Journal of Chromatography, 203 (1981) 109–116
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,351

MASS SPECTROMETRY AS A SEPARATION TECHNIQUE

ANALYSIS OF INVOLATILE SAMPLES*

FRED W. McLAFFERTY* and EARL R. LORY
Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853 (U.S.A.)

SUMMARY

For analytical applications, mass spectrometry (MS) can be considered as a separation method as well as an identification technique. Important factors affecting MS as a separation technique include resolution, speed, sensitivity, specificity, molecular weight limitations, cost and maintenance. Use of tandem mass spectrometers (MS–MS) for both separation and identification purposes appears to have particularly promising applications for the analysis of targeted molecules in complex mixtures with minimum separation of interfering materials. The applicability of MS should be extended very significantly by adequate methods for obtaining data from non-volatile molecules, for which direct solution introduction techniques appear to be particularly promising.

INTRODUCTION

The analytical mass spectrometer is best known for its capability to characterize organic molecules with uniquely high sensitivity and specificity. The great majority of mass spectral data obtained at the present time results from its use as a specific detector for gas chromatography (GC) or liquid chromatography (LC). However, the mass spectrometer can also be thought of as a separation device, and in fact its first large-scale analytical use was for the quantitative analysis of known components of complex light hydrocarbon mixtures. Recently there has been a greatly renewed interest in such applications of the mass spectrometer because of its performance characteristics for analytical separations[3].

Separation in mass spectrometry (MS) is based on the nuclidic masses of the component atoms in the species. Thus it is ideal for separation of some mixtures, such as homologs or isotopically labeled molecules, but restricted in application to other mixtures, such as those of isomers or chiral molecules. Mass spectra can contain hundreds of separated peaks, comparable to packed-column gas chromatograms, while the greatly increased number of peaks possible in a high-resolution mass spectrum can be compared to those in a capillary column chromatogram. The time

* Collisional activation and metastable ion characteristics, Part 81. For Part 80, see ref. 1.

0021-9673/81/0000–0000/$02.50

between entrance of sample molecules into the mass spectrometer and signal detection is much less than one second, which is particularly advantageous for repetitive or on-line continuous analyses.

The sensitivity and specificity of the mass spectrometer are well-known reasons for its value as a detector for chromatography. Sensitivity depends on the efficiency of ionizing the sample molecules, of ion transmission through the instrument and of ion detection; the combination of the latter two can be higher than 10%. Although the efficiency of the former is often $1/10^5$, the dynamic range of the recording system is sufficient to permit direct detection of <1 ppm concentrations in favorable cases. In chemical ionization (CI) a 10^4 excess of reagent gas is employed; it is often possible to use the major components of a sample as the CI reagent gas so that a 10^{-4} M concentration of an impurity gives a full scale signal. Analytical specificity is achieved in two major ways. A wide variety of ionization methods are available to maximize unique mass peaks from the desired compound relative to interfering substances. CI is particularly versatile in forming positive or negative ions, ions by protonation, alkali ion attachment, charge exchange and electron or negative ion capture, with differentiation by proton affinities and ion stabilities. Field ionization and low-voltage electron ionization can also be useful. However, MS competes poorly with chromatography if the separation of all components of a mixture is desired, as it is usually impossible to find ionizing conditions giving at least one peak from every component without giving many peaks from some components, which of course will not be a problem in a chromatographic separation using a universal detector.

An important new way to achieve specificity in mass spectrometer separation is to use a second mass spectrometer in tandem as a detector. Following the nomenclature commonly used for the latter application to GC and LC (GC–MS and LC–MS), this rapidly growing technique is commonly called "MS–MS"[4].

Obvious disadvantages of the mass spectrometer are its cost, maintenance and training requirements, although for routine analyses its speed can, at least in part, compensate for these. One of the main limitations in its analytical applicability is the sample volatility requirement for most common ionization methods. Although much less volatile samples can be measured by MS than by GC, quite the opposite is true for LC. Extension of the usable molecular weight range of MS is of prime importance for improving its general applicability as an analytical separation technique.

INTRODUCTION METHODS FOR NON-VOLATILE COMPOUNDS

Recently intensive research efforts in many laboratories have resulted in impressive extensions of the sample introduction capabilities of MS in higher molecular weight and more polar molecules. Probably the most used of these techniques is field desorption[5], although other methods such as desorption CI[6,7], ^{252}Cf plasma desorption[8] and flash heating[9], such as with pulsed laser radiation[10], have also given important results. Further details of such methods are given in a recent review[11].

