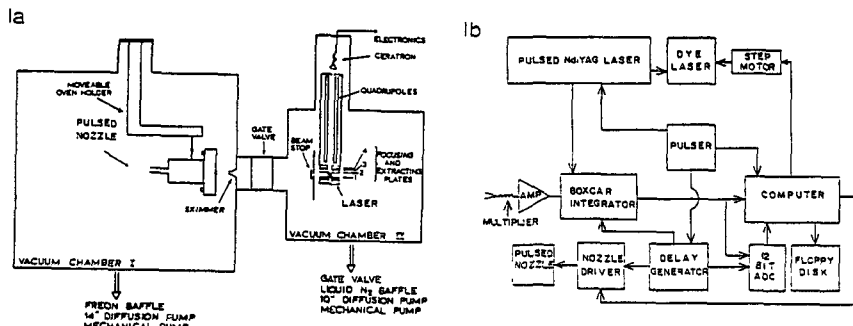


Figure 1

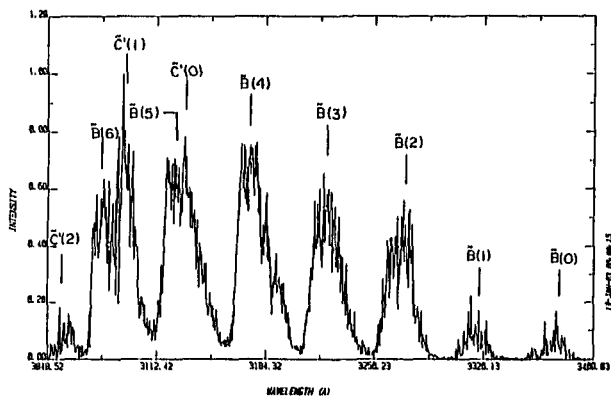


Figure 2

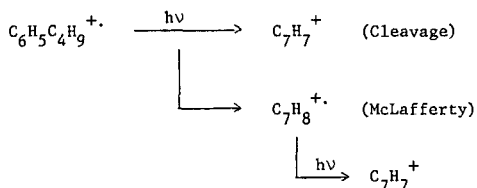
Table I
 $\tilde{C}'(2)$ Rotational Line Assignments

Feature	This work* (Å)	Glowia et al. ⁵
Q(1)	4571.9 ± 0.9	4571.5 ± 0.1
P(2)	4574.8 ± 0.9	4574.3 ± 0.1
P(3)	4575.9 ± 0.9	4575.7 ± 0.1
R(0)	4570.9 ± 0.9	4570.1 ± 0.1
R(1)	4569.0 ± 0.9	4568.7 ± 0.1
R(2)	4568.0 ± 0.9	4567.5 ± 0.1
R(3)	4566.9 ± 0.9	4566.2 ± 0.1

* The actual wavelengths have been multiplied by 1.5 to facilitate comparison with Glowia's work.

EPA 7 | FTMS STUDY OF N-BUTYLBENZENE ION PHOTO-
 DISSOCIATION:
 R. C. Dunbar, J. D. Hays and J.-H. Chen; *Case Western*
Reserve University, Cleveland, OH 44106

The direct cleavage and McLafferty rearrangement dissociation channels for n-butylbenzene ion have been compared quantitatively at several wavelengths by FTMS.



In contrast to ion beam results, only the low-energy McLafferty product is observed as a primary product at visible wavelengths, although further dissociation to C_7H_7^+ follows. At UV wavelengths, the direct-cleavage product is competitive. RRKM calculations are consistent with the results, but the possibility of direct dissociation from an excited electronic state will be discussed as a feasible alternative.

BIOCHEMICAL DIFFERENCES OBSERVED IN PYROLYSIS MASS SPECTRA OF RANGE GRASSES WITH DIFFERENT RESISTANCE TO ATTACK BY THE BLACK GRASS BUG. Willem Windig and Henk L.C. Meuzelaar, Biomaterials Profiling Center, University of Utah, Salt Lake City, Utah; B. Austin Haws, William F. Campbell and Kay H. Asay, Utah State University, Logan, Utah.

Susceptibility to insect attack plays an important part in breeding new species/hybrids of range grasses. Determining susceptibility to insect attack by measuring damage values, i.e., measuring the relative surface area of a leaf damaged by an insect under well defined conditions is time-consuming. From the literature it is known that chemical compounds may play a part in the susceptibility of plants to insect attack [1]. However, chemical characterization of plant materials is generally also time-consuming, mainly because of laborious sample pretreatment.

Since pyrolysis mass spectrometry has proven to be a fast, powerful method for chemical characterization of biological materials, which needs only minimal sample pretreatment, a feasibility study was undertaken on leaves of a range grasses with known differences in susceptibility to damage by the black grass bug, *Zabrotes hesperius* [2]. Susceptibility on the different species/hybrids studied was determined in parallel experiments by measuring damage values. Green as well as brown grass leaves of the species/hybrids listed in Table I were analyzed. Pyrolysis conditions were as follows: equilibrium temperature (Curie-point temperature of filament) 510°C, temperature rise time 5 s, total heating time 10 s. Mass spectrometric conditions were: electron energy (set value) 15 eV, mass range scanned: m/z 20-240, scanning speed 1000 amu/s, total scanning time 20 s.

The results on the brown leaves showed a better Fisher ratio (between-group variance/within-group variance ratio) than those on the green leaves, therefore, only the results on the brown leaves are shown. Typical Py-MS spectra are shown in Figure 1. Discriminant analysis

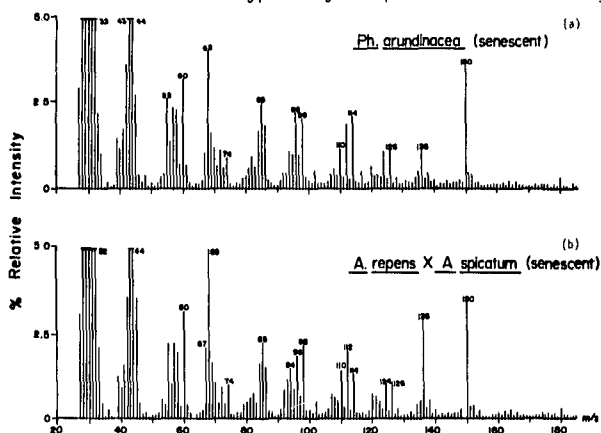


Figure 1. Typical pyrolysis mass spectra of the leaves of a grass with a low (a) and a high (b) susceptibility.

of the data set appears to reflect taxonomical relationships, as shown in Figure 2. The correlation between the pyrolysis data and susceptibility was studied by canonical variate analysis. As can be judged from Figure 3, there is a clear relationship between the two data sets. Spectral patterns correlated with the susceptibility values show a high similarity with an isoprenoid pattern for grass species/hybrids with a high damage value, and a hemi-cellulose pattern for grass species/hybrids with a low damage value. The marked isoprenoid pattern may be due to (poly)isoprenoid moieties derived from the phytol side chain of chlorophyll. This study shows that Py-MS is a possible tool for studying insect/plant relationships; taxonomical relationships are reflected and a correlation with susceptibility is found. Furthermore, the remarkably simple subpatterns obtained by multivariate data analysis yield valuable information on the chemical nature of the compositional differences in range grasses.

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TABLE I
SUSCEPTIBILITY OF GRASS SPECIES/HYBRIDS ANALYZED

Grass Species/Hybrids	Susceptibility
1. <i>Agropyron repens</i> (A.r.)	34
2. <i>A. repens</i> X <i>A. spicatum</i> (A.r. X A.s.)	35
3. <i>A. desertorum</i> (A.d.)	30
4. <i>A. repens</i> X <i>A. desertorum</i> (A.r. X A.d.)	41
5. <i>A. cristatum</i> (A.c.)	37
6. <i>A. repens</i> X <i>A. cristatum</i> (A.r. X A.c.)	15
7. <i>Phalaris arundinacea</i> (Ph.a.)	2
8. <i>Dactylus glomerata</i> (D.g.)	7

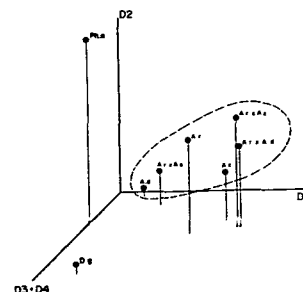


Figure 2. Plot of first four discriminant functions. Since reproducibility was very high, averaged values are used.

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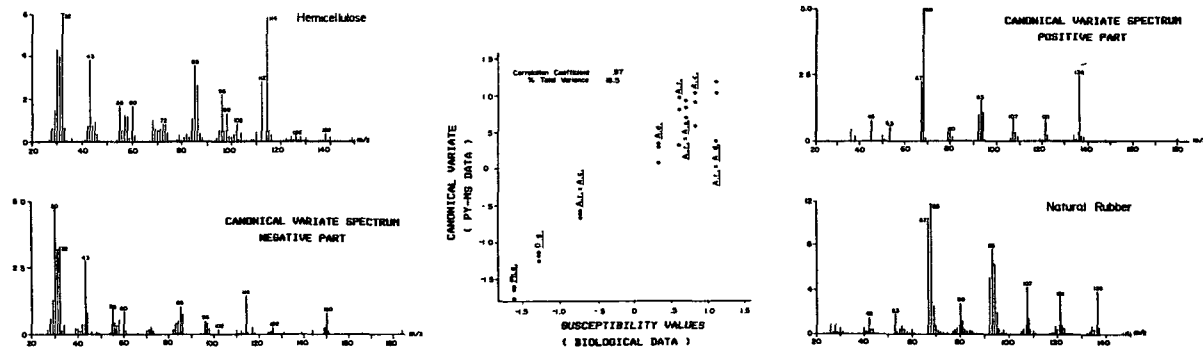


Figure 3. A high correlation between susceptibility values and Py-MS data is observed. The canonical variate spectrum shows a relatively high content of an isoprenoid pattern (compare with spectrum of natural rubber) in leaves with a high susceptibility. Grass leaves with a low susceptibility have a relatively high amount of a hemicellulose-like compound (compare with model spectrum of hemicellulose).

NEW APPROACHES TO CARBOHYDRATE SEQUENCING. Vernon N. Reinhold, Eric Coles, Christopher Coretsopoulos and Steven A. Carr, Dept. of Biological Chemistry & Lab. of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA. 02115.

This abstract summarizes a new procedure for oligosaccharide sequencing that combines HPLC purification, the sensitive techniques of fluorescence detection, and partial polymer degradation by reductive hydrosilylation with component determination by direct chemical ionization mass spectrometry (DCI-MS) (4,5). The procedure can be extended to provide linkage information using GC-MS following complete reductive cleavage. The methodology is demonstrated for the cyclic glucose heptamer, β -cyclodextrin.

We have modified the conditions of reductive hydrosilylation from earlier reports (1,2) to obtain partial depolymerization of carbohydrate polymers to a series of oligomers as outlined in Scheme 1. This procedure, which we refer to as partial reductive cleavage, produces three classes of smaller oligomers from the intact polysaccharide: terminal (non-reducing)-end, internal and reducing-end. Under the conditions chosen, glycosidic linkages which undergo cleavage yield an anhydro alditol on the terminal side of the oligomer chain and a free hydroxyl group on the reducing side.

In combination with this degradation, we have tried to amplify the sensitivity for detection of carbohydrate samples by HPLC, via the incorporation of fluorescent groups prior to chromatographic purification and isolation. Two derivatives have been employed for this purpose; the pyridinylamine (PA) group (3) which is introduced at the reducing end of the polysaccharide chains before permethylation and reductive cleavage, and the naphthoyl (Np) group which is incorporated on the hydroxyl groups generated as a result of the partial reductive cleavage. In addition to enhancing detector sensitivity, the PA moiety provides a functional group responsive to solvent acidity. This feature allows selective HPLC isolation of reducing-end fragments from the oligomeric mixtures generated as a consequence of partial reductive cleavage. Analysis of these PA- and Np-derivatized, partially reductively cleaved polysaccharides by DCI-MS (4) yields molecular weight and sequence information for each oligomer, while linkage data is obtained by complete reductive cleavage to monosaccharides followed by acetylation and structural determination of the acetylation position by electron ionization GC-MS (5).

Partial reductive cleavage of permethylated β -cyclodextrin followed by naphthoyl derivatization and HPLC yielded the chromatogram shown in Fig. 1. Seven cleanly resolved peaks were obtained which corresponded to the monomer through the open-chain heptamer. The cyclized unreacted starting material would not be detected under these conditions. Each of these peaks have been characterized by ammonia DCI-MS which provides both molecular weight and sequence information.

The extent of reductive cleavage may be followed in "real time" by analyzing the reaction mixture without chromatographic separation. Aliquots of the reaction are removed at various time intervals, acetylated and analyzed directly by ammonia DCI-MS. An example of this approach for the four-minute time interval for the partial reductively cleaved β -cyclodextrin is shown in Fig. 2. The ratio of molecular adduct ions (e.g., m/z 470, 674, 878, 1082, and 1446) reflects the distribution of oligomers (e.g., DP-2, 3, 4, 5, 6 and 7, respectively) with the last ion, [m/z 1446, (M+NH₄)⁺], being the unreacted cyclic heptamer starting material.

We have applied this new methodology to other known compounds with excellent results and are now cautiously evaluating these procedures for the structural determination of oligosaccharides in the urine of patients suffering from mannosidosis.

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PM-CYCLODEXTRIN

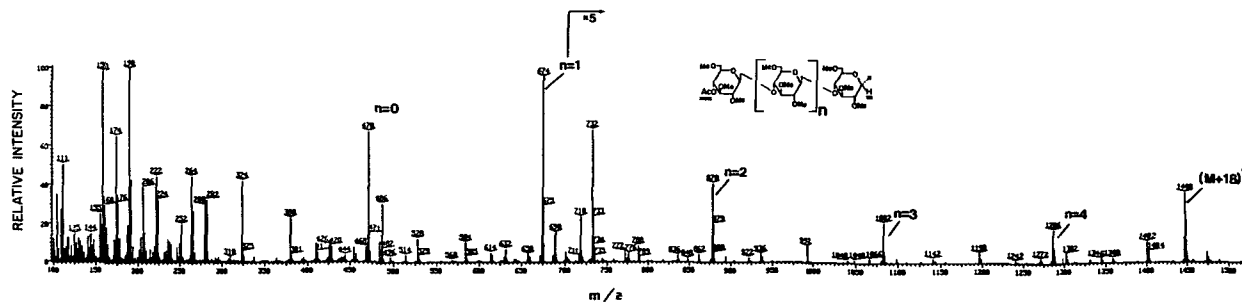
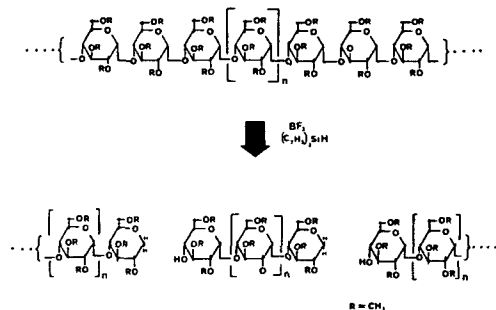


Figure 2. DCI-MS following reductive cleavage for 4 min at 0°C of PM-β-cyclodextrin. The products were acetylated prior to MS.

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Scheme 1. Generalized accounting of partial reductive cleavage on linear glucopolymers. The oligomeric series will consist of three different series (i.e., terminal, reducing and intermediate).

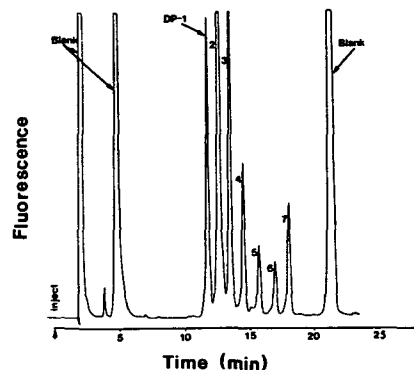


Figure 1. Mixture of naphthoylated products from partial reductive cleavage (4 minutes at 0°C) of permethylated β-cyclodextrin, approximately 0.01 μg total material. Column: C-6 Spherisorb, 25 X 4.6 mm (ID). Elution solvent: 50% to 60% acetonitrile in water (0.15M ammonium hydroxide), 1 ml/min.

Comparison of Ionisation Techniques for the Analysis of Saccharides and Natural Extracts
by Pyrolysis - Capillary GC/MS*

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Introduction

Analytical pyrolysis has become a powerful tool for studying complex and high molecular weight organic materials. These techniques show exceptional potential for differentiating between closely related organic material and in particular for rapid characterisation of saccharides. We report here results of preliminary studies into the characterisation of components in the pyrolysate of saccharides using three differing ionisation techniques, electron impact, positive (isobutane) and negative (methane/freon) chemical ionisation.

Experimental

A pyrolyser (Pyroprobe 100, Chemical Data Systems) was attached to the injector assembly of a gas chromatograph / mass spectrometer (Finnigan MAT 4500). The pyrolyser conditions were as follows: Interface (200°C); Final (750°C); Interval (10s.). The fused silica capillary column (J&W, DB-1701, 0.3mm x 30M, 1.0 μ thickness) was coupled to the injector with a 5/1 split ratio. The column outlet was led directly into the ion source region of the mass spectrometer. The temperature of the gas chromatograph was programmed from an indicated 40°C (5min.) to 240°C at 5°C/min. and then held (15min.). The mass spectrometer source temperature was 150°C and in chemical ionisation mode the source pressure was 0.7torr.

Results

The aim of these preliminary studies was to evaluate the three ionisation techniques for the characterisation of saccharide pyrolysates. A secondary objective was to evaluate the chromatograms of these differing techniques for enhanced specificity in fingerprinting differences between closely related compounds. This was feasible since the reproducibility of the pyrogram was excellent over extended periods of time.

The chromatograms when compared show major differences in selectivity and specificity which are dependent on ionisation. Unfortunately, these differences are not as great as those between individual saccharides using a single ionisation technique.

The spectra obtained were indicative of the ionisation technique (see Fig. 1). Spectra obtained by electron impact gave much structural information and evidence that many isomers were present, i.e. common ions differing only in abundance. Although this information was useful, no molecular weight information was obtained (Fig. 1A). Positive chemical ionisation (using isobutane) yielded abundant structurally significant ions as well as molecular weight information in the form of quasi-molecular ions (see Fig. 1B). This molecular weight

assignment was confirmed by the softer negative chemical ionisation technique (Cl^- attachment). Strong $(\text{M}+\text{Cl})^-$ ions were obtained with little or no fragmentation (Fig. 1C). Spectral evidence indicates that these isomers may be anhydrosugars formed by the dehydration of the parent sugars e.g. Anhydroglucose (M.W. 162).

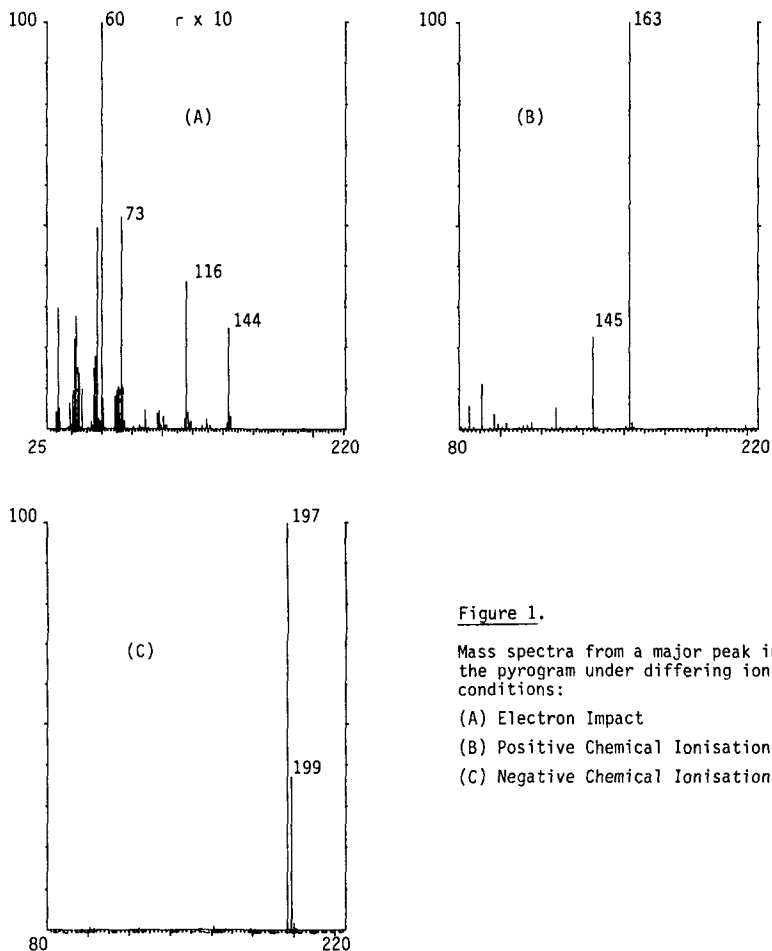


Figure 1.

