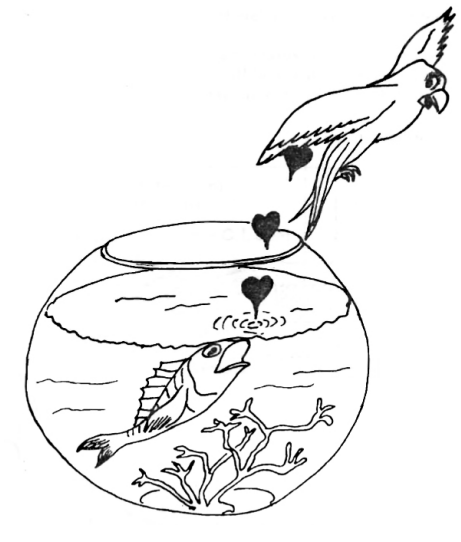


LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY: FROM IMPOSSIBLE UNION TO A MATCH MADE IN HEAVEN

ASMS History Committee: P. Jane Gale (Chair), Mariam ElNaggar, Michael Grayson, O. David Sparkman, Alfred Yergey, Kenneth Tomer



Patrick Arpino, On-Line Liquid Chromatography/Mass Spectrometry*
An old Classic
*JOC: Trends in Analytical Chemistry, Vol. 1, no. 1, 151-181.

PRELUDE

Whether a mass spectrometer is seen as a detector for a liquid chromatograph or a liquid chromatograph as an inlet for a mass spectrometer, the two techniques have become inextricably linked in an instrument serving many biologists and chemists: LC/MS.

Despite their separate origins in 1903 Russia and 1907 England in the labs of Tswett and Thomson, respectively, the courtship that would join LC and MS took place over some 70 years, facilitated by significant advances in both technologies that began to occur in the late 1950s and early 1960s.

MS STATE OF THE ART IN THE EARLY 1960s

Some commercially produced mass spectrometers were in use. These instruments had ion sources that were operated at high voltage (up to 8 kV); ionization occurred by electron ionization and mass selection was accomplished with a magnet (B), often in combination with an electric sector (E). Single-stage pumping systems were giving way in the 1950s and 60s to differential pumping systems for high analyzer vacuum. Neither instrument operation nor data acquisition was computerized. Peak masses were assigned manually to signals obtained on a strip chart recorder or visicorder. Mass spectrometers were acknowledged by users to be instruments that "almost don't work."

DEVELOPMENTS IN MS IMPORTANT TO LC

1953: Quadrupole mass filter (source operated at ground potential), developed by Wolfgang Paul, University of Bonn.

1957: Successful coupling of a GC to an MS demonstrated at Philip Morris, Inc.

1940s to 1960s: Differential pumping with low vapor pressure silicones (e.g., DC 704/705 from Dow-Corning) and perfluorinated polyethers (e.g., Fomblin from Montedison).

1966: Chemical ionization developed by Frank H. Field and Burnaby Munson at Humble Oil.

1973: Atmospheric Pressure Chemical Ionization (APCI) first reported using a ⁶³Ni foil and corona discharge by Evan and Marjorie Horning of Baylor College of Medicine.

1978: APCI with emission of ions from charged droplets demonstrated by Thompson and Irbarne of Sciex and Extraclear.

Late 1970s: Differential pumping with cryo- and turbomolecular pumps.

1979: Development of collisional dissociation on a quadrupole instrument reported by Enke and Yost at Michigan State University.

1999 OBSERVATION It is interesting to note an editorial in LC-GC in 1994 by G. Rozing (Agilent) who argued that the chasm between theoretical and practical performance of sub-microbore HPLC columns was still too large to allow the wider use of capillary-bore HPLC. In 1999, Rozing stated that he has never been so wrong in all his life.