However, these methods all are limited by the fact that the molecules in a solid sample must be separated from each other in order to form the gaseous ions to be separated in the mass spectrometer. This is particularly disadvantageous for solid samples of polar molecules with strong inter-molecular hydrogen bonds, such as many of biological importance. To vaporize the sample, the energy added to rupture these

bonds (or overcome Van der Waals' forces) can be great enough to rupture intramolecular bonds, leading to degradation or bimolecular reaction products, and thus compromising the analytical information in the resulting mass spectrum.

An alternative approach is to separate the macromolecules before ionization by dissolving them in a solvent. Dole *et al.*[12] have proposed an "electrospray technique" utilizing this concept. Very small charged droplets (*ca.* 0.1 μm) are formed by spraying a solution from an electrostatically charged nozzle. With a sufficiently dilute solution (*ca.* 10^{-6} *M*) most droplets will contain only one molecule. The droplets are directed through a differential pumping system to remove the solvent so that the resulting charged molecule can proceed into the ion source. Despite substantial technical difficulties, Dole *et al.* achieved promising results. Recent electrospray experiments by Friedman and Beuhler[13] indicate that mass spectra of 80,000 molecular weight polystyrene can be obtained by this method. A 400 kV post-acceleration ion detector and seeded molecular beam inlet were key improvements to the method by the latter authors. However, the apparatus also detects ions of high molecular weight using only low molecular weight solvents such as water in the seeded beam inlet because of the high tendency for clustering at temperatures near absolute zero. Although use of such solvents for macromolecules with this technique would thus provide a very convenient internal mass marker, clustering of solvent molecules with a polar macromolecule could cause very serious difficulties to interpreting the resulting mass spectrum.

For several years at Cornell we have attempted to develop a similar macromolecular solution introduction system[11], but to date this has not produced useful spectra of high molecular weight samples. Larger droplets of higher concentration are used, so that each droplet contains many molecules; if droplet vaporization can be made sufficiently fast, the macromolecules should not have sufficient time ($\approx 10^{-6}$ sec) to recondense into solid particles. Similar to the direct solution introduction technique for LC–MS[6,14], a CI source is utilized, with the solvent acting as the ionizing reagent gas to ionize the individual macromolecules. This should have the advantage that much less solvent must be removed by the pumping system. Also the vaporization should take place at temperatures at which the tendency for macromolecule solvation should be minimized.

The most critical feature of the system is the method for adding energy to the droplets rapidly. Three energy sources were tried: a pulsed electric discharge, continuous ion bombardment and a pulsed laser. Under normal operating conditions the LC–MS probe used produces a stream of $\approx$10-μm droplets at a $\approx$500 kHz rate traveling at a velocity of $\approx$25 m/sec. Because $\approx$400 nsec are required for the droplet to move its own diameter, approximately this time would be required for energy deposition into the droplet when it moves into a continuous energy source. On the other hand, energy deposition times for the pulsed sources should be mainly limited by the pulse rise time. The time required for energy deposition should be less than that required for macromolecule migration through the droplet by Brownian motion, estimated at 10^{-7}–10^{-6} sec; further, more rapid energy deposition should decrease the degree of solvation in the separated macromolecules.

Both the electric discharge and ion bombardment techniques exhibited discouraging experimental difficulties. For both methods the RF noise from the discharge causes severe signal interference.The most serious difficulty with the electric discharge was the erratic nature of the discharge, probably arising from local pressure

fluctuations, space charge and contamination. The ion bombardment gun also caused source contamination and raised the analyzer pressure to an extent that made resolution and sensitivity marginal.

Energy deposition from the laser source avoids the problems of additional gas load to the pumping system and RF noise. It was demonstrated[11] that a 100-nsec pulse of 9.60 and 10.58 μm radiation (CO_2 laser) could vaporize *ca.* 80 of the $\approx$10-μm droplets. The minimum energy values required for droplet vaporization agreed (within a large experimental error) with the values expected from the solvent heats of vaporization. Unfortunately, the repetition rate of the laser was $\approx 10^4$ too slow to vaporize all of the droplet stream. There are now available lasers, such as the Gentec R-200, which will give a 500-nsec pulse of 200 mJ power at a rate of 200 Hz. This should be sufficient to vaporize all the solution (100 μl/sec), assuming that the laser beam (focused to 0.4 $\times$ 5 mm) intercepts 40 droplets of 50 μm diameter. Because the energy absorption appears to be a non-resonant process, the pulse of radiation should ablate the droplet surface on a much shorter time scale, minimizing the opportunity for the solute macromolecules to agglomerate and maximizing the probability for their desolvation. Hopefully, the solvent heat-of-vaporization requirements will minimize solute pyrolysis.