Mass spectra from a major peak in the pyrogram under differing ionisation conditions:

- (A) Electron Impact
- (B) Positive Chemical Ionisation
- (C) Negative Chemical Ionisation

No one mass spectral method enables full characterisation. The preferred detection system would incorporate electron impact and chemical ionisation for maximum information. Pyrograms obtained by electron impact are more characteristic of the parent compound but spectra lack molecular weight information. Chemical ionisation gives molecular weight information but the pyrograms lack the differentiation produced by electron impact studies.

Identification of Antitumor Antibiotics in Fermentation Broths
by Fast Atom Bombardment - Mass Spectrometry

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Frederick, MD 21701

The structure of antitumor antibiotics such as toyocamycin in fermentation broths was determined by a combination of negative ion FAB-MS, high resolution exact mass measurements and mass-analyzed ion kinetic energy spectrometry (MIKES).

A. FAB-MS of Toyocamycin

The negative ion FAB mass spectrum of toyocamycin (Figure 1) yields three major ions at m/z 290 representing the deprotonated molecular ion, m/z 382 due to the glycerol adduct $(M+G-H)^-$ and the base peak at m/z 158 characteristic of the base moiety of the molecule generated by glycosidic cleavage. In some cases, the $(M+2G-H)^-$ at m/z 474 was also detected. By contrast, the $(M+H)^+$ observed in the positive ion FAB-MS constitutes the base peak and a fragment corresponding to the loss of 17 amu is clearly detected. Unimolecular MIKE spectra of the major ions in the negative ion FAB-MS of toyocamycin are shown in Figure 2. The most abundant daughter ions of $(M-H)^-$ were observed at m/z 158, 231 and 232. The loss of HCN from the base nucleus (m/z 158) is accompanied by an important kinetic energy release indicative of a rearrangement reaction. Most interesting is the MIKE spectrum of the glycerol adduct $(M+G-H)^-$ where daughter ions were obtained at m/z 318 (the metastable base peak), 322, 352 and 364 as well as m/z 250 and 290. This observation suggests that the interaction between the glycerol matrix molecule and the substrate within $(M+G-H)^-$ is more than a simple solvation. Collision-induced CAD-MIKE spectrum on m/z 382 indicates that the majority of the metastable ion current is now carried by the transition $(M+G-H)^- \rightarrow (M-H)^-$. High resolution mass measurements performed by peak matching on m/z 290 and 382 were, respectively, consistent with the deprotonated form of toyocamycin and the glycerol adduct.

B. Determination of toyocamycin in whole fermentation broth by FAB-MS

A study of the whole fermentation broth for the detection of toyocamycin showed that: 1) the minimum concentration of this antitumor antibiotic in the broth required to obtain measurable characteristic ions is 5 $\mu\text{g}/\mu\text{l}$ of whole broth; 2) this value drops to 500 ng/ μl after extraction of the fermentation broth with 1-butyl alcohol. Examination of the FAB relative responses for toyocamycin at various concentrations in whole broth suggested that the ion current generated from the former reached a limiting value at a concentration of 10 $\mu\text{g}/\mu\text{l}$. It is well known in FAB-MS that the presence of specific impurities can repress desorption of sample ions. The use of FAB-MS/MS in this case shows that this is not so here. Indeed the oscillographic out-put (Figure 3) displaying the molecular ion region of toyocamycin from an aliquot of whole broth during the preparation of toyocamycin emphasizes the problem of detecting trace amounts of the antibiotic. Figures 3b and 3c represent the unimolecular MIKE spectra obtained on m/z 290 from whole broth and the same mixture spiked with 5 μg of reference compound. Based on HPLC (Figure 4) the concentration of toyocamycin in this broth was 600 ng/ μl .

In conclusion, this study of the use of FAB-MS for the direct determination of toyocamycin in whole fermentation broth shows that the detection limit is over 5 $\mu\text{g}/\mu\text{l}$. This detection limit drops by one order of magnitude when the FAB-MS of a partially cleaned-up extract is made or if the specificity of MS/MS combined to FAB is being used directly on the whole broth. The latter data indicate that the fermentation matrix does not contribute to the total repression of sample ions and that FAB-MS/MS is adequate for the determination of toyocamycin in complex fermentation broths.

This work was supported by Contract No. N01-CO-23910, with the National Cancer Institute, NIH, Bethesda, MD 20205.

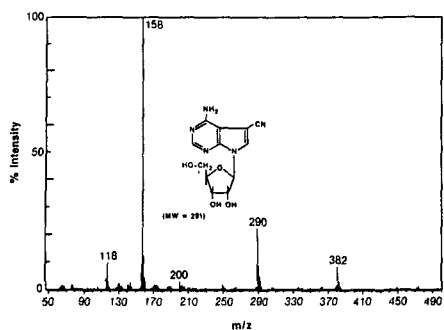


Figure 1: Negative ion FAB-MS of toyocamycin dissolved in a mixture glycerol - DMF (1-1).

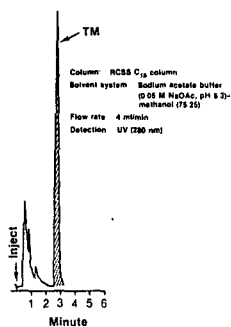


Figure 4: High-performance liquid chromatogram of toyocamycin fermentation broth.

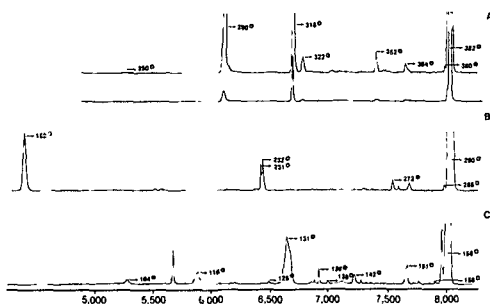


Figure 2: Unimolecular MIKE spectra of three major ions from the negative ion FAB-MS toyocamycin

- a) main beam m/z 392 ($(M + G-H)^-$)
- b) main beam m/z 290 ($(M-H)^-$)
- c) main beam m/z 158 ($(B)^-$)

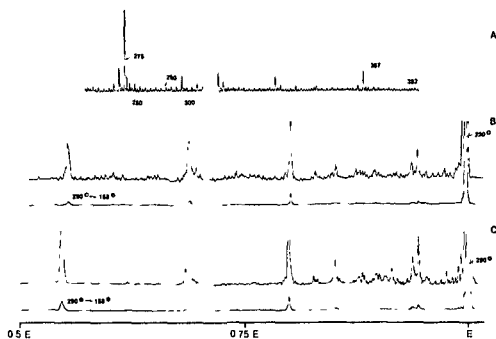


Figure 3: Negative ion FAB-MS of a whole broth mixture during the preparation of toyocamycin (MW = 291)

- a) Oscillographic out-put displaying the molecular region
- b) Unimolecular MIKE spectrum of m/z 290
- c) Unimolecular MIKE spectrum of m/z 290 from the same broth mixture spiked with 5 μ g of reference compound.

MASS SPECTROMETRY OF CYTOCHALASIN H AND ITS ACETYL AND DEACETYL ANALOGUES, D. F. Magin, R. H. Cox and W. N. Eino1f, Philip Morris Research Center, P. O. Box 26583, Richmond, VA 23261 and H. G. Cutler, USDA, SEA, Richard Russell Research Center, P. O. Box 5677, Athens, GA 30613.

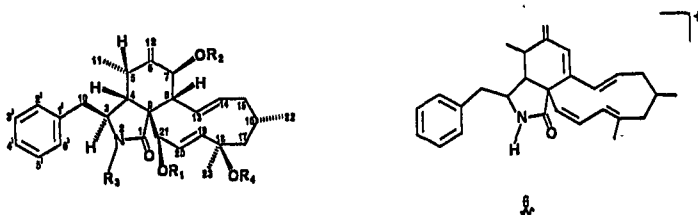
Cytochalasins are metabolites generated by a variety of molds found growing on cereal crops, and are important because of unusual biological effects such as inhibition of cell movement, inhibition of cytoplasmic cleavage, nuclear extrusion of cultured cells, toxicity to experimental animals and plant growth inhibitory activity (1).

A study was made of the electron ionization (EI) and chemical ionization (CI) mass spectra of cytochalasin H and some of its analogues for the purpose of characterizing these compounds through their mass spectra.

The CIMS experiments showed that fragmentations from the M+1 ion (either stepwise or concerted) could be followed for all of the cytochalasins to an ion at m/z 398 (6), believed to be the "backbone" (with loss of all R-groups) protonated at the nitrogen atom. In addition, the most intense peak in the CI mass spectra in the mass range 398 to M+1 results from the loss of one neutral molecule (either water or acetic acid) from the M+1 ion. Loss of a neutral molecule of 42 u was believed to be the loss of ketene from the acyl group on the nitrogen atom of compounds 4 and 5. Ions can be observed representing loss of each hydroxyl (R=H), acetyl (R₁, R₂, R₄ = COCH₃) and acyl (R₃ = COCH₃) group from the M+1 ion. Peaks can also be seen representing losses of water, acetic acid, and ketene from the M+18 (M + NH₄) reactant ion during CIMS (NH₃).

During EIMS, losses can be traced to a peak at m/z 306, which is the "backbone" mentioned above, minus the benzyl fragment. An ion at m/z 91 (benzyl) appears in all of the EI spectra. Prominent in the EI spectra are losses attributable to hydrogen atoms, water, acetic acid, radicals such as hydroxyl, acetyl and benzyl, and ketene (for 4 and 5).

(1) Cox, R. H.; Cutler, H. G.; Hurd, R. E.; Cole, R. J.; *J. Agric. Food Chem.* **31** (1983) 405



STRUCTURES OF CYTOCHALASIN H COMPOUNDS

- | | |
|---------------------------|---|
| 1 DEACETYLCYTOCHALASIN H | R ₁ = R ₂ = R ₃ = R ₄ = H |
| 2 CYTOCHALASIN H | R ₁ = COCH ₃ ; R ₂ = R ₃ = R ₄ = H |
| 3 ACETYLCYTOCHALASIN H | R ₁ = R ₂ = COCH ₃ ; R ₃ = R ₄ = H |
| 4 DIACETYLCYTOCHALASIN H | R ₁ = R ₂ = R ₃ = COCH ₃ ; R ₄ = H |
| 5 TRIACETYLCYTOCHALASIN H | R ₁ = R ₂ = R ₃ = R ₄ = COCH ₃ |

ANALYSIS OF COAL TAR AND PETROLEUM PITCHES WITH COMPUTERIZED PATTERN RECOGNITION STUDY

James G. Moncur
Wanda G. Bradshaw

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SUMMARY

Pyrolysis gas chromatography (P/GC) has been successful in differentiating pitches which are used in the manufacture of high temperature graphite materials (Ref. 1, 2). In the present study, coal tar and petroleum pitches received from five suppliers were readily differentiated using three distinct methods of polymer analysis: dynamic headspace and pyrolysis gas chromatography mass spectrometry (DH/GC/MS and P/GC/MS, Ref. 3), and pyrolysis mass spectrometry (P/MS, Ref. 4, 5). All of these methods are appropriate for the analysis and pattern recognition study of coal and petroleum products; however, methods which incorporate GC usually necessitate long analysis times and are subject to problems associated with gas chromatographic reproducibility (e.g., active sites (Ref. 6), and non-constant retention times). As a fingerprint analysis method, P/MS is rapid and ideally suited to computerized pattern recognition study of pitches due to the simple format of P/MS data (computer tabulation of normalized intensities versus masses); whereas, in the present study, the normalized peak area versus retention time data from DH/GC/MS and P/GC/MS analyses were not suitable for direct input to a computerized pattern recognition analysis program, (due to non-constant retention times and improper data format). However, P/MS did not provide positive identification of thermally evolved products (as can DH/GC/MS and P/GC/MS) due to the complexity of the mixtures of polynuclear aromatic hydrocarbons found by P/MS. Within the limits of the commercial instrumentation and computer programs used in this study, a combination of P/MS, DH/GC/MS, and P/GC/MS provided the greatest capability for the differentiation of the five coal tar and petroleum based pitches studied.

P/MS PATTERN RECOGNITION STUDY

The pattern recognition study of the P/MS spectra pitch samples was begun by first constructing a library entry (tabulated normalized intensities versus masses) for each of the five pitches. This procedure is a standard data processing algorithm present in the GC/MS data system (Ref. 7), and results in a five component P/MS library.

To test the "LIBRARY" pattern recognition program, a search of each P/MS spectrum and replicate spectrum was made to investigate the correlation between each spectrum and the five component library. In all cases examined, this search produced correct assignments for determining the identity of each of the five pitches. The results of this pattern recognition study are given in Table 1 with the similarity between P/MS sample spectra and library entry being given as a "FIT" value between 0 and 1000; where 1000 is a perfect correlation of all masses and their normalized intensities, and 0 is no similarity. Replicate analyses are given for the *samples.

Table 1

Sample	Best Match When Searching the Five Component Library	"FIT" of Best Match	Correctness of Best Match	"FIT" of 2nd Best Match
Code D*	Code D	1000	Correct	906
Code D*	Code D	996	Correct	906
A-240*	A-240	1000	Correct	751
A-240*	A-240	992	Correct	757
USS-15	USS-15	1000	Correct	974
15 VAC	15 VAC	1000	Correct	984
K-18	K-18	1000	Correct	955

Code D: Union Carbide petroleum pitch

A-240: Ashland petroleum pitch

USS15: United States Steel coal tar pitch

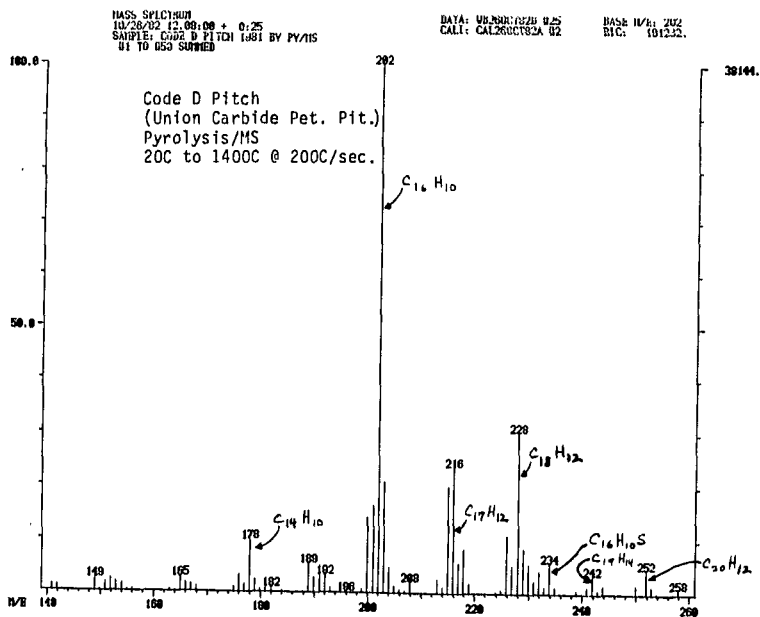
15 VAC: Allied coal tar pitch

K-18: Koppers coal tar pitch

The "FIT" value of the best matches was in all cases 992 to 1000 (out of 1000), and can be compared to the "FIT" value of the second best matches which ranged from 750 to 984. Apparently, the "LIBRARY" algorithm is capable of differentiating the five pitch samples based on differences in P/MS spectra, where a "FIT" value of 990 or higher indicated success in identifying the correct type of pitch. Replicate analyses of Code D and A-240 indicate that this differentiation is very reproducible.

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MASS SPECTRA OF STEAM-VOLATILE COMPOUNDS FROM THREE
SPECIES OF *SIPARUNA* FROM PANAMA,

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Siparuna (Monimiaceae) is a genus of small trees or shrubs that occur only in the neotropics. It ranges from Veracruz, Mexico south through tropical South America. Morphologically, the plant is considered primitive and believed to be a link between the Laurales and the Magnoliales. It is characterized by its opposite decussate leaves, unisexual flowers, and globose fruits born in the axils of the leaves or on stems. Foliage of *Siparuna* has pungent, aromatic odors reminiscent to that of species *Citrus*, particularly of lemon. In fact, one of the early names applied to the genus was *Citrosma*. Braz et al. isolated and identified sitosterol, stigmasterol, lirodenine, cassamedine (both aporphine alkaloids), and fuseine (an oxoaporphine alkaloid) from *Siparuna guianensis*; this represents the only publication found on the chemical composition.

The family Monimiaceae and specifically *Siparuna* species have a long history of use as medicinal plants in Central and South America. Antonio while living in Panama collected these plants and made a few observations on the uses as folk-medicines. *Siparuna* is used as a stimulant and carminative in Brazil, Indians in Panama drink a tea made from the leaves as a remedy for colds and snakebites, in Guatemala the Indians place the fresh leaves on the forehead to relieve headache, and the sap that exudes from leaves is placed on hot coals and applied to cuts.

The steam distilled extracts from *S. guianensis* were analyzed by gas chromatography with a Hewlett Packard series 5880A gas chromatograph equipped with a 50 m x .32 mm OV-1 fused silica column. The oven temperature was programmed from 70 to 225°C at 2° per min. The helium flow rate was 1.8 ml/min. GC/MS analysis was subsequently carried out on a Kratos MS 50 mass spectrometer equipped with a Varian Model 3700 gas chromatograph containing an OV-1 fused silica column as described above. The data were acquired and analyzed using a Kratos Model DS-55 data system. Identifications were based on the comparison of known and unknown mass spectra and were confirmed whenever possible by I_R values, that is, the retention indices relative to a series of ethyl esters of normal alkanolic acids.

The GC/MS/DA profile of the essential oil from the leaves of *Siparuna guianensis* with identified compounds as indicated is shown in Fig. 1. Of the approximately 110 compounds in the essential oil that were reported, 30 were identified. The major constituent is curzerenone (26%) and curzerenone types (42%), which together made up approximately 68% of the total. Curzerenone is a sesquiterpenoid originally isolated from *Curcuma zedoaria* (Zingiberaceae), a plant which is used to make the drug zedoary from India. Curzerene, which was found to comprise 0.36% of the oil, is probably the precursor to curzerenone and other isomers; if this is so, the plant is very efficient in carrying out the enzymatic oxidation. The next major constituent is myristicin or an isomer (8%). Myristicin is the most abundant component of the heavy oil of nutmeg, *Myristica fragrans*, and occurs in a number of other essential oils, such as that of parsley. This is the first report of curzerenone, curzerene, and myristicin in any member of the Monimiaceae. The only other compound in *Siparuna* that occurs in an amount above 1% is methyleugenol (2.8%). This compound, an insect attractant, is found in many natural sources. The mass spectral fragmentation patterns and the suggested fragmentation pattern for curzerene and curzerenone are shown in Figs. 2 and 3. The essential oil obtained from *S. domatita* and *S. griseoflavescens* proved to be a mixture of 70-80 compounds but the chief constituents did not prove very novel from the mass spectrometric viewpoint: β -citronellol, α -copaene, longifolene, farnesene, and naphthalene. These different compounds present in the three species of *Siparuna* pose both biosynthetic and chemotaxonomic challenges.

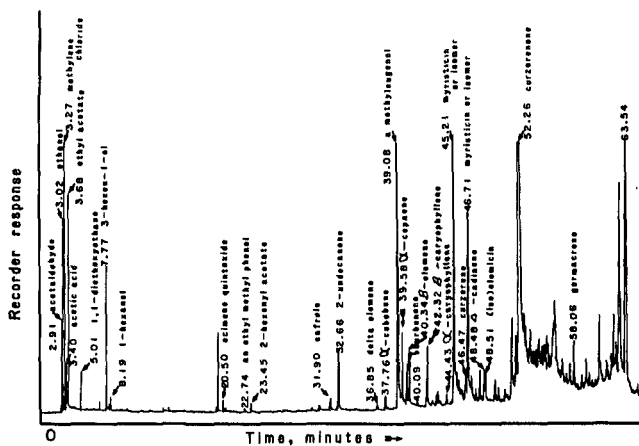


Figure 1 GC/MS/DA Profile of Essential Oil of Leaves from *S. guianensis* (Kratos)

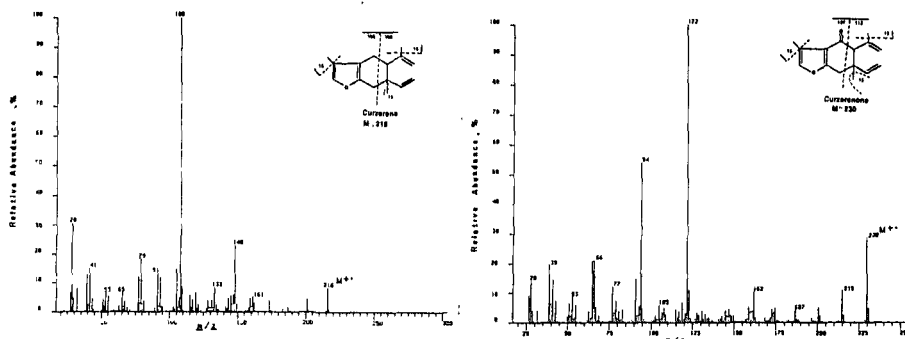


Figure 2 Mass spectrum and fragmentation pattern of CURZERENE

Figure 3 Mass spectrum and fragmentation patterns of CURZERENONE

FPA 14. QUANTITATIVE DETERMINATIONS IN MIXTURES FROM TOTAL ION-CURRENT MEASUREMENTS. Judson, C.M., Mass Spectrometry Laboratory, University of Kansas, Lawrence, KS., 66045.