LC-MALDI While LC eluent peaks have been sequentially collected in plates for off-line analysis by MALDI, the effluent has also been continuously monitored on-line with several different approaches: aerosol MALDI similar to the particle beam interface; a continuous-flow MALDI probe delivering the matrix with the LC effluent similar to continuous flow FAB; a rotational wheel or ball interface similar to a moving belt interface.

LC STATE OF THE ART IN THE 1960s AND 1970s

In the early 1960s, open columns with inner diameters >100 µm were packed in the lab. Commercial packing material was limited, and flows were gravity-induced. In the late 1960s developments in column and pumping technology to improve separations began to accelerate. With these new column packings, reverse phase solvent systems containing both volatile and non-volatile buffers prevailed. Detection was predominantly by UV.

DEVELOPMENTS IN LC IMPORTANT TO MS

1967: First Commercial LC packings developed by Horvath, Preiss and Lipsky at Yale University and commercialized as Pellosil by Northgate Labs.



1967: First commercial LC (500 psi/ 35 bar capability) pump, ALC-100, marketed by Waters.

1970: Term 'HPLC' (High Pressure Liquid Chromatography) introduced by Horvath of Yale University. As pump performance increased, HPLC became High Performance Liquid Chromatography.

1970: An ALC-100 (photo left) was installed at Harvard in 1970 in the lab of R.B. Wood-

ward, who uses it to separate isomers of a key intermediate in his synthesis of Vitamin B12. He reports on this synthesis at an IUPAC Symposium in 1971 in a talk that went on for 3 hr. and 45 min. and to an audience that had swollen to nearly 5000 by the end. (James Waters and His Chromatography People: A Personal Perspective. P.D. McDonald, pp 7-9, 2008, on http://www.waters.com/waters/en_US/Corporate-History/).

With this demonstration of utility, LC moves from research to applicable technology.

1971: Microparticulate packings developed by R.E. Majors at Varian.

1972: Chemically-bonded packings were developed by Kirkland at DuPont and Horvath and Lipsky at Northgate Lab.

1973: Small footprint HPLC pumps were produced by Waters (Model 6000) and by DuPont (Model 630).

1978: Tsuda and Novotny reported on the development of packed capillary columns with a resolution of 85,000 theoretical plates. The initial enthusiasm for the results from these capillary columns waned due to the low sub-microliter injection volumes associated with the columns and the attendant difficulty in detection at these levels.

1979-80: The groups of Scott, Kucera, and Munro, of Novotny, and of Horvath reported the development of microbore columns with 0.5 to 2.0 mm id.

1988: Karlsson and Novotny continued capillary column development and reported that plate heights of slurry-packed capillaries decreased substantially as a function of the column radius, 226,000 theoretical plates in a 1.95 m by 45 µm id column with 5 µm particles.

1989: Nanoscale columns were interfaced with Continuous Flow FAB/MS on magnetic sector MS/MS instrument. These columns had 50 nL/min flow rates and 50 µm ids by Jorgenson, University of North Carolina, and Deterding, et al., NIEHS/NIH.

THE COURTSHIP BEGINS: DEVELOPMENTS FROM 1973-1984

With the advent of high pressure, smaller footprint LC pumps, efforts to couple LC with MS began in earnest. As interest took root and grew, characteristics of the ideal LC-MS Interface were defined with regard to requirements from both techniques.

- A variety of different solvent systems and flows, including gradients, should be tolerated.
- The chromatographic integrity of the separation should be maintained.