Work by Ootvos and Stevenson (J.A.C.S. 78, 546, 1956) reported that molecular cross-sections (total-ions per molecule) can be calculated approximately as the sum of atomic cross-sections, and furthermore reported values for atomic cross-sections. Measurements based on the additivity of atomic cross-sections have been routinely used in hydrocarbon analysis, but have not much been applied to other types of compound.

Although this was not directly pointed out by Ootvos and Stevenson, the calculated cross-sections for compounds with many carbon atoms, and only a few hetero-atoms, are not only additive functions of atomic cross-sections, but are approximately proportional to molecular weights.

The equations resulting from the observations of Ootvos and Stevenson are:

- (1) $TI/m = \text{const} \times Q$, where TI = total ions, m = number of molecules, and Q = cross-section;
 - (2) $TI/(g/M) = \text{const} \times Q$, where g = grams and M = molecular weight;
 - (3) $g = \text{const} \times TI/(Q/M)$;
- and with the approximation $Q/M = \text{constant}$;
- (4) $g = \text{const} \times TI$.

In our laboratory, we frequently encounter mixtures of compounds for which we have no standard samples of pure materials, and we use integrated total-ion intensities from direct-probe or GC introductions to estimate relative quantities. We have not known very much about the accuracy of these measurements, or about the extent to which it is practical to make correct to improve the accuracy of these measurements. From the work of Ootvos and Stevenson we conclude that we ought to be able to make reasonably accurate measurements of quantities from total ion-currents (the sum of the individual ion-currents), if we can accurately measure total ion-currents, and can accurately predict molecular cross sections.

In detail, we can accurately calculate relative concentrations from relative total ion-currents, provided that:

- (1) Spectra are obtained for constituents that have been separated, or at least partly separated, e.g. by gas chromatography or by probe distillation;
- (2) Empirical formulas, from mass spectra or from other sources, are known;
- (3) Molecular cross-sections can be calculated with sufficient accuracy from atomic cross-sections and empirical formulas;
- (4) Integrated total-ion intensities can be obtained, directly or indirectly for each of the constituents;
- (5) These total-ion intensities are free from (or corrected for) errors due to mass discrimination in the ion-source, analyser or detection system;
- (6) The ion-source is operated in the E.I. mode, under conditions that provide a linear response of ion intensity to partial pressure; and
- (7) Correction is made for any molecular discrimination due to the conditions of flow of gas in and out of the source.

The experiments proposed to test the use of this procedure for quantitative measurements of samples for which we have no standard samples were:

- (1) To evaluate the validity of Ootvos and Stevenson's relationship for additivity of atomic cross-sections for "real-life" organic compounds as encountered in our laboratory, and, if necessary, to define more suitable values for atomic cross-sections.

- (2) To obtain a spectrum of a standard compound, under conditions having minimal mass-discrimination, so that comparison with the spectrum of this standard compound can be used to correct spectra used in any mass spectrometer for errors due to mass discrimination.

An attempt has been made to perform these experiments, evaluating the additivity of atomic cross-sections, and evaluating the mass discrimination of our MAT CH-5 mass spectrometer. These measurements were made using septum introduction of liquid samples to a reservoir inlet, with conditions which we expected would have minimal mass discrimination.

Gross variations (of the order of a factor of two) were observed in the ratio between the measured relative cross-sections and the relative cross-sections calculated from atomic cross-sections. These variations were observed both for total-ion intensities measured at the exit of the source slit, and

and for the sum of the mass-analysed individual ion-currents, corrected for mass discrimination of the multiplier. The compounds measured were compounds such as hexene, carbon tetrachloride, ethyl acetate, bromonaphthalene, and other compounds for which large deviations from the calculated cross-sections would not be expected.

The conditions used to minimize mass discrimination were:

- (1) A magnetic mass spectrometer was used with magnetic scanning;
- (2) The mass peaks had flat tops over the full mass range, obtained using a wide source-slit, and controlling resolution with the collector slit;
- (3) Scan-rate and bandwidth were selected to avoid peak-clipping;
- (4) Low emission current (30 uA) was used;
- (5) Absence of variation in peak ratios with partial pressure indicated linearity of ion current with partial pressure; and
- (6) Intensities were measured with a Faraday cup, or corrected for mass discrimination of the multiplier.

Although the effect of varying repeller voltage was not extensively evaluated, it did not appear that any repeller voltage could be found for which deviations from theory in the cross section measurements could be avoided.

Conclusions.

The measurements made were not sufficiently free of mass discrimination to test the additivity of atomic cross-sections.

Measurements to test the additivity of cross-sections should be made by collecting total ion-current at the repeller with the repeller made negative with respect to the ionization box, rather than from the sum of individual ion-currents, in order to ensure absence of mass discrimination errors.

A standard spectrum, to be used for correction of mass discrimination errors in routine measurements is probably best obtained by determining the mass discrimination in an instrument over a range of masses, rather than by attempting to provide conditions that completely eliminate discrimination.

Discrimination in the multiplier is easily measured for selected masses, by comparing Faraday cup currents with multiplier output currents. Discrimination in the ion-source and in the mass analyser (taken together) can be determined by comparing measured ion currents with known cross-sections for the inert gases, with extension to higher masses by measurement of ion current for individual ions and of total ion-current at the repeller for molecules of higher mass that have large molecular ions, successively bootstrapping the correction factors.

Modification of the CH-5 mass spectrometer to permit collection of total-ion current at the collector will be made, which will permit us to make the proposed test of additivity of cross-section, and the proposed measurement of discrimination corrections, and then to obtain a discrimination-corrected spectrum for a standard reference compound.

GC/MS Accuracy Criteria
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At last year's ASMS meeting, the Quality Control Protocol for the fused Silica Capillary Column (FSCC) GC/MS Determination of Semivolatile Priority Pollutants was presented (1). This protocol was discussed at the symposium on Quantitative Mass Spectrometry, and what was reported as "lively discussion" ensued (2).

This protocol is currently employed in U.S. EPA programs which routinely utilize FSCC GC/MS in the characterization of hazardous waste sites throughout the U.S. The protocol requires, among other things, that the proportionality between ion current and pressure (or injected weight) be demonstrated, and that response factor (RF) values for a minimum of five labelled, unlabelled internal standard, analyte pairs have a value of 1.00 ± 0.20 with RSD's of 10 percent or less. Interlaboratory RF data acquired within the domain of this protocol is presented which demonstrates that mean RF values for many organic compounds determined in this fashion at 70 eV on quadrupole mass spectrometers can be, on average, independent of scan or cycle time, sample volatilization (split/splitless vs. on column) and ion optics (3). Interlaboratory RF values are shown to be well correlated with the fractional ion abundance of the quantitation m/z values for many organic compounds, thereby proving that this spectroscopic parameter is the principal determinant of the RF value. These observations suggest that the establishment of a correct set of RF values is practical.

In studying multi-analyte, interlaboratory GC/MS data, it has become apparent that the establishment of a true set of RF values (i.e., accuracy criteria) is needed. It can be shown that a single RF value encodes both the qualitative and quantitative components of the GC/MS measurement process. A series of RF values, therefore, provides a concise code of the complete standardization process for all analytes of interest.

To demonstrate the power of RF value monitoring, a set of interlaboratory RF data is presented. The ability to quickly determine errors related to analytical standards, data processing/transcription and instrumentation is demonstrated. RF values and related indicators of data quality are shown to be capable of segregating spectroscopic and nonspectroscopic (i.e., chromatographic) errors.

The establishment of a set of true RF values for organic compounds amenable to FSCC GC/MS would greatly aid quantitative GC/MS studies for technical and practical considerations. It is suggested, therefore, that the ASMS (via a Committee of Quantitative GC/MS) formally consider the establishment of GC/MS accuracy criteria via the response factor.

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FPA 18 A COMPARATIVE EVALUATION OF THE EFFECT OF ION SATURATION AND CALIBRATION RANGE LIMITS ON QUANTITATION FOR CAPILLARY AND PACKED COLUMN GC/MS ANALYSIS; A. E. Rosecrance and J. J. Zoldak, Mead CompuChem, Cary, Illinois, 60013.

Quantitative methods for GC/MS analysis require the use of reference standards for calibration. Calibration is generally accomplished by analyzing standards at varying concentration levels. A calibration curve is established from standard response values using external or internal standard techniques. Quantitation of unknowns is performed within the concentration range of the standards where the accuracy of quantitation depends on the degree of variation in the calibration curve. Two parameters that will effect quantitation are saturation of the quantitation ion and areas or response factors exceeding the calibration range limit. This presentation will provide a comparative evaluation of the effect of ion saturation and responses exceeding the calibration range limit on quantitation for capillary and packed column GC/MS analyses.

FPA 19 | A SUCCESSFUL APPLICATION OF QUALITY ASSURANCE PRACTICES TO A GC/MS ANALYSIS TASK; B. N. Colby and P. W. Ryan; S-CUBED, Box 1620, La Jolla, CA 92038

A series of quality assurance activities was applied during a four month study involving quantitative isotope dilution GC/MS determinations of 18 pollutants spiked into an activated sludge pilot plant waste water treatment system. These activities included initial establishment of a series of measurement quality goals, followed by evaluation of performance with respect to those goals. This presentation will address how goals were established in order to test data quality in a useful and cost effective fashion and how a completely unanticipated problem was identified and corrected.

COMPARISON OF GC/MS AND LC/MS FOR ROUTINE ANALYSIS OF ETORPHINE IN EQUINE URINE, Thomas Covey, Elizabeth A. Dewey, and George Maylin, New York State College of Veterinary Medicine, 925 Warren Drive, Ithaca, NY 14850

Etorphine (Fig. 1) is a narcotic analgesic structurally related to morphine. It is one of the most pharmacologically potent opiate-like alkaloids. The substance has high abuse potential in both man and the equine racing industry. GC/MS and LC/MS procedures were compared with regard to their sensitivity, specificity, and ease of sample preparation for the determination of etorphine in equine urine. Diprenorphine (Fig. 1) was included in the experiment as an antagonist to the biological effects of etorphine.

EXPERIMENTAL

Sample preparation. Ten ml of urine from a horse injected 2 hours prior with .5 mg etorphine and 1 mg diprenorphine was adjusted to pH 9 with 1M NH₄OH and extracted with n-butylchloride following hydrolysis of the glucuronide with β -glucuronidase. The concentrated extract was subjected to thin layer chromatography (silica gel G, CHCl₃/MeOH/propionic acid - 72/18/10) and the R_f region corresponding to standard was scraped, eluted, and dissolved in 50 μ L EtOAc.

GC/MS. A Hewlett-Packard Model 5992A was used for the GC/MS analysis. An aliquot of 1.25 μ L of the sample was silylated on column with BSTFA to produce the monotrimethylsilyl derivative. A 1 meter 3% OV-101 column was used temperature programmed for 200-260°C at 16°C/min. The total ion current trace is shown in Fig. 2. EI Mass spectra of approximately 25 ng etorphine-TMS was obtained in the 2 hour extract free from interference representing a level of 125 ng/mL urine (Fig. 3). The M⁺ of the derivative (483) appeared at 30% of the base peak.

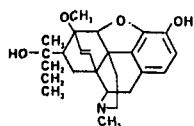
LC/MS. A Hewlett-Packard Model 5985B mass spectrometer was used for this work. A 25 cm Whatman ODS-3 microbore column was used to perform the separation isocratically with MeOH/H₂O (84/16) at 40 μ L/min with a Waters Model 6000A HPLC modified to deliver micro flow rates. The MS interface consisted of a direct liquid introduction probe constructed in the lab (1). The mass spectrometer was run in the chemical ionization mode with the LC solvent used as the reagent gas. The drugs were observed in a 5 μ L injection of the 2 hr. urine extract at approximately the 100 ng level. Figure 4 shows the total ion and selected ion current traces. The (M+H)⁺ appeared at 10% of the base peak, the base peak being the loss of H₂O (Fig. 5).

CONCLUSION

Determination of etorphine in equine urine is best done on a routine basis by GC/MS. Analysis times are shorter (4 min. vs 10 min.) with enhanced sensitivity. The DLI LC/MS procedure has the advantage of being able to analyze the sample without prior derivatization but the longer analysis times, decreased sensitivity and more involved instrumentation negate this advantage for routine work. Improved sample clean up and chromatographic conditions would help overcome these disadvantages.

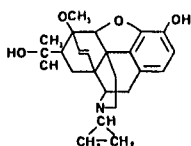
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ETORPHINE

M.W. = 411.52



DIPRENORPHINE

M.W. = 425.57

Figure 1

ETORPHINE EI SPECTRUM

+ TMS

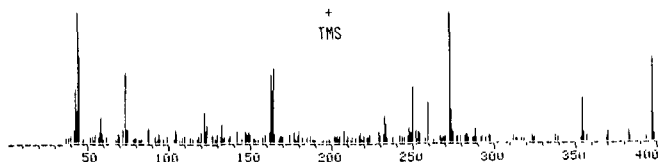


Figure 2

Figure 3

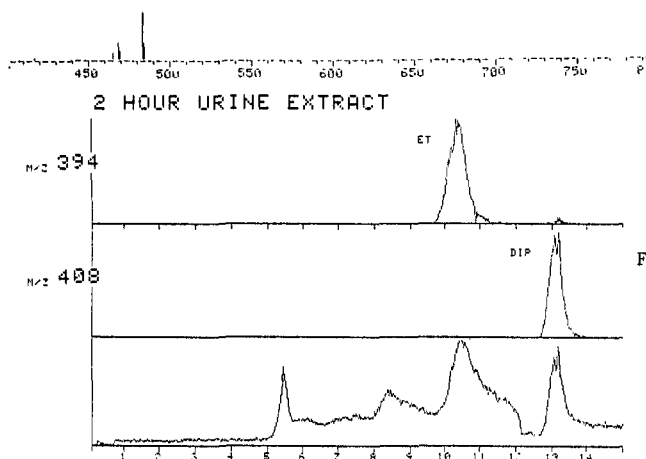


Figure 4

ETORPHINE CI SPECTRUM

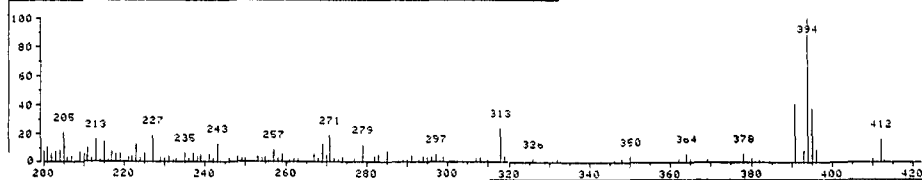


Figure 5

CONFIRMATION OF SORBIC ACID, SODIUM BENZOATE, AND PARABENS IN FOOD PRODUCTS USING ON COLUMN DERIVATIZATION GAS CHROMATOGRAPHY/MASS SPECTROMETRY; Tung Sun; USDA, Food Safety and Inspection Service, P. O. Box 6085, Athens, Georgia 30604

Sorbic acid and sodium benzoate are effective antimycotic agents at the level of about 50 to 1200 ppm in food products (1), but they are not approved to be used as food preservatives for fresh meat and poultry food products in the United States.

A simple and rapid method was utilized to monitor the presence of these compounds. The sample was acidified and extracted with ethyl acetate and dried over sodium sulfate (2). The extract was injected simultaneously or with a constant time delay (DT) using N,O-Bis(trimethyl silyl) acetamide (BSA) into a gas chromatograph for GC/MS confirmation by monitoring the GC retention times with the molecular ion (M^+) and other important characteristic ions, e.g., $(M-CH_3)^+$, $(M-CH_3-CO_2)^+$, $(M-OSiMe_3)^+$, $(M-COOSiMe_3)^+$. As DT was utilized longer than 2 minutes, it was found that the intensities of the mass spectra fragments dropped slightly, and the trimethyl silyl (TMS) sorbate was almost masked by the reagent peak. The on column derivatization GC method can also be applied to other food preservatives such as methyl, ethyl, n-propyl, and n-butyl parabens.

Traditionally, sorbic acid, benzoic acid, and the parabens were analyzed using thin layer chromatography (TLC) and ultraviolet (UV) detection (3). The sensitivities of these approaches were quite poor, with benzoic acid and sorbic acid having nearly the same R_f values. Zonneveld (4) then used a spectrophotometric method to estimate benzoic and sorbic acid at 227 nm and 255 nm, respectively. Again, there was interference superimposed on the benzoic acid peak, and the sensitivities for both acids were poor. Zygmunt (2) used a GC method for the simultaneous determination of sorbic acid and sodium benzoate in table syrup. This method provided good sensitivity, but required a specially prepared GC column, e.g. 9% SP-1200 and 2% phosphoric acid on Chromosorb W. Graveland (5) analyzed sorbic and benzoic acids in rye and margarine with a polar GC column, 5% Carbowax 20M-terephthalic acid. When this author attempted to use this method, the benzoic acid showed GC tailing and required a higher GC column elution temperature.

Perfetti et al. (6) developed a high pressure liquid chromatograph (HPLC) method with 254 nm UV detector for the determination of methyl and propyl parabens in comminuted meats. They had success with the spiked sample extracts. Yet their LC chromatograms showed some peaks which might interfere with the sorbic acid, benzoic acid, and methyl paraben. Daenens and Laruelle (7) analyzed parabens in food using TMS derivatives on a commonly available GC column 3% OV17. The GC peaks were very symmetrical. This method usually required freshly prepared sample derivatives to avoid the problems of sample stabilities and artifacts.

In 1962, Anders and Mannering (8) developed a peak-shift technique to prepare derivatives of alkaloids and steroids directly on the GC column by following the injection of the parent compounds with the derivatizing reagents. This author found it was very useful and convenient for preparing derivatives of compounds discussed in this paper.

GC/MS/DS System

A Pye Unicam model 104 gas chromatograph equipped with any one of the common liquid phases OV1, OV3, OV7, and OV17 was used to separate the TMS derivatives, and all worked well. A Kratos AEI MS-30 double beam mass spectrometer either in EI/EI or in CI/EI mode was used. Data acquisition was accomplished with DS-50S software with a Data General NOVA 2 computer. Accurate masses of the full mass spectra were measured first by checking with the standards. Since the EI spectra of these derivatives were quite simple and the concentration of the food preservatives were usually very high compared to the other co-extracting interference materials, the confirmations were usually successful by using GC/EI MS at low resolution with multiple peak monitoring. In case significant coelution GC interference peaks showed up, the resolution could be increased.

Results

(A) TMS esters of sorbic acid and benzoic acid:

(1) GC operational parameters

Column: 3 ft x 4 mm ID glass packed with 3% OV7
 Column temperature: Isothermal 120°C
 Carrier gas: Helium at 20 cc/min
 Delay time for BSA injection: 10 sec
 Retention times: TMS sorbate - 4 min, 4 sec
 TMS benzoate - 5 min, 37 sec

(2) EI mass spectra

TMS ester	Relative Intensities				
	M	M-CH ₃	M-CH ₃ -CO ₂	M-OSiMe ₃	M-COOSiMe ₃
Sorbic acid	8	100	30	68	30
Benzoic acid	5	98	40	100	60

(B) TMS ethers of parabens:

(1) GC operational parameters

Column: 1.5 ft x 4 mm ID glass packed with 3% OV3
 Column temperature: 120°C (1 min) programmed at 7°C/min to 200°C
 Carrier gas: Helium at 20 cc/min
 Delay time for BSA injection: 10 sec
 Retention times: Methyl paraben TMS ether (5 min)
 Ethyl paraben TMS ether (6 min)
 n-Propyl paraben TMS ether (8 min)
 n-Butyl paraben TMS ether (11 min)

(2) EI mass spectra

TMS ethers of parabens	M	M-CH ₃	Ions and Relative Intensities			
			SiMe ₃	C ₁₀ H ₁₃ O ₂ Si	C ₉ H ₁₁ O ₃ Si	C ₁₀ H ₁₄ O ₃ Si
Methyl-	52	100	41	32	--	--
Ethyl-	70	100	10	71	19	2
n-Propyl-	30	52	71	100	57	47
n-Butyl-	26	38	78	91	84	100

Other common weak intensity ions are m/z 75, 89, 91, 121, 135, 151, 177, and 179.

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FPA 22 | DETERMINATION OF PHOSPHOROTHIOATE RESIDUES
IN FOODS BY NEGATIVE ION CHEMICAL IONIZATION (NICI) GC/MS;
THOMAS L. BARRY, AND GLENN PETZINGER; FOOD AND DRUG ADMINIS-
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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 insecticides or industrial chemicals found and to identify unknown contaminants in these products. The overall trend in recent years has been to replace many organochlorine compounds with organophosphates. This trend has taken place overseas, as well as in this country, resulting in the detection of a greater number of organophosphorus residues or their metabolites in foods.

This study reports the application of CH_4 NICI GC/MS for the confirmation and identification of a particular group of organophosphate residues, namely the phosphorothioates $(\text{RO})_2\text{-P(S)-O-R'}$. EI, PICI, and NICI spectra were recorded for a number of these compounds and a comparison made as to the sensitivity and fragmentation patterns obtained. NICI was found to be a sensitive technique and produced spectra with very little fragmentation. A mixture of phosphorothioates were also examined using various source temperatures to determine its effect on sensitivity and the degree of fragmentation.

The two major ions produced in NICI when R' is a pyrimidinyl group are, the $(\text{M-l})^-$ ion, and the fragment ion corresponding to $(\text{RO})_2\text{-P(S)-O}^-$. In those cases where R' is a nitrophenyl group, a molecular ion M^- is observed along with a major fragment ion, $(\text{S})\text{R}'^-$.

NICI has been successfully applied to the determination of Fenitrothion (0,0-dimethyl 0-(3-methyl-4-nitrophenyl) phosphorothioate) residue in figs, Pirimiphos Methyl (0,0-dimethyl 0-(2-diethyl-amino-6-methyl-4-pyrimidinyl) phosphorothioate) in dried beans, pigeon peas, and dehydrated vegetables, and Diazinon (0,0-diethyl 0-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate) in kiwi fruit.

Simultaneous Measurements By GCMS Of The Metabolic Products Of The Anti-Arrhythmic Drug Mexiletine For The Determination Of Interindividual Differences in its Metabolism; Frank Hatch, Gordon Hansen, Jamie Graham and Susan Hattox; Boehringer Ingelheim Ltd., Ridgefield, CT 06877

Mexiletine (Figure 1) is an effective anti-arrhythmic drug for prophylaxis and control of ventricular arrhythmias such as those occurring after myocardial infarction. It has the advantages of being effective when administered orally as well as intravenously, and a long duration of action.

Interindividual variations in plasma elimination rates have been documented in both healthy volunteers and patients.(1,2) Several explanations have been offered for this variation, including urinary pH (3,4), weight (2,5) and genetic differences (6). A genetically-determined difference in the rate of oxidation of some drugs is known to exist in humans. This difference results in differing kinetics of elimination and therefore variations in the blood levels of some drugs metabolized by the cytochrome P450 monooxygenase system. The major route of elimination of mexiletine is known to occur by oxidative metabolism. In vitro tests carried out at higher than expected therapeutic concentrations indicate that this may be carried out by that monooxygenase system which is subject to a defect in oxidizing capacity (7).

The method described has been developed to probe the question of whether the metabolism of mexiletine is genetically controlled in human volunteers. If mexiletine elimination is dependent on genetically determined rates of metabolism, blood level monitoring will be essential to ensure maximal efficacy and minimal side effects.

Several sensitive methods have been reported for the determination of mexiletine in biological fluids utilizing HPLC or GC with flame ionization, electron capture and nitrogen selective detection (8-10). In one case both mexiletine and its 2-hydroxymethyl metabolite (11) were quantitated in plasma (10). The majority of these methods demonstrate the difficulty with which mexiletine is extracted from aqueous solutions. Extraction efficiencies range from 45 to 80% and vary significantly with ionic strength. We wish to report a method utilizing capillary column GC/ammonia CIMS for the simultaneous quantitation of mexiletine and its four major metabolites in urine which requires no sample preparation other than enzymatic hydrolysis.

Experimental

Patient urine, 100 μ L, was mixed with 100 μ L of internal standard solution (10 μ g A, 20 μ g B, 2.5 μ g C), 10 μ L of β -glucuronidase Type IX (SIGMA, 2000 units/mL) and 10 μ L of sulfatase Type H-2 (SIGMA, 2000 units/mL) and taken to a volume of 800 μ L with distilled water. Enzymatic hydrolysis was carried out at 40°C, overnight (17 hours). Two hundred microliters of the hydrolysis solution was taken to dryness and derivatized with 300 μ L of N-methyl-N-TMS-trifluoroacetamide (MSTFA, Pierce) at 75°C for 2 hours.

All analyses were carried out on a Finnigan 4023 GC/MS/DS tuned for unit resolution (5% Valley) over the entire mass range. The standard EI/CI source was modified to optimize CI operation by decreasing the ion exit (lens 1) and electron entrance aperture to 1 mm diameter. The collector assembly was eliminated. Typical operating pressures with NH_3 reagent gas were 2.0 Torr (250°C) and analyzer pressures of $\sim 2 \times 10^{-5}$ Torr. Sensitivity was maximized with an electron energy of 190 volts and emission current of 0.1 ma. The electron multiplier was held at 1.1 Kv and the conversion dynode at -3.0 Kv. Total scan cycle time was 0.5 seconds.

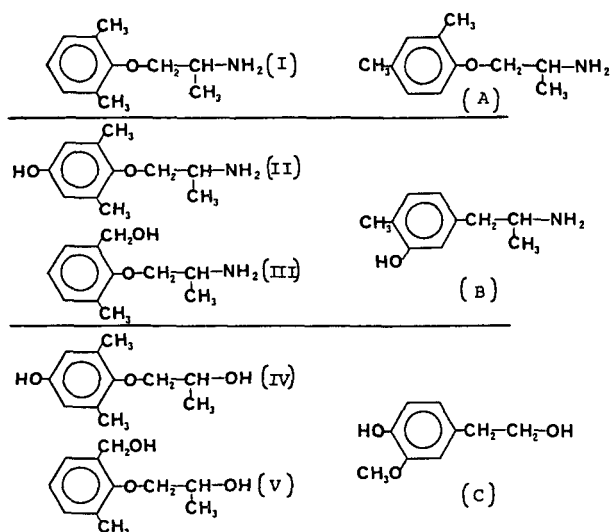
The $(M+H)^+$ species were monitored for the amine compounds (I, II, III, A, B) and the $(M+NH_4)^+$ species were monitored for the neutrals (IV, V, C). Gas chromatography was carried out on a 15 m x 0.3 mm ID DB-1 fused silica column (J+W Scientific) interfaced directly to the MS ion source. Helium carrier gas ($u = 80$ cm/sec) was employed. Temperature program conditions were: 140°C hold .5 minutes, 140-240°C at 12°C/min. Direct injections of 0.5 μ L with a fused silica needle syringe were carried out at 225°C. Compounds II and III were found to be thermally sensitive with metal needle syringes. No significant interferences were observed over the concentration range studied (minimum .2 μ g/mL urine).

Results

Standard curves were constructed from six sample/internal standard concentrations covering ranges of 1-40 μ g/mL urine for metabolites IV and V, 2-80 μ g/mL urine for mexiletine and metabolite III, and 8-320 μ g/mL urine for metabolite II. The average correlation coefficients ($n=3$) for the standard curves of compounds I, IV and V were better than 0.9985 and better than 0.996 for compounds II and III. The total assay C. V. calculated for 5-replicate sample (0-2 hour patient urine ~ 5 μ g/mL urine) each injected 5 times was better than $\pm 4.0\%$ for each compound.

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Figure 1:



Mexiletine (I) and
Major Metabolites
(II-V)

Source: Boehringer
Ingelheim, KG,
Ingelheim/Rhein,
FRG

Internal Standard
(A-C)

Source: Aldrich (B)
Regis (C)

Application of Headspace GC/MS for Automatic Analysis
of Industrial Streams, Polymers and Environmental Samples

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In recent years gas chromatographic headspace analysis has become an important branch of chromatography. Combination of headspace/GC with Mass Spectroscopy can substantially increase the sensitivity of GC/MS as well as the breadth of problems to which GC/MS can be applied. It is a powerful method both for trace analysis of ppb level volatiles in water and for the sampling of volatiles in "difficult" matrices such as soils, sludges and polymers since the sample matrix injected into the GC is always a mixture of volatiles, air and carrier gas.

The Headspace/GC unit used is a Perkin-Elmer F-42 with a 30 sample auto sampler. At the end of the GC column, part of the effluent from the column (packed or capillary) is spit to an FID detector and the remainder to a heated transfer line which goes through a shut-off valve into the third port of the Finnigan 4000 GC/MS interface. The experiment itself is simple. A measured amount of sample is placed in a septum vial, sometimes with salt added to increase the volatility of polar compounds. The sample is allowed to equilibrate with the headspace above it, usually at elevated temperature. For aqueous samples equilibration at 50°C requires 45 minutes with sample shaking or stirring. The sampling needle is inserted into the vial and the vial is pressurized to the GC operating pressure. The carrier gas is temporarily interrupted and the vial acts as a carrier gas source, delivering the sample to the column. This method eliminates the need for traps, which can become contaminated or can exhibit break-through. Also the vial used is disposable, resulting in less cross contamination and less need for blanks.

Headspace GC/MS is an equilibrium method. The fraction of non-polar volatiles in the headspace can approach 100%, which is why the storage of such samples without any headspace is so important. The concentration factor can still be significant for more polar compounds since one can inject 1000X more headspace than liquid sample. The concentration of polars is also enhanced by the addition of salt to the sample. The liquid/headspace equilibrium constant is also an estimate of the purging rate and efficiency for purge methods.

Our initial application was the analysis of volatiles in environmental water samples. The prescribed EPA method for this analysis is purge-and-trap. If purging and trapping efficiency were complete, purge-and-trap analysis would always be more sensitive with the same sample temperature. However in a parallel analysis with a commercial laboratory we found sensitivities to be comparable in most cases, and that headspace was significantly better for very light compounds probably because of poor trapping efficiency. Headspace/GC is also easily automated and has low blanks because there is no cross contamination from glassware or traps. Foaming is also not a problem.

Headspace/GC/MS is an ideal method for the analysis of contaminated soils. One can sample the volatiles in a soil without any kind of complicated sample handling which might result in losses. In our work, the soil is mixed with water, equilibrated and the headspace sampled. Some soils we have examined were so contaminated that even with water dilution there is still probably a separate organic phase. In such cases target compounds are best quantitated by standard addition.

Headspace GC/MS of polymers is as simple as putting the polymer in a septum vial. In one case analysis of a S/AN copolymer indicated that substantial styrene monomer was present. The presence of a butadiene dimer showed that the polymer was rubber reinforced. 2-Ethylhexanol, an additive degradation product which would contribute to odor in a finished product, was also observed. Quantitation of an equilibrated sample is possible in some cases, but in others depolymerization takes place at elevated temperatures, or equilibration takes days, weeks or years. In such cases the polymer can be dissolved or swelled in a non-volatile solvent to reduce diffusion restrictions for evaporation from the solid matter.

Another problem, that would have been a difficult analysis by any other technique, was to verify the presence of benzene at the ppm level in a glue-like prepolymer. The prepolymer consisted of 70% oligomer and 30% of a light organic solvent. Using MID benzene was indeed identified and was quantitated by standard addition to be one ppm.

Sensitivity for trace volatiles in liquid organic matrixes can be enhanced if there is a significant difference in volatility or polarity between the target compounds and the matrix. In our example sensitivity for benzene and furan in a polar solvent was enhanced by diluting the sample 1:100 in water and sampling the headspace. The water increased the volatility of the benzene and furan while decreasing that of the solvent.

In summary we believe that Headspace GC/MS has many advantages. It is cost effective, the method has low blanks, and it is applicable to a wide variety of problems where high sensitivity is needed or difficult matrices are encountered.

GC/MS ELUCIDATION OF PHOTOLYTIC
DISCOLORATION OF BENZOTRIAZOLE STABILIZERS
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Introduction

The phenomenon of discoloration of commercial products caused by low levels of intensely chromophoric impurities or degradation products is a recurrent problem in industrial chemical analysis. The combination of GC/MS following thin layer chromatographic (TLC) purification and visualization proves to be a valuable problem solving approach for organic chromophores. The success of this method relies heavily on the sensitivity and specificity of the chromatographic and spectral analyses.

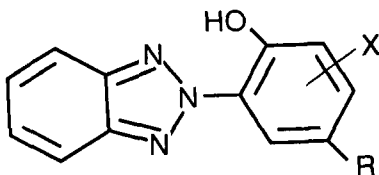
The particular application arose from the need to elucidate the cause of discoloration due to weathering in sunlight and air of certain fabricated plastic products. Silica TLC of organic extracts from "good" and "bad" plastic samples revealed that particular components discolored with time upon exposure to both light and air, analogous to the behavior of the discoloring product. The discoloring components were purified by TLC and identified by fused silica capillary gas chromatography and quadrupole mass spectrometry utilizing both methane chemical ionization and electron impact techniques. Further confirmation of proposed structures was obtained from high resolution accurate mass measurement of purified samples.

The discoloring components were identified as two different 2-(2H-benzotriazol-2-yl)-4-alkyl-phenols. These substituted benzotriazoles are commercial additives used as ultraviolet light absorbers to stabilize plastics against discoloration and deterioration.

Results and Discussion

Silica gel TLC of crude extracts revealed a major intermediate R_f, UV absorbing, band which upon standing in air and light slowly turned yellow-brown. Elution and repeat TLC showed predominantly the original band, which again discolored with time, and a new permanently yellow-brown band at lower R_f. Improving TLC resolution showed that there were in fact two discoloring components and two corresponding yellow-brown products. Analogous TLC of standard substituted benzotriazoles I and II (see figure) evidenced the same retention and discoloring behavior, likewise producing yellow spots (III and IV).

Substituted
Benzotriazoles:



<u>Compound</u>	<u>R</u>	<u>X</u>	<u>MW</u>	<u>Formula</u>
I	CH ₃	H	225	C ₁₃ H ₁₁ N ₃ O
II	tC ₈ H ₁₇	H	323	C ₂₀ H ₂₅ N ₃ O
III	CH ₃	NO ₂	270	C ₁₃ H ₁₀ N ₄ O ₃
IV	tC ₈ H ₁₇	NO ₂	368	C ₂₀ H ₂₄ N ₄ O ₃

Benzotriazole stabilizers I and II were weakly detectable by GC/MS in crude sample extracts. Preparative TLC purification of the discoloring extract bands provided cleaner samples for better mass spectral characterization of the isolated benzotriazoles I (MW225) and II (MW323) by CI-CH₄ and EI GC/MS (see spectral data).

Spectra Data

I: CI m/z 226(M+H), 254(M+C₂H₅), 266(M+C₃H₅), 135, 120
 EI m/z 225(M), 196(M-COH), 168(M-COH-N₂), 154, 120, 93, 77
 65, 51, 39

II: CI m/z 324(M+H), 352(M+C₂H₅), 364(M+C₃H₅), 252(MH-C₅H₁₂),
 212(MH-C₈H₁₆), 113(C₈H₁₇), 120
 EI m/z 323(M), 252(M-C₅H₁₁), 133, 120, 105, 91, 77, 65, 57, 41

III: CI m/z 271(M+H), 299(M+GH₅), 311(M+C₃H₅), 241(MH-NO), 149, 131, 120
 EI m/z 270(M), 240(M-NO), 212(M-CNO₂), 167 (C₁₂H₉N), 115, 105, 91,
 77, 65, 51, 39

IV: CI m/z 369(M+H), 397(M+C₂H₅), 409(M+C₃H₅), 297(MH-C₅H₁₂),
 267(MH-C₅H₁₂-NO), 257(MH-C₈H₁₆), 149, 131, 120, 113(C₈H₁₇)
 EI m/z 368(M), 338(M-NO), 297(M-C₅H₁₁), 267(M-C₅H₁₁-NO) 251, 148,
 120, 105, 91, 77, 65, 57, 41

Further preparative TLC purification of the permanently yellow bands at lower Rf allowed GC/MS characterization of benzotriazole derivatives III (MW270) and IV (MW 368) (see spectral data). The molecular weights of the yellow products were 45 higher than the commercial stabilizers, having changed from odd (N₃) to even (proposed N₄). Fragmentation data was characteristic of nitro substitution and indicated that the benzotriazole moiety remained intact. Hence it was proposed that yellow products III and IV were simple nitro derivatives, apparently on the phenol ring of the substituted benzotriazole (see figure).

The identification of the yellow products from extracts as benzotriazole nitro derivatives was validated by exposure of standard benzotriazoles I and II to the same conditions of silica, light and air to again obtain the respective colored nitro species III and IV, compared by TLC Rf, GC retention time, and MS. Moreover, exposure of standard benzotriazoles in an atmosphere enriched in NO_x produced the same nitro derivatives in higher yield. In fact, at high concentrations of NO_x, after several days no unreacted benzotriazole stabilizer remained. The products included a complex mixture of very polar yellow-brown species, probably due to extensive nitration.

Final confirmation for the nitro derivative identification was provided by high resolution accurate mass measurement by peak matching with purified samples.

Compound III:	calculated	measured	ppm	mmu
M ⁺ C ₁₃ H ₁₀ N ₄ O ₃	270.0752	270.0752	0	0
M-30 C ₁₃ H ₁₀ N ₃ O ₂	240.0772	240.0767	2	0.5
M-58 C ₁₂ H ₁₀ N ₃ O	212.0823	212.0812	4	1.1
M-103 C ₁₂ H ₉ N	167.0735	167.0726	5	0.9
Compound IV:				
M-71 C ₁₅ H ₁₃ N ₄ O ₃	297.0987	297.1004	6	1.7

Conclusions

Benzotriazole stabilizers can discolor upon exposure to light and air (containing NO_x), demonstrating behavior similar to the discoloration of certain plastic products under investigation. Strongly yellow-colored species were purified by TLC and identified by GC/MS and high resolution MS as nitro derivatives of the commercial benzotriazoles. These nitro derivatives can have such a strong yellow chromophore that one can visually see on a silica TLC plate a quantity not detectable by GC/MS. Due to the extremely low concentrations involved, the yellow nitro compounds were not detected directly in the crude extracts which contained large amounts of other constituents of the plastic samples. Upon TLC, either contact with silica and/or increased surface exposure to light and air (containing NO_x) apparently catalyzed or enhanced the rate of conversion of benzotriazoles to yellow nitro derivatives. The nitro benzotriazoles can be an offending chromophore in discolored plastic products, however exposure to varying concentrations of NO_x or the presence/absence of synergists or catalysts for nitration can alter the rate or extent of such discoloration.

A Dual Column Fused Silica Capillary Column GC/MS Technique For Identification of Positional and Geometrical Isomers of Aliphatic Compounds

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INTRODUCTION

The identification of positional and geometrical isomers of aliphatic compounds is important in many industrial processes as well as in the studies of natural products. Although several analytical methods using gas chromatography (GC) or GC/mass spectrometry (GC/MS) that are capable of resolving and identifying these isomeric compounds have been reported, difficulties do remain. High resolution capillary gas chromatography has proved to be a most valuable technique in the separation of these compounds. However, it is often not possible to separate all of the isomers of interest on one column. Recently, dual column GC methods have been reported where two fused silica columns coated with different liquid phases have been connected to a single injector. In this way the analysis is carried out simultaneously on both columns with the effluent from each column going to a separate detector. Unfortunately, GC detectors do not provide the specificity which is sometimes necessary. The use of GC/MS systems has provided the specific detection needed for most analyses. However many isomers of aliphatic compounds have the same mass spectral pattern. A dual fused silica column GC/MS technique which aids in the identification of these compounds is presented here.

EXPERIMENTAL

GC/MS data were obtained using a Finnigan Model 3300 GC/MS system which has been modified to facilitate high resolution capillary GC. Chromatography was done using two columns, one coated with OV-101 and one with Carbowax 20 M, which were prepared in this laboratory from identical lengths of fused silica tubing 0.25 mm I.D. x 25 meters long. For dual column analyses, both columns were connected to the injector of the GC/MS system using a 1/16 in. graphite ferrule having a single 1 mm hole. The detector ends of the columns were connected directly to the ion source through the transfer line using a 1/16 in. graphitized vespel ferrule (15%) in which two holes were drilled.

The analyses were done in the splitless mode at an initial temperature of 60° followed by rapid programming at 30°/min to 80° and then 4°/min to 220° using hydrogen as carrier gas at a linear velocity of 50 cm/sec.

RESULTS AND DISCUSSION

Three mixtures of aliphatic isomers were prepared to test the technique. Sample 1 contained positional isomers of octadecene, sample 2 positional isomers of hexadecenal while sample 3 contained both positional and geometrical isomers of octadecene with all components at a concentration of 10-20 ng/ μ l. All analyses were made using methane positive ion chemical ionization (CI) mass spectrometry as the CI spectra provided molecular ion and some structural information.

Five replicate analyses were made on each sample, first using the OV-101 column, then the carbowax 20 M column system and finally using the dual column system. Retention times relative to the internal standard (RRT) were calculated for each isomer in all analyses. The mean RRT and standard deviation was then calculated for each component from these data and is shown in Tables 1 to 3 representing samples 1-3 respectively. These data show good agreement between most of the RRTs of components eluted on the single column systems and those eluted using the dual column system.