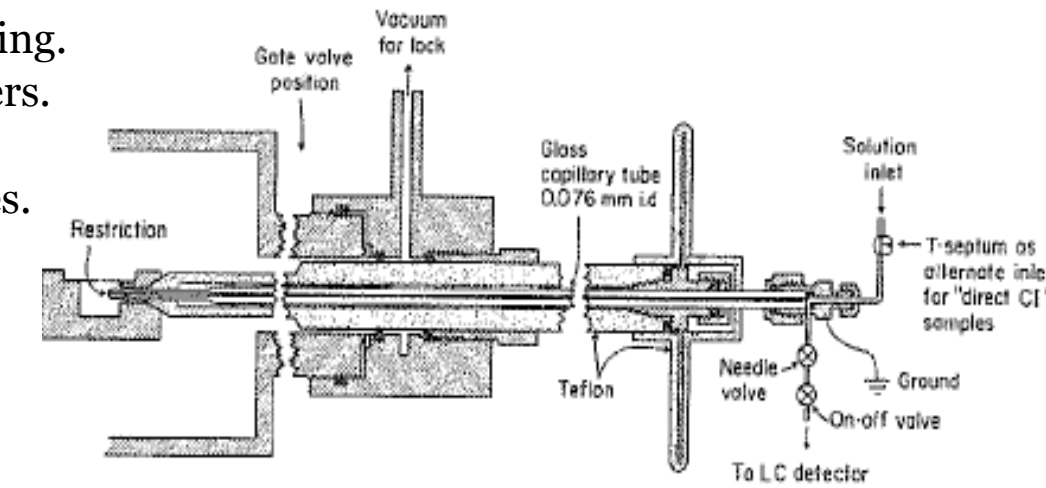
- Transfer of the analyte to the source should be efficient.
- Partial pressure due to transfer of solvent should be within the operating limits of the source.
- MS operation should not be affected.

INTRODUCTION (DLI)

- 1968:**
- Viktor Tal'rose, Institute of Chemical Physics of the Soviet Academy of Sciences, reported the direct (non-chromatographic) introduction of water into an EI source at high vacuum (10⁻⁷ torr).
 - Malcom Dole, University of Chicago, reported the formation of a beam of macromolecular ions (including multiply-charged ions) by spraying a dilute solution through a charged needle into an evaporation chamber and analyzing via a mass spectrometer. This paper remained relatively obscure for almost 20 years.

1973-4: DLI with chemical ionization reported by Baldwin and McLafferty, Cornell University. In a second paper, Arpino, Dawkins & McLafferty introduced the effluent from an LC column directly into the source.

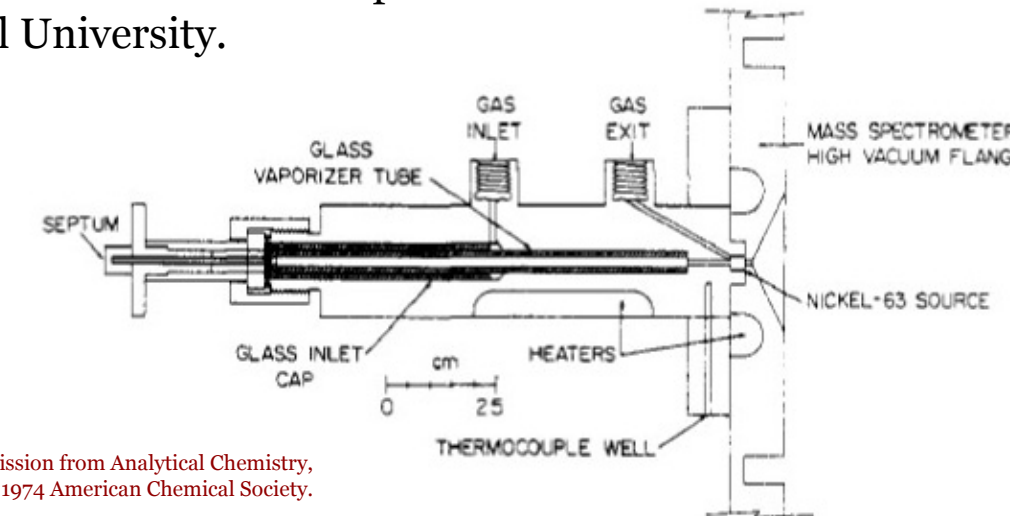
- Compatible with normal and reverse phase solvents.
- Only CI spectra.
- Suffered from clogging.
- Difficulty with buffers.
- Difficulty with non-volatile analytes.



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1974: Corona discharge at atmospheric pressure. DLI with effluent introduced directly from an LC column with APCI using ⁶³Ni foil/corona discharge source, reported by Evan and Marjorie Horning, Baylor College of Medicine.

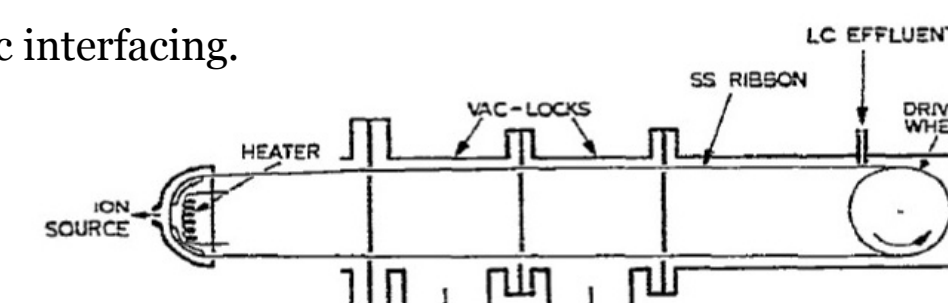
- Corona discharge became basis of APCI instruments.
- 1980 - DLI using micro LC columns reported by Henion, Cornell University.



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MECHANICAL TRANSPORT

- 1976:** Moving Belt
- Interface was introduced by McFadden, Schwartz & Bradford of Finnigan, Corp.
 - True chromatographic interfacing.
 - EI spectra.
 - Good sensitivity.
 - Difficulties with non-volatile labile analytes and LC buffers.
 - Unreliable performance.

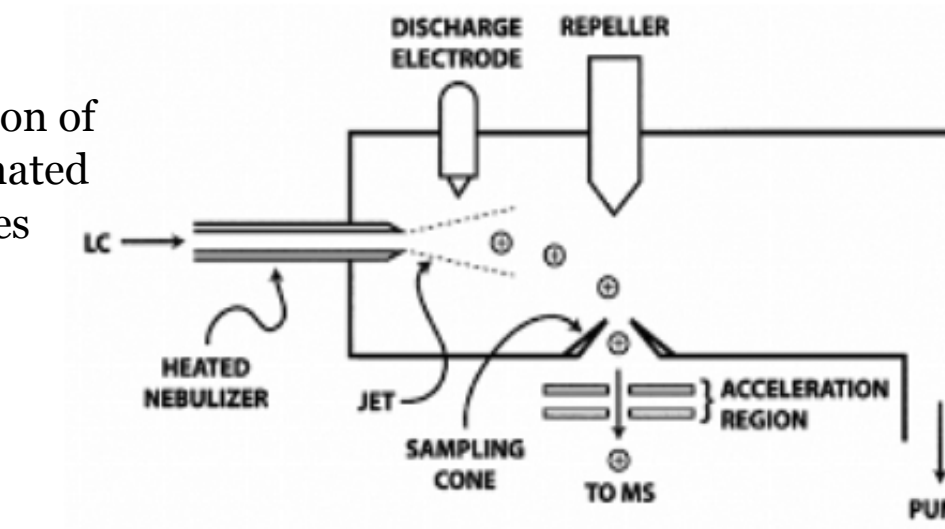


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THERMOSPRAY (EVOLVED FROM DLI)

1984: Thermospray, an off-shoot of DLI, developed by M. Vestal, University of Houston.