Figures 1, 2 and 3 show the reconstructed total ion chromatograms obtained from Samples 1 to 3 respectively. Figure 1 shows (Z)-4-methyl-5-octadecene is completely resolved from all the other isomers in the mixture using the OV-101 column but co-elutes with (Z)-4-octadecene on the carbowax 20M column. (Z)-6, (Z)-7 and (Z)-8 octadecene co-elute on the OV-101 while only the (Z)-8 and (Z)-9 isomers co-elute using the Carbowax 20M.

Figure 2 demonstrates the ability of the polar carbowax 20M phase to separate the (Z)-4, (Z)-5 and (Z)-6 isomers of hexadecenal which do not separate on the OV-101 phase. The separation of cis and trans isomers is demonstrated in Figure 3. (Z)-8 and (E)-8 octadecene co-elute on OV-101 but are well resolved on carbowax 20M.

Component	Mean Relative Retention Time and Standard Deviation (sec) on			
	Temp. Col. OV-101	Temp. Col. Carbowax 20M	Dev. Col. OV-101	Dev. Col. Carbowax 20M
Z3-18 H	955 (.001)	1.081 (.001)	929 (.000)	1.082 (.000)
Z4-18 H	969 (.001)	1.043 (.001)	963 (.001)	1.043 (.001)
Unk Z5-18 H	1.012 (.001)	1.058 (.003)	1.014 (.001)	1.000 (.002)
Z5-18 H	982 (.001)	1.044 (.001)	982 (.001)	1.044 (.001)
Z6-18 H	977 (.002)	1.034 (.001)	976 (.001)	1.034 (.001)
Z7-18 H	971 (.001)	1.032 (.002)	970 (.001)	1.024 (.002)
Z8-18 H	971 (.001)	1.018 (.002)	970 (.001)	1.018 (.001)
Z9-18 H	971 (.001)	1.018 (.002)	970 (.001)	1.018 (.001)

TABLE 1

Component	W	Mean Relative Retention Time and Standard Deviation (sec) on			
		Temp. Col. OV-101	Temp. Col. Carbowax 20M	Dev. Col. OV-101	Dev. Col. Carbowax 20M
Z4-16 old	221 (21)	1.034 (.001)	960 (.001)	1.033 (.002)	
Z5-16 old	874 (.002)	1.026 (.002)	874 (.001)	1.024 (.001)	
Z6-16 old	874 (20)	1.022 (.001)	874 (.001)	1.019 (.001)	

TABLE 2

Component	Mean Relative Retention Time and Standard Deviation (sec) on			
	Temp. Col. OV-101	Temp. Col. Carbowax 20M	Dev. Col. OV-101	Dev. Col. Carbowax 20M
Z4-18 H	952 (.001)	1.044 (.001)	953 (.001)	1.060 (.001)
Z4-18 H	988 (.001)	1.054 (.002)	988 (.001)	1.050 (.001)
Z8-18 H	943 (.001)	1.017 (.003)	970 (.002)	1.014 (.001)
Z8-18 H	976 (.001)	1.028 (.001)	977 (.001)	1.028 (.001)

TABLE 3

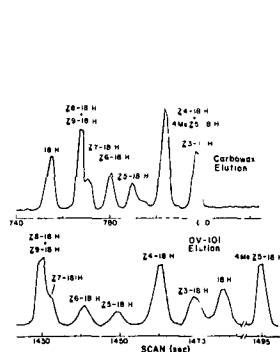


FIGURE 1

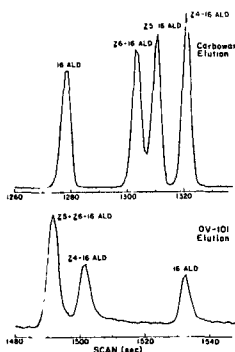


FIGURE 2

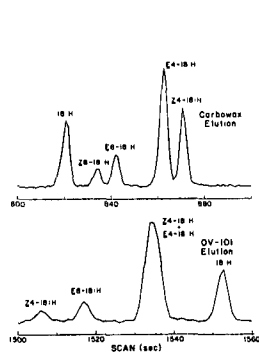


FIGURE 3

CONCLUSIONS

The dual column GC/MS system offers two main advantages over conventional single column systems. First of all, retention data on two columns coated with different liquid phases can be obtained in a single analysis. By determining RRT's using two high resolution columns of different polarity, compound identifications can be made with a much greater degree of confidence, particularly when the mass spectrum of the compound is also provided in the analysis. The dual column system provides very similar data with no greater variance than that obtained using two single column systems and therefore saves GC/MS down time required to change columns.

Secondly, the use of the dual column system often provides chromatographic separation on the one column that is not possible on the other column and vice-versa. This increased separating power is particularly useful in the analyses of complex mixtures of aliphatic positional and geometrical isomers.

* A detailed paper describing this study has been submitted to the Journal of Chromatographic Science for publication.

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Business Meeting of the
American Society for Mass Spectrometry

Tuesday, May 10, 1983, 5:12 P.M.
Grand Ballroom, Boston Sheraton

President Fenselau called the meeting to order and introduced H. Fales as the Parliamentarian for the Business Meeting to the approximately 250 assembled members of the Society.

The Secretary began his report by calling for a motion to dispense with the reading of the revised minutes from the 30th Annual Conference on Mass Spectrometry and Allied Topics, so moved, seconded and passed unanimously. He then introduced the Student Travel Stipend Awardees; C. Dass, T. Francl, D. Hunton, B. Larsen, J. Marasco, D. Stauffer, and H.-Y. Tong. Brief mention was made of the deaths of two members of the Society and the Secretary requested those assembled to inform him if they were aware of any other losses. The results of this year's election were announced and Fenselau introduced the successful candidates.

Vice President for Programs	M. A. Grayson	341
	M. I. Levenberg	265
Secretary	H. S. Hertz	358
	A. Yergy	244
Member-At-Large	D. M. Desiderio	269
	R. E. Finnigan	341

The Secretary concluded his report by stating the changes in the Bylaws made by the Board of Directors. The changes define the terms of membership and voting rights of members. The revised Constitution will be published in this year's Abstracts and he pointed out that the most recent version was last published in 1981.

Treasurer DeCorpo then summarized the present membership in the Society (1734), contrasting it with the 1982 membership (1808). He pointed out that we have historically had a membership turnover of 10-15% annually and that the number of student members had increased this year. He then reported on the financial stability of the Society and contrasted the income and expenses of the last three Annual Meetings. Briefly, Minneapolis with 1102 attendees made \$2200, Hawaii with 789 registrants lost \$1400, and Boston was scheduled to break even at 925. The registration as of 4 P.M. stood at 1130 assuring that this year's Conference would not lose money.

He then gave a breakdown of the 1983 Conference expenses and the consolidated 1982 financial report which showed that the Society had a net \$2000 loss. It was pointed out that approximately \$14 of the annual dues goes toward the printing of the Abstracts and the cost of our leased computer services had increased in 1982. The balance on hand as of May 1, 1983 was \$93,978.76

DeCorpo then announced the composition of the Audit Committee as F. Saalfeld, H. Fales, and B. Wyatt. In response to a question, he said that the present arrangement with ASTM regarding the printing of the Abstracts would continue.

Vice President for Programs Cooks then summarized the 31st Annual Conference, and acknowledged the Review Committee. This year's Conference had 532 papers (305 oral and 227 poster) versus 511 (277 oral and 234 poster) in 1982. He thanked the Review Committee which consisted of Hites, McIver, Glish, Busch, Freiser, and Sweeley for their efforts.

Cooks gave an overview of the Vice President for Programs' efforts and said that the skeleton of the next Conference is fairly well set by August. He requested that any suggestions concerning the 1983 Conference be made in writing to him by mid-July. He then solicited comments regarding the number of parallel sessions; protected lectures (Introductory, Plenary, Policy or Historical); Friday afternoon sessions; number of

papers; overhead versus slide projectors; and workshops. The consensus was that the posters should be displayed for longer periods of time, even without the author being present. This year's evening poster sessions were deemed to be a success and there was some discussion of limiting the total number of oral papers.

Vice President for Arrangements Compson was then introduced. He began by apologizing for the conditions of the hotel and explained that we had not been informed that the construction had started. He pointed out the problems with either two concurrent poster or just two oral sessions is that the facilities must have two very large rooms. Compson outlined the future meeting plans:

- 1984 San Antonio: Hyatt, Menger and Mansion as the principal hotels with the Convention Center also reserved.
- 1985 San Diego: Harbor Island Sheraton
- 1986 Cincinnati: Stouffer and Weston as the principal hotels
- 1987 Denver: Several hotels under consideration, the main hotels had not yet been picked.
- 1988 West Coast: Suggestions should be sent before August to Mike Grayson

Compson announced that due to the success of the Fall Workshops on FAB and MS/MS, the Board of Directors had proposed a workshop this year on LC/MS to be held on the West Coast in November.

Member-At-Large Lampe presented the deadlines for the extended abstracts, workshops and committee reports for inclusion in the Abstracts. He stated that the correct citation form for work presented at the Conference could be found in the Abstracts.

Member-At-Large Vestal reported on the two shorts courses presented prior to the Conference which had a combined registration of 20 students. He thanked H. Hertz and the Washington Discussion Group for their efforts. He requested that suggestions for future short courses or workshops be mailed to him by the middle of July for consideration at the August Board Meeting.

Fenselau then introduced Judith Watson of Professional Association Management as the Society Office person. She thanked M. Gross for his efforts in organizing the MS/MS workshop and helping make it a success. She then recognized the Nominating Committee (W. Fite, H. Fales, B. Colby, M. Bowers, and A. Burlingame).

President Fenselau introduced the Presidents of two other Mass Spectrometry Societies present, A.-M. Andreani and D. Games.

She then called W. Fite to the podium. Fite announced the sponsorship by Extranuclear of an ACS award in mass spectrometry. The award, named the "Frank H. Field - Joe L. Franklin Award", will be presented in recognition for advances in mass spectrometry. He stated that the award will be presented first in 1985 for fundamentals and then alternate between applications and fundamentals annually.

President Fenselau thanked the out-going Board members, T. Ashe, K. Compson, and F. Lampe. She announced that T. Aczel would be leaving the Board in December when his term as Chairman of ASTM Committee E-14 expires.

There being no new business, the meeting was adjourned at 6:12 P.M.

Respectfully submitted,

T. R. Ashe
Secretary

Revised Minutes of the 30th Annual Meeting of the
American Society for Mass Spectrometry

Wednesday, June 9, 1982, 5:30 P.M.
Coral IV, Hilton Hawaiian Village

President Munson introduced Henry Fales as the Parliamentarian for the business meeting to the approximately 90 assembled members of the Society. He then asked for corrections to the Minutes of the previous year which were then approved as published in the "Abstracts of the 29th Annual Conference on Mass Spectrometry and Allied Topics."

The Secretary then introduced the recipients of the travel fellowships (W. Bone, D. Burinsky, K.W.S. Chan, R. Cody, G. Gellene, J. Gillman, G. Groenewold, R. Voyksner). He then exhibited the election results and introduced the successful candidates.

Vice President for Programs	<u>R. Graham Cooks</u>	316
	<u>Mike Gross</u>	233
Treasurer	<u>Jim DeCorpo</u>	262
	<u>Flote Vane</u>	242
Member-At-Large	<u>Fred Abramson</u>	229
	<u>Marvin Vestal</u>	307

Brief mention was made of the deaths of three members and the Secretary requested those assembled to inform him if they were aware of any other losses.

President Munson then expressed the Society's appreciation of the efforts of the departing Board Members. Vice President for Programs Fenselau presented President Munson and Past President McCloskey with tokens of the Society's appreciation of their efforts.

Treasurer Frisch then gave the financial picture of the Society. The balance on hand as of May 28, 1982 was \$116,408.20. In light of the lower registration and projected Conference deficits, the Treasurer advised that the registration fees would probably be increased for the 1983 Conference.

Vice President for Programs Fenselau presented an overview of the Honolulu program, and thanked the review committee for its work and acknowledged the committee members: L. Powell, T. Aczel, R. Cotter, C. McKewn, and R. McIver. She then presented the demographic distribution of authors, for 1981 and 1982 noting that the Conference had increased contributions from overseas.

	<u>1981</u>	<u>1982</u>
Canada	31	33
U.S.	336	353
Aus./NZ	1	17
Japan	10	64
Other	62	63
	<u>440</u>	<u>530</u>

Munson then thanked several Corporate Members for making extraordinary one-time financial contributions for the success of the Conference. The Corporate Members acknowledged were:

Colorado Group (In Memory of Glenn Kerfoot)
Extranuclear-Laboratories
Hewlett-Packard
Kratos Scientific Instruments
Nicolet Instruments
Phrasor Scientific
Scientific Glass Engineering
Sciex

Vice President for Arrangements Compson then discussed future meeting sites. The schedule is as follows: Boston, MA, May 8-13, 1983; San Antonio, TX, 1984; San Diego, CA, 1985; East Coast, 1986. Compson announced that negotiations are underway with both Cincinnati and Pittsburgh for 1986. Compson asked for a straw vote of the attendees concerning a mid-week social affair in Boston. Nothing had been planned for Honolulu due to variety of activities available. The clear consensus of those attending was that a formal social event was desirable. Compson solicited suggestions from the membership for possible 1987 meeting sites.

Member-At-Large Fred Lampe then discussed the Conference Abstracts which will again be produced with the aid of Judith Watson (ASMS Staff) and ASTM's publishing facilities. Lampe urged that all authors citing work in the Conference Abstracts conform to the citation notice printed in the Conference Abstracts.

Munson praised the technical success of the FAB Workshop held in Baltimore and discussed the new workshop "Theory and Applications of Multiple Analyzer Mass Spectrometers" proposed for Fall, 1982. He then acknowledged the efforts of the Nomenclature Committee and urged authors to conform to their recommendations.

Munson then introduced Judith Watson (PAM) to the attendees. One of the Society Office's functions is to act as a clearing house for employment. Prospective employees can obtain a list of available openings by contacting the Society Office, resumes will not be circulated.

The meeting was adjourned at 6:31 P.M.

Respectfully submitted,

J. R. Ashe
Secretary

IN MEMORIAM

P. D. BEIRNE

W. BRYANT

G. KERFOOT

AMERICAN SOCIETY FOR MASS SPECTROMETRY

CONSTITUTION AND BYLAWS

ARTICLE I. NAME

The name of this Society shall be "The American Society for Mass Spectrometry".

ARTICLE II. PURPOSE

It is the purpose of this Society to promote and disseminate knowledge of mass spectrometry and allied subjects.

ARTICLE III. MEMBERSHIP

Membership in the American Society for Mass Spectrometry shall be open to any individual, or with approval of the Board of Directors, to any corporate entity with a bona-fide interest in mass spectrometry.

ARTICLE IV. MANAGEMENT

The management of the Society shall be vested in the Board of Directors which shall consist of the following members: the officers of the Society hereafter listed, two elected members-at-large, the immediate Past-President, the Chairman of ASTM Committee E-14 on Mass Spectrometry.

The Officers of the Society shall be:

President
Vice President for Programs, who shall be President-Elect of the Society
Vice President for Arrangements
Secretary
Treasurer

ARTICLE V. DUTIES OF OFFICERS

Section 1. The President shall be the chief executive officer of this Society; he shall preside at all meetings of the members and directors; he shall have general and active management of the business of this Society; he shall see that all orders and resolutions of the Board of Directors are carried out; he shall execute all bonds, mortgages, and all contracts of this Society and cause or direct the affixing of the corporate seal thereto; he shall have general superintendence and direction of all other officers of this Society and see that their duties are properly performed; he shall submit a report of the operations of the Society for the fiscal year to the Board of Directors and members at their annual meeting, and from time to time shall report to the Board of Directors all matters within his knowledge that may affect this Society; he shall be 'ex-officio' a member of all committees except the Nominating and Audit Committees and shall have the power and duties and management usually vested in the office of President in a corporation; he shall appoint all committees, except as may be otherwise provided; and he shall send, or cause to be sent by the Secretary, notices of any meetings of the Board of Directors.

Section 2. The Vice President for Programs shall be vested with all the powers and shall perform all the duties of the President during the absence of the latter and shall have such other duties as may, from time to time, be determined by the Board of Directors. The same shall be true of the Vice President for Arrangements who shall be next in the line of succession. In the event that the President shall be absent at any meeting, the Vice President for Programs shall preside, and if neither is present at a meeting, the Vice President for Arrangements shall preside.

Section 3. The Secretary or a deputy appointed by the President shall attend all meetings of the Board of Directors and all general meetings of the Society and act as a clerk thereof; he shall record all votes and minutes of all proceedings; he shall perform such other duties as may be prescribed by the Board of Directors or the President; and he shall be the custodian of the corporate seal and all of the books and records of the Society except as may be otherwise provided.

Section 4. The Treasurer shall have charge of the funds of the Society and shall deposit them in the name of the Society in depositories designated by the Board of Directors; he shall arrange for the payment of all valid Society bills and debts from Society funds, and shall make a complete and accurate report of the finances of the Society at each annual meeting of the members, or at any other time upon request, to the Board of Directors.

ARTICLE VI. DUTIES AND POWERS OF THE BOARD OF DIRECTORS

Section 1. The property and business of the Society shall be managed by the Board of Directors.

Section 2. In addition to the general powers of the Board of Directors by virtue of their office, the powers and authority expressly given by law, by terms of the Charter of this Society, and elsewhere in these bylaws, the following specific powers are expressly conferred on the Board of Directors: To purchase or otherwise acquire for the Society any property, right or privilege which it is authorized to acquire at such price or consideration and upon such terms as they deem expedient; To appoint, to remove or suspend subordinate agents or servants, to determine their duties and affix their salaries; To remove or suspend any subordinate officer or servants; To determine who shall be authorized on behalf of the Society to sign bills, notices, receipts, acceptances, endorsements, checks, releases, contracts and any other instruments; To delegate any of the powers of the Board to any standing committee, special committee, or to any officer or agent of the Society, with such powers as the Board may deem fit and proper to grant; Generally to do all such lawful acts and things as are not by law, or by Charter, or by these bylaws directed or required to be done by members of the Board of Directors.

Section 3. Any action which may be taken at a meeting of the Board of Directors, may be taken without a meeting by a letter ballot sent by and filed with the Secretary of the Society and provided all members of the Board of Directors file ballots.

Section 4. In the event that the office of any elected officer or member of the Board of Directors shall become vacant, the Board of Directors shall immediately fill the vacancy for the remainder of the unexpired term; however, no member who thus fills a vacancy in the office of Vice President for Programs shall automatically succeed to the Presidency, but the Society shall in such event elect its next President. A President who vacates office before the conclusion of his term shall not retain membership on the Board of Directors as Past-President as described in Article IV.

Section 5. The ballot of the election to membership on the Board of Directors of the Society shall be by closed, written ballot.

Section 6. Any member shall be eligible to hold office in the Society.

ARTICLE VII. FIDELITY BONDS

The Board of Directors may require such officers or agents to be bonded as it shall deem necessary; for any amount or amounts as it may deem requisite, at the expense of the Society.

ARTICLE VIII. LOCATION

The principal office of this Society shall be located in the City of Pittsburgh, Commonwealth of Pennsylvania.

ARTICLE IX. DISSOLUTION

In the event of either voluntary or involuntary dissolution of the Society, the funds or assets of the Society, remaining after discharging all just debts of the Society or its officers in the name of the Society, shall be distributed without encumbrances to a non-profit group, organization or institution of learning engaged in the field of mass spectrometry within the contemplation of Section 170 (c) (2) of the Internal Revenue Code (1954). The selection of the recipient or recipients shall be made by the majority of the Board of Directors in office at the time of dissolution but in no event shall the assets be distributed to any member or members of the Society.

ARTICLE X. SOCIETY SEAL

The Society shall have a seal, upon which shall be inscribed the name of the Society, the year of its creation, and the word "Pennsylvania", the state of the Society's incorporation.

ARTICLE XI. AMENDMENTS

Section 1. A proposed amendment to the Constitution and Bylaws of the Society, signed by at least three members of the Board of Directors or by fifty members of the Society must be submitted to the Secretary at least fifteen weeks before the regular annual business meeting at the Annual Conference of the Society. The Secretary shall send or cause to be sent the proposed amendment to each member within a reasonable time and at least prior to thirty days before the Annual Conference. After the amendment has been discussed at that meeting, the Secretary will send a letter ballot on the proposed amendment as amended at the meeting to all Society members; if two-thirds of the votes obtained by letter ballot within six weeks are in favor of the proposed amendment, it shall be adopted.

Section 2. The Board of Directors shall also have the power to amend the Bylaws by a two-thirds vote of its membership.

Section 3. The Board of Directors is authorized to renumber articles and sections of the Constitution and Bylaws to correspond with any changes that may be made.

Section 4. Proceedings for removal of members of the Board of Directors shall be conducted as outlined above for amendments to the Constitution (Article XI, Section 1). If the request for removal is supported by two-thirds of the letter ballots received within six weeks, the Board member or members in question shall be removed from office and a replacement selected by the Board of Directors in accordance with Article VI, Section 4.