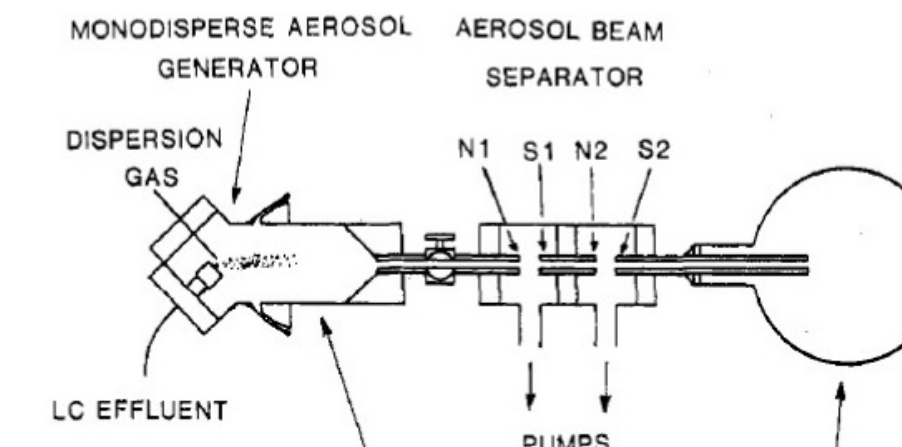
- EI-type fragmentation sometimes observed.
- Significant fragmentation of protonated or deprotonated molecule was sometimes observed.
- Low sensitivity for non-volatile analytes.
- Limited range of LC conditions.
- Became the primary LC/MS interface until the 1990s, primarily due to the observation of EI-type fragmentation and to limited analyte volatility range of alternatives.



Simplified diagram of a thermospray source: https://www.researchgate.net/publication/25729197_Coupling_Mass_Spectrometry_with_Liquid_Chromatography_for_Petroleum_Research/figure

PARTICLE BEAM

- 1984:** Interface based on incorporation of jet separators for removal of solvent vapors. Willoughby & Browner, Georgia Institute of Technology.
- Library searchable EI-type spectra.
 - Analytes limited in volatility.



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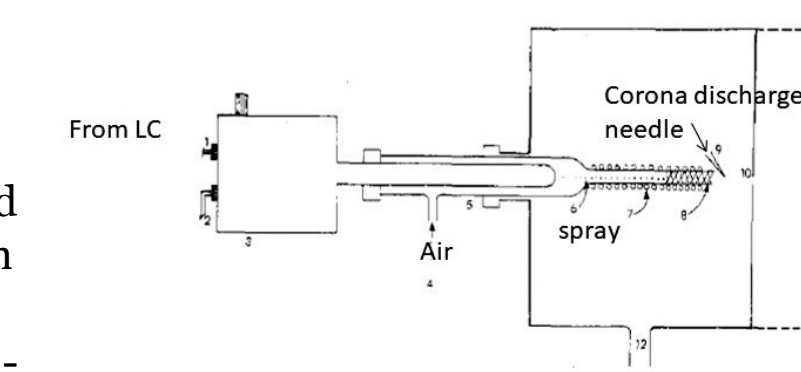
CONTINUOUS FLOW FAB

1985: Takeuchi, Watanabe, Kondo, Goto, Ishii, Nagoya Univ, Japan: Caprioli, Fan, Cottrell, Univ., Texas Med. School at Houston.

- Molecular ions of large molecules were observed.
- Fragment ions observed.

IONSPRAY

- 1986:** IonSpray (Pneumatic Electropray) – Bruins, Covey, Henion, Cornell and Sciex.
- Detection levels of one hundred picograms, 2 orders better than previous techniques.
 - Surprisingly, especially in hindsight, these results did not initiate wide commercialization and widespread utilization of APCI.



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THE WEDDING DANCE: A TANGO FOR TWO

Atmospheric Pressure Ionization

At this point in time, although a number of approaches had been successful in interfacing LC with MS, all had serious drawbacks. It seemed to many that the field needed a breakthrough in technology to make LC/MS a practical methodology. This breakthrough would occur from developments in atmospheric pressure sources and instrumentation developments.



The flying elephant above was taken from the poster announcing Alpha Proteomics' grant success. ©Global Foundations

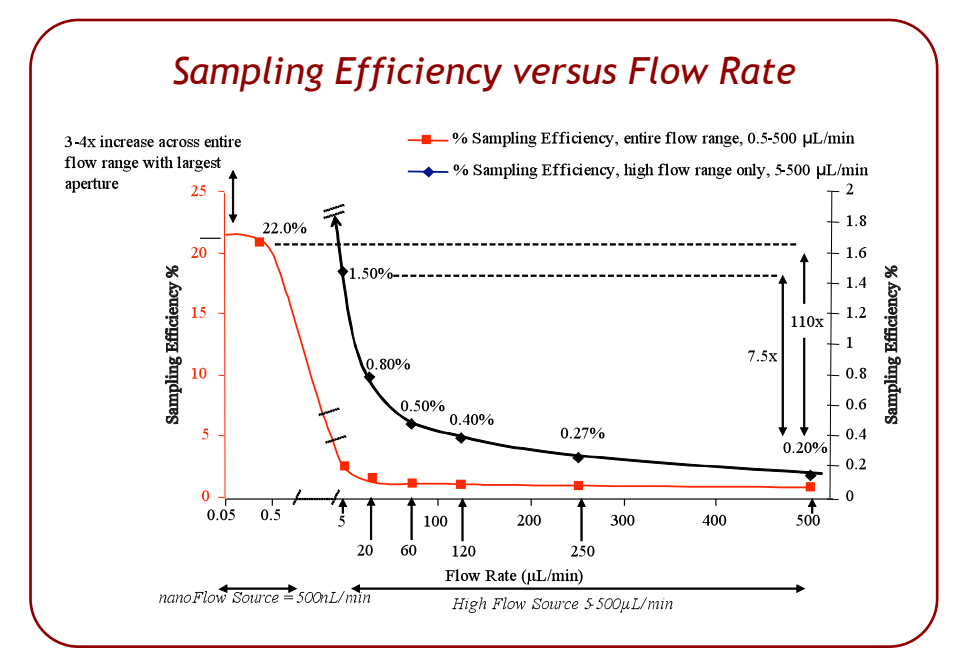
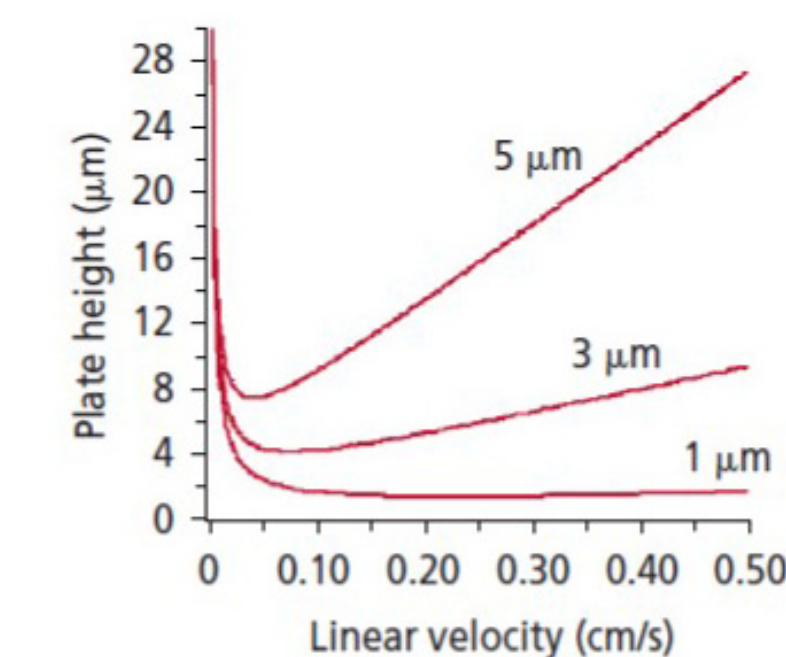
1984: John Fenn, Yale University, and independently, Lidia Gall, Institute of Analytical Instrumentation in the National Academy of Science at St. Petersburg, developed an electro-spray ionization (ESI) source that produced intact, high molecular weight, multiply protonated or deprotonated ions. Prof. Fenn received the Nobel Prize in Chemistry in 1992 for this discovery. His acceptance speech was titled "When Elephants Fly".