ARTICLE XII. ARCHIVIST

The Board of Directors shall appoint a Society Archivist whose duties shall be the collation and preservation of all documents deemed to be of historical interest. All records will be reproduced on microfilm from time to time and all of the original documents will be reviewed by the Archivist before they are disposed of. The Archivist shall report his activities to the Board of Directors annually and will report them to the general membership whenever appropriate.

The Archivist shall be nominated by the President and appointed to office by a two-thirds vote of the Board of Directors. The incumbent shall be reviewed annually and may be reappointed at the pleasure of the Board.

Expenses incurred by the Archivist on Society business shall be reimbursed.

BYLAWS

1. Membership

1.1 Application for membership shall be made in writing, on a form to be specified by the Board of Directors. The membership year shall commence January 1 and end December 31.

1.2 Membership becomes effective upon receipt by the Treasurer of dues and a properly executed application.

1.3 Failure to pay dues by four (4) weeks prior to the Annual Conference shall result in the requirement to pay nonmember registration fees for the Annual Conference in that year. Membership in the Society shall lapse if in arrears dues are not paid by December 31. Payment of current dues shall suffice for reinstatement; however, payment of nonmember registration will reinstate membership for that year.

1.4 Membership may be terminated without prejudice by written notice to the Secretary.

1.5 Members of record shall be defined as those members who have paid their dues prior to December 31 the preceding year and new members who have paid their dues prior to February 1 the present year.

1.6 Every member of record as of February 1 shall have the right to vote and to hold office.

1.7 The books, accounts and records of the Society shall be open for inspection to any member of the Board of Directors at any time. Members of the Society may upon written request to the Board of Directors, inspect such books, accounts and records of the Society at such reasonable times as the Board of Directors may designate.

1.8 The Secretary will prepare or cause to be prepared at least one annual membership report to the Board of Directors.

1.9 Members whose dues are in arrears shall not receive the Abstracts from the Annual Conference.

2. Collections and Disbursements

2.1 Membership classes and dues within such classes shall be determined by the Board of Directors.

2.2 The Board of Directors is authorized to determine separately a fee for each general meeting to cover the expenses of holding the meeting. Payment of this fee is a requirement for attendance.

2.3 Disbursements shall be made by approval of a majority of the Board of Directors. Disbursements in excess of \$2500.00 shall be made by check signed by two members of the Board.

2.4 The fiscal year of the Society shall be from January 1 to December 31.

2.5 The Treasurer will prepare or cause to be prepared an annual financial report to the Board of Directors.

3. Terms of Office and Election

3.1 The elected officers and directors-at-large shall hold office for a term of two years, except as otherwise provided in Article VI of the Constitution, with their term year commencing on July 1 and ending June 30.

3.2 Upon assuming office, the President shall appoint a nominating committee consisting of five members representing diverse areas of mass spectrometry. He shall not be a member of this committee. This selection shall be approved by the Board of Directors by a two-thirds vote. The committee shall serve two years concurrent with the tenure of the President. It shall nominate a slate of at least one candidate for each of three offices to be filled in alternate years according to the following rotation:

Even Years - Vice President for Programs

- Treasurer
- Member-at-large

Odd Years - Vice President for Arrangements

- Secretary
- Member-at-large, who shall serve in the capacity of Editor of the Conference Proceedings.

The committee will report in writing to the Board of Directors before June 30 each year. These reports will include a statement of each candidate's consent to serve and a professional resume for each candidate. Each slate of candidates and the resumes will be distributed to the members by the Secretary before September 15.

3.3 Any Society member can nominate another member for any office except President by submitting to the Secretary before January 15 of the election year:

- a. signatures of at least 50 Society members endorsing the nomination;
- b. signed statement of the nominee indicating his agreement to hold office if elected;
- c. professional resume of the nominee.

3.4 The election results shall be made known at the next annual business meeting and the new officers shall assume office as specified in paragraph 3.1 of the Bylaws.

4. Committees

4.1 The President shall appoint such committees as are necessary to accomplish the purposes of the Society. The terms of these appointments shall not exceed the term of office of the President. He may terminate a committee at any time. Members and chairmen may be reappointed by the incoming President.

5. Meetings

5.1 The Society shall meet at least once a year in order to present scientific papers and conduct business. Fifty members shall constitute a quorum for the transaction of business.

5.2 The time and place of the general meeting shall be determined by the Board of Directors.

5.3 Meetings of the Board of Directors may be called by the President or, in his absence, by the Vice President for Programs. Alternatively, two-thirds of the Board of Directors may call a meeting by written request to the President. Five directors shall constitute a quorum of the Board of Directors.

5.4 A simple majority of those present shall be required to pass any motion at any meeting of the members or Board of Directors, unless otherwise provided (see Article XI, Section 3).

5.5 Each Director shall be entitled to two weeks notice of any meeting of the Board of Directors.

5.6 Notice of the annual general meeting shall be sent to each member at least thirty days before the meeting.

5.7 The President shall, in response to a petition bearing the signatures of not fewer than twenty members and requesting that the Board of Directors consider a specified question, place this item on the agenda of the first meeting of the Board of Directors which is scheduled to convene on a date not preceeding the thirtieth day following receipt of the petition. The member whose signature appears first on the petition shall be entitled to two weeks notice of such meeting, and the same member or a member designated by him shall be entitled to attend such meeting and discuss the specified question with the Board of Directors.

5.8 Whenever any notice is required to be given by the provisions of the Bylaws, a waiver thereof in writing, signed by the person or persons entitled to such notices whether before or after the time stated therein, shall be equivalent to the required notice.

5.9 The President may appoint any member of the Society, not a member of the Board of Directors, to be parliamentarian for the duration of a given business meeting.

6. Compensation

6.1 No member of the Board of Directors of the Society shall receive salaries or fees for his services.

6.2 Members of the Board of Directors of the Society shall be reimbursed for all reasonable expenses incurred in connection with their official duties.

7. Miscellaneous

7.1 The incoming President shall cause the financial records of the Society to be audited within the first six months of his term of office.

7.2 The business of the Society shall be conducted in accordance with the latest edition of Roberts' Rules of Order unless otherwise specified herein.

ASTM E-14 1983 BUSINESS MEETING MINUTES SUMMARY

The Business Meeting of ASTM Committee E-14 on Mass Spectrometry was held on May 5, 1983, at the Boston-Sheraton Hotel. It was attended by 20 individuals representing industrial and governmental interests. The meeting was opened by Chairman, Thomas Aczel, who introduced the current Officers and ASTM Staff Manager:

Chairman: Thomas Aczel, Exxon Res. and Eng. Co., Box 4255, Baytown, TX 77520

Vice-Chairman: P.C. Price, Union Carbide Corp., Bldg. 770-120, Box 8361, S. Charleston, WV 25303

Secretary-Treasurer: B.N. Colby, S-CUBED, Box 1620, La Jolla, CA 92038

Minutes of the previous Business Meeting were accepted as published in the 1982 ASMS Abstracts. Current membership remains constant at 56 reflecting 7 resignations and 7 new members since June, 1982. The E-14 Financial Report indicates a balance of \$3,925.21 as of January 1, 1983.

The Chairman updated the members on the past years activities including last years Oil Shale Symposium, support of the ASMS Board of Directors and a \$500 contribution to the general Boston ASMS Program.

The ASTM Staff Manager provided a staff report. Included was an update on the status of Standards and Ballot Actions.

- 1) E-137 Revision - Approved July, 1982
- 2) E-899 New Standard - Approved September, 1982

Also presented were the next years schedule of events which included February 3, 1984 as the date Ballot items are due at ASTM for printing and mailing.

Subcommittee Reports were then heard.

Dallas Winter (for M.C. Hamming), Chairman of Subcommittee E-14.04 on Data and Information, indicated that there had been no activity during the past year.

Douglas Cameron, Chairman of Subcommittee E-14.10 on Definitions and Terms indicated that there would be a joint workshop later in the week with ASMS. He asked for volunteers to help with new definitions and terms in new areas. He has also been working on a new set of terms for balloting.

Jim Litton, Chairman of Subcommittee E-14.13 on GC/MS indicated that David Parees, who has agreed to take over as Chairman of E-14.13, and John McGuire had been working on the GC/MS Practice. D. Parees described how it had been difficult to get concurrence on various aspects of the practice when they were detailed. He indicated that in his opinion, only a "frame work" document would be possible. There was a general discussion of this approach and concurrence that it would be appropriate. John McGuire pointed out that ASTM Committee D-19 had prepared a GC/MS method for water analysis, that it had become a rather general document and that one of the problems they had encountered was a lack of input from "E" Committees.

Phil Price, Chairman of Subcommittee E-14.4 on General Practices, indicated that activities they had been working on were now complete and he requested new ideas. Some he suggested were: library search use, chemical ionization setup, environmental methods and drug methods. There was some question as to whether an "E" Committee could prepare a "Method"; the Staff Manager agreed to check on this as there could be overlap with D19, D34 or D2.

Douglas Cameron, Chairman of Subcommittee E-14.15 on Instrumentation, indicated that he had been working with ASMS in this area. There was a request for an approach for setting up a series of standard parameters through which mass spectrometers could be compared. This will be looked into.

Thomas Aczel, Chairman of Subcommittee E-14.41, Liaison, reported on D2 activities.

A report from the E-14 Officers Nominating Committee was provided by Dallas Winter through Mynard Hamming. Current nominees are:

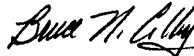
Chairman	- Phil Price
Vice Chairman	- Gil Greenwood
	- Dallas Winter
Secretary-Treasurer	- John McGuire

A second candidate for Secretary-Treasurer has yet to accept nomination.

No new business was discussed and the meeting was adjourned.

An informal Executive Committee Meeting was held by the Officers prior to the Business Meeting. They discussed the above topics and the status of interaction with ASMS.

Respectively Submitted,



Bruce N. Colby
Secretary-Treasurer

Proceedings of ASTM Committee D-2
Research Division IV, Section M on Mass Spectrometry

The annual meeting of ASTM D-2, Research Division IV, Section M on Mass Spectrometry was held at 1:15 p.m., May 9, 1983, in Boston, Massachusetts. Approximately 28 people attended including the ASTM representative, Ms. Louise Neall. (Names and affiliates are available from the Chairman.) The meeting was chaired by Thomas Aczel of Exxon Research. Items discussed and/or approved are summarized below.

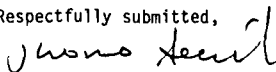
- Minutes of the 1982 meeting were approved by acclamation.
- T. Aczel reported on the status of the five D-2 methods, D-1658, D-2424, D-2498, D-2567, D-2601, that were proposed for discontinuation in 1982. This process is in its last stage and is being handled by ASTM headquarters.
- Gil Greenwood of Phillips reported on the status of the shale oil analysis. Fifty-five gallons of Paraho shale oil have been separated into 60-400°F and 400-1000 cuts (N content, respectively 1.03 and 1.28%) for HRMS and GC/MS analyses. The samples are being distributed to interested parties (contact G. Greenwood for list, or if interested in participating). Gil also discussed methods for chemical class separation and olefin analysis.

It was decided that available separation methods be evaluated by a small task group, and only consensus separated samples be distributed to round robin participants, so as to assure reasonably uniform samples. G. Greenwood will be in charge of this effort.

- Analysis of saturate samples was discussed by M. Story of Finnigan. Preliminary tests indicated some lack of accuracy when pure cyclohexane was added to a petroleum fraction; T. Aczel pointed out that this was not quite a fair test, as cyclohexane is too low boiling a compound. It was decided that performance of new instruments be compared not only by matrix analysis, but also by comparing the spectra mass by mass. T. Ashe suggested that samples be sent to all instrument manufacturers for this purpose. Although T. Aczel had a set of samples ready, it was decided that the sample to be used be the one analyzed by the Section in 1981-82, of which there is still ample supply in G. Greenwood's lab. T. Aczel will write to all manufacturers on the availability of this sample.

The meeting was adjourned at 2:10 p.m.

Respectfully submitted,



THOMAS ACZEL
Exxon Research and Engineering Co.
BARD Chairman

TA:mgm

COMPUTER APPLICATIONS COMMITTEE REPORT

Wednesday May 11, 1:15 PM

This year will probably go down as the year when computers took control of all phases of mass spectrometry. I, for one was quite impressed with the number of different applications presented at the meeting, and was glad, for example to see people finally using data systems to collect FAB spectra. The Computer Applications Committee contribution to A.S.M.S. consisted of sponsoring a "mini-symposium" on Inter Computer Communications (several posters Wednesday morning which seemed to be well attended). However, most suprising (and gratifying at least to your chairman) was the fact that a record number of people sought out our committee meeting on Wednesday afternoon, sucessfully negotiating the construction and finding the "Liberty G" room. For the first time in my memory the meeting was S.R.O. (roughly 50 people according to reliable estimates).

Unlike some previous meetings the major topic of discussion was NOT "Should the Computer Applications Committee continue to exist another year?" After a (very quick) review of past activities, shortened by the lack of an overhead projector (thanks to faulty preparation by your chairman), discussion commenced on what activities should be planned for next year. Everybody seemed to be involved in data processing from a different point of view, so no clear consensus emerged. Thirteen different topics were proposed, and subsequent discussion narrowed that down to three or four.

There was a general consensus that there should be some sort of "Introductory Lecture" next year on the subject of data processing. The overwhelming majority of mass spectrometry users are engaged in some sort of computer operations, and many at the meeting expressed an interest to know more about what data systems actually do (both good and bad) to their data. This was felt to be particularly important in light of the fact that many of the more recent entrants into mass spectrometry have probably never used a mass spectrometer without a data system. The general feeling was that such a lecture should encompass computer hardware and mass spectrometer interfaces, the flow of data within a typical data system, the use of high level languages, and the manner in which data systems have altered the way mass spectrometers are used. Particular emphasis should be placed on historical developments and user impact. (That should be sufficiently broad to allow the speaker to cover anything at all!)

In addition, Bob Minard volunteered (well, actually your chairman twisted his arm a bit) to organize a workshop on the general topic of "Personal Computers in Mass Spectrometry". As was evident from this year's meeting, Personal Computers are starting to gain a foothold in mass spectrometry, and this trend should continue given their low cost and increasing capabilities. So anyone with any ideas on this topic should write or give Bob a call. (Department of Chemistry, Penn State University, University Park, Pa. 16802, 814-865-2202)

The suggestion was also made that the Good Laboratory Practice committee be approached about the possibility of some sort of joint session on "Computers and G.L.P. Regulations" or a similar topic. Anyone with any other ideas is welcome to contact me at any time.

The meeting was formally adjourned at 2:00, although informal discussion continued among many of the participants for at least another half-hour.

Respectfully submitted


D. Thomas Terwilliger

ASMS EDUCATION COMMITTEE REPORT

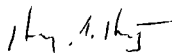
The Education Committee sponsored two short courses on the weekend preceding the Annual Conference on Mass Spectrometry and Allied Topics. The courses were held at the Sheraton Boston Hotel. The topics of the two courses were Interpretation of Mass Spectra and Quantitative Mass Spectrometry. The faculty for the two courses consisted of:

Dr. Patrick Callery, University of Maryland at Baltimore, Baltimore MD.
Dr. Robert Cotter, Johns Hopkins University School of Medicine, Baltimore, MD.
Mr. Peter Dreifuss, Bureau of Foods, F.D.A., Washington, D.C.
Dr. Henry M. Fales, National Institutes of Health, Bethesda, MD.
Dr. Harry S. Hertz, National Bureau of Standards, Washington, D.C.
Dr. John Strong, National Cancer Institute, N.I.H., Bethesda, MD.
Dr. Edward White V, National Bureau of Standards, Washington, D.C.
Dr. Alfred L. Yergey, National Institutes of Health, Bethesda, MD.

Based on comments received from the students, both courses were extremely successful, with the students achieving their education goals. The students recommended continuation of these courses with ASMS sponsorship, as a complement to the courses currently offered by a number of the mass spectrometer manufacturers.

In a committee meeting following the courses, a recommendation was made to add to the short courses offered at future meetings. The additional courses would focus on advanced topics, be offered on a rotating basis, and would be intended for the continuing education of members of the Society. It was also recommended that the Society reintroduce students half-priced tuition for the short courses. These short course slots would be made available after all full-paying students are accommodated. Finally, it was also recommended that if slots were still available after all tuition-paying students are accommodated, unemployed mass spectrometrists and other scientists with hardships be offered tuition-free enrollment.

Respectfully submitted,



Harry S. Hertz
Education Chairman

ENVIRONMENTAL COMMITTEE REPORT

Workshop: Sampling and Analysis of Hazardous Wastes

This workshop consisted of four discussions:

"Mass Spectrometry and the Love Canal", William L. Budde, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

"Dynamic Range Problems Associated with Hazardous Waste Analyses", B. Mason Hughes, Monsanto Research Corp., 1515 Nicholas Rd., Dayton, OH 45418.

"Emotional and Other Issues Related to the Analysis of 2,3,7,8-TCDD", Denis C. K. Lin, Environmental Testing and Certification Corp., 284 Raritan Center Pkwy., Edison, NJ 08837.

"Hazardous Waste: Sticking to Measurements and Avoiding Controversy", Larry R. Hilpert, National Bureau of Standards, Bldg. 222, Room A113, Washington, D. C. 20234.

The workshop was attended by about 120 people, discussion was active, and useful information was exchanged.

Committee Meeting

The committee meeting was held just after the workshop; it was attended by about 12 people. Discussion centered on symposia and workshops for the 1984 meeting. Two topics were suggested: "Dioxins - An Update" and "Alternate Ionization Methods for Environmental Analysis".

Submitted by

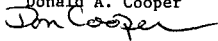
Ronald A. Hites
Indiana University
Bloomington, IN 47405

ASMS Committee on Forensic Applications

The committee convened with a small group in attendance (8).

It has been noted in previous years that attendance at the annual conference by the forensic community has been poor. This year that comment was again made and, therefore, constructive suggestions were solicited. It was pointed out that the obvious first step must be to create an awareness of what ASMS can offer the forensic mass spectrometrists, and that the only avenue available is mailings of the appropriate literature. Considering the manpower requirements entailed in this type of an endeavor, help was requested. Dr. Tom Munson (FBI Research and Training Facility, Quantico, Va.) volunteered his assistance in this task.

A discussion ensued concerning a perceived need for the placement of quality assurance programs in forensic mass spectrometry facilities. A consensus was not obtained on either the nature of such a program or methods of implementation.

Donald A. Cooper


GOOD LABORATORY PRACTICES WORKSHOP/COMMITTEE MEETING

The Good Lab Practices (GLP) Workshop was held on Monday, May 9, at 12:30. Even though scheduled across the lunch hour, 75-85 people attended.

Tony DeStefano, from Procter and Gamble, presented a brief history of efforts within ASMS to foster "good mass spectrometry lab practices". An ad hoc ASMS committee was formed in 1977; its work resulted in a document published in the bound conference proceedings (1), and in G. R. Waller's book (2). With editorial changes, this document had been issued as an ASTM method (3). Louise Neal, a staff representative from ASTM, attended the workshop and provided information on ordering copies of method E 899-82.

Don Cooper, of the Federal Drug Enforcement Agency, presented information on some forensic aspects of GLP. In addition to written records, he stressed maintaining a clear chain of custody for samples supporting any legal work. His lab encourages the use of registered mail, and heat welded sample pouches with tamper proof seals over the seams. Sample results are interpreted by comparison with spectra, collected on the same day, of authentic standards. This type of evidence holds up very well in court.

John Russell, from Mead Compuchem, gave a basic description of the EPA guidelines (4) for hazardous waste analyses, as practiced at his company. He noted that their Quality Assurance Officer checks the qualifications and experience of each instrument operator. There was some discussion of archiving and magnetic media. He suggested examining and exercising all tapes every three to five years.

Bill Budde, with the EPA's R&D lab in Cincinnati, provided some speculations on future directions of GLP. He felt that the next big push would be in the area of dioxin analyses; "even state governors are talking about high resolution mass spectrometry in news conferences". He recommended that more attention be paid to statistical work up of low level quantitative data, and suggested (5) and recent work by Ron Haas in Analytical Chemistry as relevant.

At the close of the session, I solicited requests for future topics to be discussed at the GLP meeting in San Antonio. Four areas recommended were: false positives and negatives during sub ppm analyses; library searching GLP's; nuclear chemistry and NRC aspects of GLP; pros and cons of chemist/mass spectroscopist licensing procedures. If there are more suggestions for topics, please contact me at (304) 747-5594.

Respectfully submitted,

Phil Price

Philip C. Price

1. 26th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 28-June 2, 1978. pp. 761-770.
2. G. R. Waller, Ed., Biochemical Applications of Mass Spectrometry, First Supplement, 1980. Appendix IV.
3. Standard Guide for Records Management in Mass Spectrometry Laboratories Performing Analyses in Support of Nonclinical Laboratory Studies, ASTM Method E 899-82, 1982. ASTM, 1916 Race St., Philadelphia, PA 19103.
4. EPA Test Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, EPA-600/4-82-057, July 1982. USEPA, 26 W. ST. Clair St., Cincinnati, OH 45268.
5. "Trace Analyses for Wastewaters", Environmental Science and Technology Vol. 15, 1426 (1981).

COMMITTEE REPORT

HIGH TEMPERATURES AND INORGANIC SOLIDS

Following a workshop on "Absolute Pressure Measurements," chaired by M. A. Frisch, our committee held a brief meeting. About 35 people stayed for this meeting. There was a general agreement about the success of the preceding workshop and about the usefulness of workshops in general. A number of suggestions were made about the need for a workshop on the electron impact ionization cross sections, since these play a crucial role in the thermochemical data derived from mass spectrometric measurements. Other suggestions for special Symposia were made, and we agreed to maintain contact about such possibilities. Attendance at the workshop was augmented by a group of 10 scientists representing the *Association Francophone de Spectrométrie de Masse des Solides* under the leadership of its president, Mme. Am. Andreani. Because of the unusually early date of the meeting this year, some of our colleagues were unable to attend because of a competing High Temperature Symposium sponsored by the Electrochemical Society and held in San Francisco during the same week.

Edmond Murad
Chairman

Committee Report

Ion Physics and Instrumentation

The committee sponsored a workshop on "Detection of High Mass Ions", held Tuesday, 10 May at 12:30 p.m. Panel members for the discussion were R. Beuhler (Brookhaven), J. Campana (Naval Research Laboratory), J. Gray (Galileo Electro-Optics) and K. Standing, who served as Chairman.

Topics discussed included the effects of postacceleration (Beuhler), detection of large alkali halide clusters (Campana) and proposed improvements in electron multipliers (Gray). Recent results from Orsay on the use of CsI coated surfaces for electron emission (le Beyec) were reported by the Chairman. O. Echt (Konstanz and Penn. State) reported on detection of large gas clusters and N. Milleron described a novel detector using superconducting films.

About 200 attended the workshop. It was followed by a short business meeting.

Respectfully submitted
K.G. Standing
University of Manitoba
Chairman.

Committee Report
Isotope Ratio Measurements

The 1983 ASMS meeting was extremely successful for the Isotope Ratio Committee. A total of 37 contributed papers were presented in oral and poster sessions under the headings of Isotope Ratios and Elemental Analysis by Mass Spectrometry. In addition, two workshops were held, resulting in a rather full three day schedule. The workshops, each of which attracted approximately 100 people, addressed the topics of Resonance Ionization Mass Spectrometry (RIMS) and the State of Systems Calibrations for Automated Mass Spectrometers. The workshops are summarized elsewhere in this volume. Jack Fassett, John Travis, and Ernest Garner are to be thanked for providing excellent speakers.

A number of suggestions for future programs were made at the Isotope Ratio Business meeting. One problem which surfaced was the co-scheduling of the Isotope Ratio and Solids sessions. Program Chairman, Graham Cooks, has indicated that an effort will be made in 1984 to avoid such conflicts whenever possible. Tentative plans for 1984 (pending ASMS Board approval) include workshops on statistical analysis of data and multiple collector systems for thermal ionization mass spectrometers, as well as a symposium on the rapidly advancing technique of Resonance Ionization.

Respectfully submitted,

Lura J. Powell
Chairman

ASMS COMMITTEE REPORT - QUALITATIVE ORGANIC TECHNIQUES

Technical Activities

WORKSHOP 1: FRAGMENTATION MECHANISMS IN SOFT IONIZATION MODES

The workshop was held on Tuesday, May 10, from 12:30-2 PM. About fifty people attended. The format was an informal discussion centered on fragmentation mechanisms for soft ionization mass spectra that have been suggested in the literature and in oral or poster presentations. Participants were asked to provide their own observations which supported or contradicted these mechanisms, or to contrast results obtained for samples which had been analyzed by more than one ionization method. Aspects of the discussion which evoked the most response were fragmentations from cluster ions, the likelihood of free radical and oxidation/reduction processes initiated by particle bombardment, and effects of varied matrices on the spectra observed. Some consideration was given to establishing criteria for acceptance by journal reviewers of postulated mechanisms. It was suggested that a tutorial-style session be held next year on the same general subject matter, with a few prearranged presentations and the opportunity for dialogue among participants.

WORKSHOP 2: PHOTOPLATE USERS DISCUSSION GROUP

The workshop was held on Wednesday, May 11, from 5:00-6:15 PM. About twenty-five members attended. The session provided an opportunity for this small interest group to compare common problems, to exchange procedural details, to assess the future needs of photoplate users, and to discuss these areas with invited guest Joe Masters, the producer of Ionomet plates, who is a local resident.

Business Meeting:

A brief business meeting was held at the end of the Tuesday workshop, attended by the captive participants. Suggestions for future workshops and candidates for chairing the committee were requested. Interest in further exploration of the workshop topic was expressed by several members.

Respectfully submitted,

Catherine E. Costello

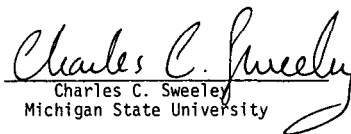
Catherine E. Costello

Mass Spectrometry Facility
Department of Chemistry
M.I.T.

ASMS Committee on Quality Assurance in Quantitative
Mass Spectrometry

The committee sponsored a symposium entitled Quality Assurance in Quantitative Mass Spectrometry on Wednesday, May 11, 1983. This was a joint session with the ASTM Committee E-14 on Mass Spectrometry, which was organized by Stuart Scheppele and Charles Sweeley. The speakers were Terrence Ashe (Exxon), Stuart Scheppele (U.S. Department of Energy), John Hayes (Indiana), Roger Foltz (Utah), Dennis Lin (Environmental Testing and Certification Corp.) and Harry Hertz (National Bureau of Standards). Attendance averaged 150 during the symposium.

The committee also sponsored a work-shop on Quantitation of Isotopic Enrichment in Gases Derived from Biological Samples, which was organized by Dwight Mathews (Washington University). Dr. Mathews has provided a separate report of this work-shop.


Charles C. Sweeley
Michigan State University

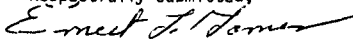
ASMS COMMITTEE REPORT
Workshop on Automated Mass Spectrometry

The Workshop on Automated Mass Spectrometry was held on May 12, 1983 and attended by 75 to 100 registrants of the meeting. The purpose of this workshop was to provide a forum for assessing the state-of-the-art in calibrating measuring circuits of automated mass spectrometers. Brief presentations were made by Larry Callis of the Argonne National Laboratory and Bill Boyce of the Savannah River Plant. The presentation by Mr. Callis focussed on the instrument modifications and the procedures developed for a systems calibration accurate to $\pm 0.02\%$. The presentation by Mr. Boyce took a slightly different direction and identified some of the problems in calibrating a mass spectrometer under plant or field conditions. This work indicated a level of accuracy in the calibration between 0.1% and 0.25%.

The presentations were followed by a lively and thorough discussion addressing accuracy requirements of automated mass spectrometry. Although needs in this area were not clearly identified, there was a general consensus that field measurements required less accuracy than the state-of-the-art (0.02% accuracy). Participants felt that more work in this area could lead to specifications for high accuracy laboratory needs as well as the day-to-day needs of the nuclear plant.

In addition to a number of regular attendees of the ASMS Meeting, this workshop also drew a number of "old timers" who had not been active for a number of years. This response was very gratifying and is additional evidence of revitalization of the isotope ratio community.

Respectfully submitted,



Ernest L. Garner
Inorganic Analytical Research Division
Center for Analytical Chemistry
National Bureau of Standards

WORKSHOP REPORT ON CARBOHYDRATE SEQUENCING

With the growing physiological interest in carbohydrate biopolymers and associated glycoconjugates, and the increased involvement of mass spectroscopists in the efforts to resolve these problems, a workshop was organized and convened for sharing current problems and progress.

Panel Members Present:

Dr. Steven Carr, Harvard Medical School, Boston, MA. 02115

Dr. Anne Dell, Imperial College of Science and Technology, London, England.

Dr. Karl-Andres Karlsson, University of Goteborg, Goteborg, Sweden.

Dr. Charles Sweeley, Michigan State University, East Lansing, MI. 48824.

The chairman summarized the recent physiological interest in glycoconjugates and carbohydrate polymers and discussed information that was needed to resolve problems of structure. Dr. Sweeley related interesting early history of glycolipid structural determination of which he had been a part and discussed the limitations and advantages of the current analytical approaches. Further comments were provided on the GC analysis of permethylated oligosaccharide alditols using short (15 m) fused silica capillary columns.

Dr. Dell discussed the progress to date with polysaccharide analysis using FAB-MS and related details of matrix preparation with ammonium thiocyanate, glycerol, and thioglycerol. The solvent in which the sample is added to the matrix was also suggested to strongly influence the results. Some rationale for fragmentation was presented along with complications due to the misassignment of sequence structure. These problems were observed in the FAB spectra of underivatized samples. For samples up to approximately 4000 μ , Dr. Dell commented that FAB-MS was adequate and the samples could be analyzed directly. To gain the maximum in structural information however, polymers up to 7000 μ were usually derivatized by acetylation or permethylation.

Dr. Karlsson outlined the experimental approach for the analysis of glycolipid materials using "in beam" EIMS and commented very convincingly on the advantages of this procedure in preference to FAD analysis. Several comments and contributions were taken from the participants relative to glycolipid analysis. Dr. Carr continued the glycolipid analysis discussion by describing studies of these materials using ammonia DCI-MS. The DCI spectra show greater structural detail than FAB, but are simpler than those obtained by EI-MS. The samples studied, however, were 2000 μ or less and it was unclear if these advantages could be observed at higher mass.

The topic next on the agenda was the analysis of chondroitin sulfates by FAB-MS which was presented by Dr. Carr. This approach yielded molecular weight information as well as considerable structural detail including sequence and, from comparative studies, positional assignment of the sulfate residue. The need for FAB to analyze these compounds directly in their underivatized state was stressed since chemical manipulation causes loss of the sulfate residue.

Dr. Coles contributed a few comments and discussed the importance of HPLC purification of carbohydrate oligomers previous to mass spectral analysis. The limitations of carbohydrate detectability in HPLC effluents required that the samples be derivatized and he described pre-derivatization approaches with fluorescent groups.

The last topic discussed at the workshop was a new approach to carbohydrate sequencing centered around partial reductive cleavage of permethylated oligomers. The procedure appears to avoid some of the limitations of hydrolytic depolymerization and the products are amenable to further analysis by DCI. This discussion was shortened because details were to be presented in Poster FPA #9.

The meeting was attended by 27 people and concluded after two hours.

Vernon N. Reinhold, Ph.D.
Harvard Medical School

Workshop Report

Internal Energy Effects in Collisional Activation - Part II

This workshop was a continuation of that held at the last ASMS meeting in Honolulu. It was fitting that it took place on the evening of Fred McLafferty's 60th birthday, an event that enabled all the participants (80 - 100) to make a suitable vocal contribution at the start of the workshop.

As in the previous year, the various prepared contributions provoked animated discussion although there was perhaps a wider measure of agreement on the factors over which one must take care in interpreting spectra of fragments produced by collision-induced decompositions. A continuing difficulty is that of devising methods of producing ions of a single, known structure but it was agreed that if this can be achieved and if only high energy processes are considered, internal energy effects are rare and spectra are reproducible to within $\pm 5\%$. Exactly how one defines the lower energy processes still appears to be controversial: if it is set too low, internal energy effects may appear but if it is set too high, the percentage of the total ion current used for structure determination may be unacceptably low. In cases for which there are large barriers both to isomerisation and to fragmentation, it is possible to prepare ions with a wide range of internal energies and internal energy effects on the spectra can then be observed since in these cases, all fragmentations are what would normally classify as high energy processes.

The situation is less clear-cut when two or more structures may be present in the ion beam. An apparent internal energy effect may then be more correctly attributed to a change in the relative abundances of the different isomers although to be certain that this is the correct interpretation, it is of course desirable that spectra be obtained for each isomeric form of the ion. A number

of practical problems were discussed and it emerged that although the majority of groups adjust the collision gas pressure to alternate the beam by a given fraction, a sizeable minority use a constant collision gas pressure.

Finally, the subject of angle-resolved mass spectrometry was discussed and it was soon apparent that, at present, there is no general agreement on the fundamentals of this topic. The translational energy converted into internal energy on collision rises as the scattering angle increases but complications arise from the release of internal energy as translational energy of the fragments. The angle of observation is then not readily related to the scattering angle and at present, it is not clear if a simple relationship is to be expected between E and θ . Work in progress in several laboratories is expected to throw some light on this in the near future and this topic would form the basis of a future workshop.

Keith Jennings

Lasers for Isotope Ratio Measurements: Practical Aspects and Limitations

The Isotope Ratio Committee sponsored the second workshop in two years on the use of lasers for isotope ratio measurements. This year's workshop was held on Wednesday, May 11, with approximately 100 people in attendance. The emphasis of the workshop was on RIMS, an acronym referring to Resonance Ionization Mass Spectrometry, a technique which weds atomic spectroscopy and mass spectrometry. In contrast to the previous year's workshop in which experimental results were limited, four or five laboratories have made experimental breakthroughs in the past year. Representatives from three of these laboratories made presentations on key aspects that affect the long-term utilization of lasers for isotope measurement in mass spectrometry.

Professor Nicholas Winograd from Penn State University led off the session by discussing the atomization process with the emphasis on the pulsed sputtering source used in his experiments. The sputtering process and the use of energy discrimination to distinguish secondary ions (SIMS) from laser-produced ions (RIMS) were reviewed. Critical aspects of timing in order to achieve maximum sample utilization were discussed. Results were presented for a model system in which In surfaces were examined. In reply to questioning from the audience concerning the use of laser ablation to produce atomization from a solid sample surface, Professor Winograd expressed the opinion that the sputtering process is capable of providing a more controlled input of energy, permitting the study of either surface or bulk properties, and was better suited for surface science investigations.

The second speaker at the workshop was Dr. David Donahue from Oak Ridge National Laboratory. Dr. Donahue discussed the use of a thermal vaporization source combined with a pulsed laser system. He reviewed the fundamentals of RIMS and defined the duty cycle when a continuous atom source and a pulsed laser are combined. He also outlined and briefly discussed competing processes that could occur in thermal vaporization and that could complicate spectroscopy and measurement in RIMS. It is now obvious that low-lying excited states are significantly populated in vaporization and metastable, as evidenced by the transitions observed which originate from these low-lying energy states.

The final presentation was made by Drs. Nicholas Nogar and Charles Miller from Los Alamos National Laboratory. Dr. Nogar, who participated in the previous year's workshop, discussed theoretical calculations which compared the relative merits of CW versus pulsed lasers in terms of maximum sample utilization when applied to a thermal (continuous) source. Dr. Miller punctuated the theoretical discussion with recent experimental results which demonstrated sample utilization efficiencies (ions detected divided by atoms loaded) on the order of 10^{-5} for the element Lu. This demonstration surpassed the theoretical prediction, though still about 10-fold less sensitive than thermal ionization (but with spectral selectivity).

The workshop served to emphasize the rapid advances made in the combination of mass spectrometry and laser spectroscopy. As Dave Donahue commented, it is making a lot of mass spectrometrists relearn optical spectroscopy (and vice versa). And, as Nick Winograd commented optimistically, it may significantly impact measurement technology in a number of fields. The analytical chemistry and surface science applications discussed in the workshop are already truly exciting.

Submitted by

J. D. Fassett
J. C. Travis

Workshop Organizers

ASMS NOMENCLATURE COMMITTEE WORKSHOPS

Boston, 1983

Two workshops were held this year. The first was on Monday, May 9, and the second on Thursday, May 12. The attendance was low for both workshops, no more than 30 people at any one time, but this was a sufficient number to make some progress and still have a wide cross section of ideas and opinions. There was a general consensus that this committee should be focusing its attention to the more current areas in the field of mass spectrometry in an effort to avoid unnecessary confusion where possible. A significant portion of the second workshop was devoted to this ideal.

The attached list is divided into two parts. The first section consists of terms that were agreed upon at the workshops and are being sent to the ASMS Board for approval. The second section is a list of terms for consideration at next year's meeting. In addition to this section there are the definitions on secondary ion mass spectrometry and ion mass that were listed in the 1982 Bound Volume that are to be considered next year.

All comments on this year's list of definitions or suggestions for future consideration should be sent to:

Doug Cameron
Science and Technology Division
Union Oil Company
P.O. Box 76
Brea, CA 92621
(714)528-7201

Doug Cameron

Doug Cameron,
Chairman

DEFINITIONS FOR APPROVAL

ANALYZERS

Electrostatic Analyzer. A velocity focusing device composed of means for producing an electrostatic field perpendicular to the direction of ion travel. Effect is to bring to a common focus all ions of a given kinetic energy. Usually used in combination with a magnetic analyzer for mass analysis.

Magnetic Analyzer. A direction focusing device composed of means for producing a magnetic field perpendicular to the direction of ion travel. Effect is to bring to a common focus all ions of a given momentum with the same mass to charge ratio.

Quadrupole Analyzer. A mass filter consisting of means of creating a quadrupole field of a constant component and a varying component in such a manner as to allow transmission of only a selected mass-charge ratio.

Time of Flight Analyzer. A device consisting of a means to measure the flight time of particles with an equivalent kinetic energy over a fixed distance.

Wien Analyzer. A velocity filter composed of means for creating crossed homogeneous electric and magnetic fields such that only ions of a fixed velocity are transmitted.

Mass Resonant Analyzer. A mass analyzer composed of means for mass dependent resonant energy transfer and measurement of the resonance frequency, power or ion current of the resonant ions.

(The following are standard instrumental configurations utilizing one or more of the above techniques.)

Double Focusing Analyzer. The combination of a magnetic analyzer and electrostatic analyzer in either sequence to effect direction and velocity focusing.

Ion Cyclotron Resonance Analyzer. A device to determine the mass of an ion by measuring its resonant frequency.

Ion Trap Analyzer. A mass resonance analyzer composed of means for creating a three dimensional rotationally symmetric quadrupole field capable of storing ions at selected masses.

Mass Spectrometer Configurations. Multianalyzer instruments should be named for the analyzers in the sequence in which they are traversed by the ion beam, where B is for a magnetic analyzer, E for an electrostatic analyzer, Q for a quadrupole analyzer, TOF for time of flight analyzer, and ICR for an ion cyclotron resonance analyzer. For example, we have a BE mass spectrometer ("reversed" geometry double focusing instrument), BQ mass spectrometer (hybrid sector and quadrupole instrument), EBQ (high resolution followed by a quadrupole). Note that a triple quadrupole which has two mass analyzers is a QQ mass spectrometer. Problem: Time of flight, simultaneously or sequentially with other mass analyzers.

IONIZATION

Desorption Ionization (DI). General term to encompass the various procedures (secondary ion mass spectrometry, fast atom bombardment, californium fission fragment desorption, thermal desorption) in which ions

are generated directly from a condensed phase sample by energy input.

Note: Intent is to establish a broad term analogous to chemical ionization which also encompasses a group of related ionization processes.

SAMPLE INTRODUCTION

Sample Introduction System. This is a system used to introduce sample to a mass spectrometer ion source. (Sample Introduction System, Introduction System, Sample Inlet System, Inlet System, and Inlet are synonymous terms.)

Reservoir Inlet. This is an Inlet System having an enclosed volume (the reservoir), with provision to evacuate the reservoir, to admit sample to the reservoir, and to allow gas or vapor from the reservoir to flow through a leak to the mass spectrometer ion source. A complete description of the Reservoir Inlet should include a description of the method by which the sample is introduced into the reservoir.

Batch Inlet. This is the historic term for a Reservoir Inlet. Reservoir Inlet is preferred because a Direct Inlet Probe is also a form of Batch Inlet. Batch Gas Inlet or Batch Vapor Inlet is, however, a completely descriptive term.

Dual Viscous-Flow Reservoir Inlet. This is an inlet having two reservoirs, used alternately, each having a leak that provides viscous flow. This inlet is used for making precise comparisons of isotope ratios in two samples.

Continuous Inlet. This is an inlet in which gas or vapor passes continuously into a mass spectrometer ion source, as distinguished from a Reservoir Inlet or a Direct Inlet Probe.

Non-Fractionating Continuous Inlet. This is a Continuous Inlet in which gas flows from a gas stream being analyzed to the mass spectrometer ion source without any change in the conditions of flow through the inlet or by the conditions of flow through the ion source.

Direct-Inlet Probe. This is a rod having a sample holder at one end, which is inserted into the vacuum system of a mass spectrometer through a vacuum lock, placing the sample near to, at the entrance of, or within the ion source, so that the sample can be vaporized after introduction to the vacuum system by heat from the ion source or by heat applied to the probe from an external source. (Direct Inlet Probe, Direct-Introduction Probe or Direct-Insertion Probe are synonymous terms. The use of DIP as an abbreviation for these terms is not recommended.)

Vacuum-Lock Inlet. This is an inlet in which a sample is placed in a chamber, the chamber is pumped out, and a valve is opened so that the sample can then be introduced to the mass spectrometer ion source. A Vacuum-Lock Inlet commonly uses a Direct-Inlet Probe which passes through one or more sliding seals, but other kinds of Vacuum-Lock Inlets are possible.