ESI sensitivity was observed to be analyte-concentration-dependent. Peaks eluting from capillary columns are significantly sharper than from wider bore columns, leading to greatly increased sensitivity with lower injection volumes.

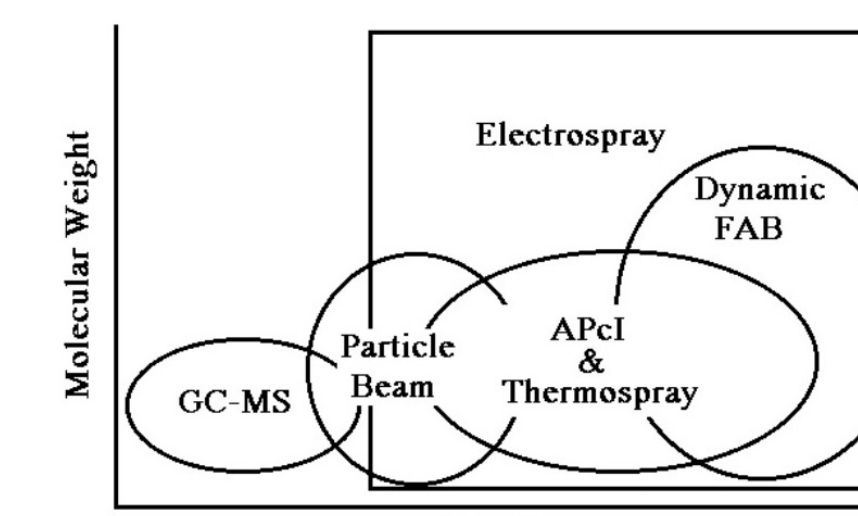
1986: Ion Spray, developed by Bruins, Covey, and Henion, combined pneumatic nebulation assisted by an electric field with a high voltage on the spray capillary.

1994: To take advantage of the concentration sensitivity of ESI, Caprioli and Mann independently published papers within days of each other describing nanoflow (nanoliters per minute) ESI (Emmett, & Caprioli, JASMS, 5, 605-13, 1994.) for flowing liquid introduction and nanospray ESI for static liquid introduction (Wilm & Mann, J. Mass Spectrom. Ion Processes, 136 (1994) 167-180). Both approaches are now commonly termed nanospray.

2000s: After ESI and nanoESI became prevalent, studies in both LC and ESI led to further improvements. A study of plate height vs. linear velocities of the solvent (below left) determined that 1 µm diameter packing provided a broad range of stable velocity with greatly decreased plate height and improved separation efficiency. (Jerkovich, Mellors, and Jorgenson, University of North Carolina). The lower flow rates of the columns, when combined with a nanoflow ESI source, led to an improved sampling efficiency (below right). At flow rates of .05 µl/minute, the sampling efficiency reached 22% compared to a sampling efficiency of 1.5% at a flow rate of 5 µl/min.



WHY IS ESI SO DOMINANT?



Thomas R. Sharp; Pfizer Global Research and Development

THE IMPONDERABLES

The relationship between LC and MS precluded in fits and starts with opportunities seized as well as missed. This raises some questions:

- How soon would the coupling of LC and MS have proceeded without the development of commercial bench top LC pumps?
- How fast would LC/MS (and MS itself) have developed if tandem MS had been available when the atmospheric pressure studies (APCI and ionspray) were first reported?
- How soon would capillary LC have become widespread without MS detection?
- Where would the field be without ESI?

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