Extended Direct-Inlet Probe. This probe provides for insertion of a sample on an exposed surface (such as a flat surface or a wire) into (rather than up to the entrance of) the ion source of a mass spectrometer. (This term is synonymous with Direct-Exposure Probe.)

Crucible Direct-Inlet Probe. With this probe, the sample is held in a cup-shaped device (the crucible) rather than on an exposed surface. A Direct-Inlet Probe is assumed to be a crucible type unless otherwise specified.

GC/MS Interface. This is an interface between a gas chromatograph and a mass spectrometer which serves to provide continuous introduction to a mass spectrometer ion source of effluent gas from a gas chromatograph during the period for which the effluent gas is to be analyzed.

Direct GC/MS. This is an interface in which the entire effluent from the gas chromatograph passes to the mass spectrometer ion source during an analysis, without any splitting of the effluent.

Splitter GC/MS Interface. This is an interface in which the effluent from the gas chromatograph is divided before admission to the mass spectrometer, without enrichment of sample with respect to carrier gas.

Separator GC/MS Interface. This is an interface in which the effluent from the gas chromatograph is enriched in the ratio of sample to carrier gas. (Separator, Molecular Separator, and Enricher are synonymous terms.) A separator should generally be defined as an Effusion Separator a Jet Separator, or a Membrane Separator.

Effusion Separator (or Effusion Enricher) This is an interface in which carrier gas is preferentially removed from the gas entering the mass spectrometer by effusive flow (e.g. through a porous tube or through a slit).

Jet Separator. This is an interface in which carrier gas is preferentially removed by diffusion out of a gas jet flowing from a nozzle. (Jet Separator, Jet-Orifice Separator, Jet Enricher and Jet-Orifice are synonymous terms.)

Membrane Separator. With this separator, the gas or vapor passes to the mass spectrometer through a semi-permeable membrane (e.g. a silicone membrane) which selectively transmits organic compounds in preference to carrier gas. (Membrane Separator, Membrane Enricher, Semi-Permeable Membrane Separator, and Semi-Permeable Membrane Enricher are synonymous terms.)

Solvent-Divert System. This system is used in conjunction with an interface which permits temporary interruption of the flow from a gas chromatograph to a mass spectrometer by opening a valve to a pumping line, so that an effluent present at a high concentration (usually solvent) does not enter the mass spectrometer ion source at a high concentration.

Liquid Chromatograph/Mass Spectrometer (LC/MS) Interface. This interface is between a liquid chromatograph and a mass spectrometer which serves to provide continuous introduction to a mass spectrometer ion source of the effluent from a liquid chromatograph during the period for which the effluent is to be analyzed.

Moving Belt (Ribbon or Wire) Interface. With this interface, all or a part of the effluent from a liquid chromatograph is continuously applied to a belt (ribbon or wire), which passes through two or more orifices, with differential pumping, into the mass spectrometer vacuum system; after which heat is applied, to remove the solvent, and then to evaporate the solute into the ion source.

Direct Chemical Ionization Interface. With this interface, all or a part of a liquid chromatograph effluent passes continuously to the mass spectrometer, in which the solvent is used as a chemical ionization agent for ionization of the solute.

ION/MOLECULE REACTIONS

Collision-Induced Dissociation (CID). The fragmentation of a polyatomic ion due to the collision of the ion with a target, usually a neutral gas molecule. This is brought about by conversion of part of the ion's translational energy to internal energy during the collision. (Collision Activated Dissociation (CAD) is a synonymous term, but Collisional Activation is not recommended.)

Collisional Activation (CA). This refers to the increase in internal energy of an ion as the result of a collision between the ion and a target, usually a neutral gas molecule or atom. (The ion may decompose subsequently.)

Note: The definitions for the terms collision-induced dissociation and collisional activation are modifications of the definitions printed in the 1981 "Bound Volume."

Definitions for Consideration:

Nominal Ion Mass. The mass of an ion for a given empirical formula calculated using the integer masses of the most abundant isotope of each element, e.g. C=12, H=1, O=16.

Monoisotopic Ion Mass. The mass of an ion for a given empirical formula calculated using the exact mass of the most abundant isotope of each element, e.g. C=12.00000, H=1.007825, O=15.9949.

Average Mass. The mass of an ion for a given empirical formula calculated using the atomic mass of each element, e.g., C=12.011, H=1.00797, O=15.999.

Preformed Ions. Vapor phase ions that existed as ionic species in the condensed state.

Thermal Desorption. The removal of ionic or neutral species from the condensed state by the input of thermal energy into the condensed state. The mechanism of energy input should be specified.

Dynamic Headspace Gas Chromatography/Mass Spectrometry. The distillation of volatile and semivolatile compounds into a continuously flowing stream of carrier gas and onto a gas chromatographic column. This is followed by mass spectrometric analysis of compounds eluting from the gas chromatograph.

WORKSHOP: ORGANIC THIN FILM ANALYSIS

This workshop focussed on the task of characterizing organic films using mass spectrometry, an area in which continued advances should usher in the day when organic layers several micrometers thick may be depth profiled while retaining molecular information. Bill Katz from G.E. summarized depth profiling studies performed on a Cameca IMS-3f with O_2^+ bombardment. Rather than attempt to detect molecular species, Bill examined a two layer film in which one part was deuterated by obtaining H^- and D^- profiles. Such experiments successfully reproduced the actual film profile for samples as thick as seven μm , with charge compensation achieved by first coating the sample with gold. Depth profiles of heat treated samples showed that the two organic layers diffused into each other, highlighting the wealth of information obtainable in atomic ions alone.

Mark Ross of NRL discussed the use of a liquid gallium/indium matrix for molecular analysis on a Cameca IMS300. He noted that indium cationized molecules dominated $(M + Ga)^+$ ions even though Ga^+ was more intense than In^+ , and that molecules with carbonyl groups failed to yield molecular ions. Both these effects point to strong interactions between this matrix and the sample. It was clear, though, that molecular ion detection and oxygen bombardment are not mutually exclusive. Joe Campana, also of NRL, described an ion beam neutralizer that produces focussed beams for insulator analysis. One advantage of the device, which involves neutralization of the ions as they pass by an electron-emitting filament, is its adaptability to virtually any kind of mass spectrometer system. Gary Byrd of NBS closed out the session by outlining the characterization of thin organic layers absorbed on carbon particles using laser desorption/TOF techniques. He concluded that film analysis was possible in the LD experiment if one took data with the laser grazing the sides of the particles.

Committee: **Quantitative Organic Analysis**

Workshop: **Quantitation of Isotopic Enrichment in Gases Derived from Biological Samples**

Panel Members: D.E. Matthews, Washington University School of Medicine
D.A. Schoeller, University of Chicago School of Medicine
J. Santrock, Indiana University
A. Barrie, VG Isogas
M. Jeevanandam, Sloan-Kettering Cancer Center

Dwight Matthews introduced the workshop by defining the primary use of isotope ratio mass spectrometry (IRMS) in biomedical research, i.e. the measurement of exogenously administered stable isotopes to serve as metabolic tracers in vivo. Although GCMS is used extensively for this purpose, the sensitivity of GCMS for measuring isotope dilution is limited (ca. 0.1 atom % excess). In contrast, IRMS has been developed over the years to measure specifically very small differences in isotopic content. IRMS is required to measure the very high dilution of tracer when the tracer enters either pools of very high flux or pools with very slow turnover. Many of the important principals of IRMS summarized in this workshop were discussed in detail in an earlier talk by Professor John Hayes (WOB3).

Dale Schoeller described his methods for preparation of CO₂ for ¹³C and ¹⁸O measurement: dried, organic material is converted to CO₂ by placing samples in small quartz tubes containing small amounts of combustion catalyst, sealing the tubes, and heating to 850° C to force combustion. The resulting CO₂ is easily removed from the tubes and separated from the other combustion products for ¹³C measurement by IRMS. The ¹⁸O in the organic sample is lost in this procedure. The water ¹⁸O in physiological fluids can be directly captured via exchange of CO₂ and water oxygens. Fluid samples (ca. 1 ml) are placed in Vacutainer tubes containing CO₂ and equilibrated at a constant temperature. Because the amount of starting CO₂ is minor compared to the amount of water, the equilibrated CO₂ reaches an ¹⁸O content equal to the starting water ¹⁸O.

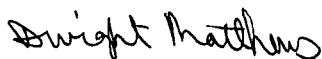
Jeff Santrock described a new method for pyrolyzing organic material while conserving the oxygen isotopic content of the sample. This work is described in more detail in the abstract by Santrock and Hayes appearing in this volume (TOB8). Briefly, the organic sample is pyrolyzed without the addition of extra oxygen. The resulting mixture of CO and CO₂ is reduced to CO and, then, oxidized with I₂O₅ to CO₂. The entire process is continuous and automated. Because of significant sample memory and background, an iterative correction procedure has been developed to account for and remove these factors.

Andy Barrie described a method to measure simultaneously ²H and ¹⁸O and to measure the ¹⁸O directly as water. The system, offered commercially by VG Isogas, contains two mass spectrometers: one for measuring ²H from H₂ gas and another for measuring ¹⁸O as water. The two mass spectrometers share a common heated inlet system into which μ l amounts of water are injected. The water flows both directly into the one mass spectrometer for ¹⁸O measurement and into a uranium reduction furnace to convert water to H₂ as it enters the second mass spectrometer for ²H measurement. The single inlet is used alternately for sample and reference water aliquots. Because of inlet memory, each sample is injected in replicate.

Malayappa Jeevanandam described methods for converting organic samples to N₂ gas and the pitfalls associated with measurement of ¹⁵N from N₂. In contrast to the methods for ¹⁸O, most of the procedures for ¹⁵N preparation and measurement were developed over 40 years ago. The primary methods for N₂ preparation remain either direct combustion or Kjeldahl digestion to NH₃ followed by hypobromite reduction. One novel method is the direct reaction of urea with hypobromite: the reaction is monomolecular for urea, conserving the urea di-¹⁵N isotopic content in the N₂ gas. Because the nitrogen preparation apparatus and the mass spectrometer are surrounded by a sea of N₂, leaks are critical to the ¹⁵N measurement and must be kept to a minimum.

Because of the limited time available, the Quantitative Organic Analysis Committee did not convene a formal meeting after the workshop. Rather, the committee chairperson, Charles Sweeley, requested that interested parties send suggestions concerning the future direction of the committee directly to him.

Report submitted by:

A handwritten signature in dark ink, reading "Dwight Matthews". The signature is written in a cursive style with a large, prominent 'D' and 'M'.

Dwight E. Matthews
Metabolism Division
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WORKSHOP - STANDARD OPERATING CONDITIONS FOR THE ACCUMULATION OF LIBRARY MS/MS SPECTRA AT LOW COLLISION ENERGIES - PART II

As reflected in the title, the workshop was extended to include hybrid (BQQ) and other instruments besides triple quadrupoles so long as they operate with low (0-100 eV) collision energies. About sixty people participated in the workshop (despite the noontime scheduling) showing a steady growth of interest in low energy MS/MS. The workshop began with a rapid review by the chairperson of developments since Part I last year, summarizing the results of the first round robin, which had already been presented in more detail in a poster session (see abstract WPA 15). The reasons for the revised proposals were outlined. These attempt to set the collision energy and amount of target gas (target thicknesses) to an arbitrary standard condition by specifying ratios of daughter ion yields such as 92/91 and 65/91 from the amylbenzene parent.

A general and rather invigorating discussion then took place. It was again necessary to emphasize that there is no intent to impose operating conditions for targetted analysis or to limit the range of instruments. Obviously, optimum conditions for the targetted problem at hand are explored and then utilised. The intent is firstly to have a means of comparing through "standardisation" the operating conditions in different laboratories so that spectra can be transferable. Secondly, there are clear advantages in building up transferable libraries of spectra when analysis is investigative rather than targetted i.e. with spectra accumulated under known conditions (perhaps eventually with several options to suit various compound classes).

The points of view expressed in the meeting can be (unfairly ?) summarized in three groups (1) the optimists: - if the MS/MS technique at low collision energies is to develop its full potential, it is essential that an attempt be made to establish the limits of transferability. The use of MS/MS in investigative analysis is inhibited by the lack of libraries. (2) the pessimists: - the aim is very noble, but chances of success are small (zero ?) because there are too many variables to be controlled (method of ionization, set-up of the quadrupole instrumentation etc.). Should one not attempt (or have to attempt) to specify all these factors? (3) the nihilists: - everyone should be content in his/her own microcosm and comparing spectra is an unnecessary exercise.

The intriguing question of multidimensional libraries and library searching was raised. This would make use of the power of low-energy MS/MS with, for example, collision energy as a third dimension. The present chairperson prefers to postpone multidimensional standardisation efforts for future holders of that position.

The discussion concluded with the suggestion that the value of continuing the efforts could best be measured by the willingness of laboratories equipped with MS/MS to participate in a second round robin using the revised proposals. Over 25 groups have expressed their interest so far, a very satisfactory response. The round-robin will be organised in the next few months and we should soon at least know what the present limitations are. The present proposal is far from ideal and other possibilities should be considered. One needs a parent which dissociates to give (a) two daughters similar in mass whose yield ratio depends largely on energy and not on the amount of target gas and (b) two other daughters whose ratio depends mostly on the amount of target gas.

If the transferability of spectra proves possible within useful limits, the question remains as to who will make the effort (and provide the resources) to build up a library of a significant size.

P.H. Dawson
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HIGH TEMPERATURE INORGANIC COMMITTEE WORKSHOP REPORT

Absolute Pressure Measurement by Mass Spectrometry

Tuesday, May 10, 1983 12:30 P.M.

Panel Members:

David W. Bonnell	NBS
Jean D. Drowart	Vrije University of Brussels
Margaret A. Frisch	IBM Research
Donald L. Hildenbrand	SRI International
Kenneth A. Lincoln	NASA, Mountain View

The workshop was attended by about 50 persons. Jean Drowart started the discussions by laying down the fundamental principles which must be considered, namely ionization cross sections, ion extraction efficiency, transmission through the analyzer and multiplier efficiency. Calibration of the total system is usually done by evaporating a known amount of a substance, e.g. silver and integrating the ion signals during the course of the heating. This method leads directly to a calibration value which does not depend on making estimates on the above parameters. However, reasonable estimates of calibration constants for more complex systems can be derived in a relative manner through ratioing the above parameters. In the discussion that followed, using the additivity method for estimating molecular cross sections was found not satisfactory. If desperate, the square root resulted in a better estimate. Also, experiments which attempt to measure absolute cross sections give results which are uncertain by at least 20%.

Margaret Frisch showed experimental results on the calibration of O_2 from the decomposition of $RuO_2(s)$, using the direct method by weight measurement. Her data was consistent over the course of several experiments within $\pm 10\%$. Dave Bonnell presented experimental results where Knudsen effusion and high pressure transpiration measurements were in very close agreement. Ken Lincoln then discussed possible methods he has been exploring to calibrate pulsed laser vaporization of surfaces using time of flight mass spectrometry. He presented some data on a technique where the vacuum valves are closed and the pulse of gas is expanded into the chamber. Don Hildenbrand, in absentia, submitted some charts on their methods and experience at SRI. They use calibration standards (Au, Sn) and determine K at a specific electron energy. These constants are related to other atoms using cross sections from J. B. Mann. Don stressed that they generally evaluate pressures from parent ion signals measured at low electron energy and apply a correction for fragmentation where applicable.

A short committee method followed the workshop. Because of the IUPAC meeting next May and the summer Gordon Conference on High Temperature Chemistry, it was decided only to have a workshop for the 1984 ASMS meeting. For the 1985 meeting at San Diego the group felt a symposium on a subject which covers two or more disciplines would be appropriate. One of the allied topics suggested would be on laser vaporization and the deviations from equilibrium.

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WERNESS STEPHEN C	WARNER LAMBERT	2800 PLYMOUTH RD	ANN ARBOR	MI	48106
WESDEMOTIS CHRYS	709 TPIPHAMMER RD		ITHACA	NY	14850
WESTMORELAND D G	ROHM & HAAS CO		SPRING HOUSE	PA	19477
WESTON A F	OCCIDENTAL CHEMICAL	RESEARCH CTR	GRAND ISLAND	NY	14072
WHITE E	NBS	A113 CHEMISTRY	WASHINGTON	DC	20234
WHITEHOUSE CRAIG M	YALE UNIV	ENGIN DEPT MASON LAB9	NEW HAVEN	CT	06520
WHITNEY J O	131 TERESITA BLVD		SAN FRANCISCO	CA	94127
RHITTAKER N F	NIH	BLDG 4, RM B2-37	BETHESDA	MD	20205
WICKHAM MARGARET E	MIDWEST RES INST	425 VOLKER BLVD	KANSAS CITY	MO	64110
WILKES P S	FDA	60 EIGHTH ST NE	ATLANTA	GA	30309
WILKINS CHARLES L	UNIV OF CALIFORNIA	CHEMISTRY DEPARTMENT	RIVERSIDE	CA	92521
WILLES EMERY	LAW LIV NAT'L LAB	L-232	LIVERMORE	CA	94550
WILLIAMS R M	RESEARCH CORP	405 LEXINGTON AVE	NEW YORK	NY	10174
WINOGRAD NICHOLAS	CHEMISTRY DEPT	PENN STATE UNIV	UNIVERSITY PK	PA	10802
WINTER DALLAS L	CONOCO INC	RM 314 RDE	PONCA CITY	OK	74602
WISHNOK JOHN S	MIT	NUTRITION & FD SCI	CAMBRIDGE	MA	02139
WITTSTUCK THOMAS A	NUCLIDE CORP	AGV DIVISION	ACTON	MA	01720
WOLFE MARTA H	MASS GENERAL HOSP	MASS SPEC LAB	BOSTON	MA	02114
WOLSTENHOLME W A	13840 W OAK GLEN RD		VALLEY CENTER	CA	92082
WONG C M	LLL	PO BOX 808, L-310	LIVERMORE	CA	94550
WONG L K	UNIV OF PITTSBURGH	SCHOOL OF PHARMACY	PITTSBURGH	PA	15261
WOOD DAVID	150 DAVENPORT DRIVE		STAMFORD	CT	06902
WOOD G M	2 LITTLE BLUFF RD		NEWPORT NEWS	VA	23606
WOOD KARL V	PURDUE UNIV	FUELS ANAL LAB	WEST LAFAYETTE	IN	47907
WOOD NEVILLE	BASF WYANDOTTE	140 NEW DUTCH LN	FAIRFIELD	NJ	07006
WOODS J W	8 RICHARDS RD		LYNNFIELD	MA	01940
WOODWELL BOB	HONEYWELL INC	13350 US HWY 19 S	CLEARWATER	FL	33516
WOZNIAK T J	INDIANA UNIV	CHEMISTRY DEPT	BLOOMINGTON	IN	47401
WRIGHT CHARLES J	1210 MAJESTIC WAY		WEBSTER	NY	14580
WRIGHT LYNN H	US EPA	EMSL MD-78	RES TRI PARK	NC	27711
WRONKA J	5011 GARFIELD AVE		PENNSAUKEN	NJ	08109
WU RICHARD	WRIGHT STATE UNIV	BREHM LAB	DAYTON	OH	45431
WYAIT J R	NRL	CODE 6110	WASHINGTON	DC	20375
YATES JOHN R	UNIV OF MAINE	AUBERT HALL	ORONO	ME	04469
YEE D Y	102 GRIFFITH DR		WHIPPANY	NJ	07981
YELLE LAUREN M	ARTHUR D LITTLE INC	15 ACORN PARK	CAMBRIDGE	MA	02140
YELTON R O	116 WALDEN FARM CIR		UNION	OH	45322
YERGEY ALFRED L	NIH	BLDG 6, RM 136	BETHESDA	MD	20205
YERGEY JAMES A	JOHNS HOPKINS UNIV	PHARMACOLOGY DEPT	BALTIMORE	MD	21205
YOST R A	UNIV OF FLORIDA	CHEMISTRY DEPT	GAINESVILLE	FL	32611
YOUNG S E	2455 THADDEUS		MOUNTAIN VIEW	CA	94043
YOUNGLESS TIM L	GULF RES & DEV	PO DRAWER 2138	PITTSBURGH	PA	15230
YOUSSEFI I	UNION CARBIDE	PO BOX 670	BOUND BROOK	NJ	08854
YU K O	AFAMRL-THI		WRIGHT PATT AFB	OH	45433
ZACCHEI A G	MERCK SHARP & DOHME	RESEARCH LABORATORY	WEST POINT	PA	19486
ZAKETT DONALD	DOW CHEMICAL CO	ANAL LABS 574 BLDG	MIDLAND	MI	48640
ZEBRE LEAH E	CHEMISTRY DEPT	MASS SPEC LAB	BERKELEY	CA	94720
ZIMMERMAN MICHAEL L	PHILIP MORRIS CO	3109 MAURICE AVE	RICHMOND	VA	23224
ZINK DEBORAH	MERCK & CO	PO BOX 2000	RAHWAY	NJ	
ZUBYK ROSALIE M	230 SUMMIT HOUSE		WEST CHESTER	PA	19380

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