Vestal and McCloskey[15] have recently proposed an LC–MS interface in which a droplet stream of the sample solution formed at atmospheric pressure is partially vaporized through a differential pumping system on its way to the mass spectrometer ion source. Here the droplets, which have a velocity of $\approx$50 m/sec, collide with a heated metal plate to complete the vaporization process. This thus should produce vaporization of a 10-μm droplet in $\approx 2 \cdot 10^{-7}$ sec if the heat transfer is sufficiently efficient. Apparently no deleterious effects of the surface such as solute degradation have been observed; excellent CI spectra had been obtained of thermally labile compounds such as nucleotides. However, with the direct solution–introduction interface[14] Arpino[16] finds that if the droplets strike the ion source walls this essentially destroys the capability of the system to produce CI spectra of low volatility compounds such as disaccharides.

TANDEM MASS SPECTROMETRY (MS–MS)

The coupling of chromatographic separation devices to a mass spectrometer detector giving GC–MS and LC–MS systems has provided revolutionary approaches for the analysis of complex organic mixtures. Substituting a mass spectrometer separator to give MS–MS provides a complementary "separator–identifier" system whose similar analytical potential is indicated by the explosive growth of research in this area. Comprehensive reviews of this field have appeared recently[17,18] to which the reader is referred for further details and key references.

Analogous to GC–MS and LC–MS systems, the basic parts of an MS–MS system are (a) the "separator" (MS-I), which includes the sample ionization as well as component ion separation; (b) the interface region, which effects fragmentation of the separated component ion and transmission of these products to MS-II; and (c) the "identifier" (MS-II), which separates and detects these products. MS–MS measurements have been carried out with a wide variety of MS instrumentation, such as the reversed-geometry double-focusing mass spectrometer or the normal-geometry instrument with "linked scanning". However, this discussion will be limited to two

quite different approaches. The tandem quadrupole instrument developed by Yost and Enke[19] is particularly promising for routine analysis, and the tandem double-focusing mass spectrometer developed at Cornell[20] appears to have the highest performance capabilities of any MS–MS instrument developed to date.

Several research groups have now shown the great promise of the tandem quadrupole for MS–MS, and three companies have announced the commercial availability of such instruments. A conventional quadrupole mass spectrometer is used as MS-I. The separated ions are made to undergo low energy collision ($\approx$10 eV) with a higher molecular weight collison gas such as nitrogen in an RF-only quadrupole field which serves to transmit the fragmentation products with high efficiency (20–100%) to the MS-II quadrupole. Some pressure-dependent ion–molecule reactions are observed in addition to the collision-induced unimolecular fragmentations. The quadrupole advantages which have led to its domination of the GC–MS instrumentation field also are beneficial for MS–MS. These include ease of computer control, simplicity and potentially low cost. The secondary mass spectra obtained by this low energy collisional activation (CA) are surprisingly similar to the CA spectra obtained by high energy (>2 kV) grazing collisions and also to normal electron-ionization mass spectra. As discussed above, the characteristics of MS separation should make such instruments particularly suitable for routine analysis of targeted components in complex mixtures, with particular advantages for MS–MS in speed of analysis, specificity, sensitivity, unit mass resolution of MS-II and automation. If such attributes lead to sufficient demand, the cost of such microprocessor-controlled quadrupole instruments could approach the cost of a high-quality gas chromatograph.

We have recently made operational at Cornell[20] an MS–MS instrument which, although it is much more complex and expensive than other such systems, has much higher capabilities of resolution, specificity and mass range, and for specific analyses should also have higher sensitivity. MS-I is capable of 50,000 resolution, approximately 2 orders of magnitude greater than the reversed-geometry double-focusing or tandem quadrupole MS–MS instruments. A new interface greatly increases the sensitivity as well as operational flexibility in that up to 20 kV energy can be added to the separated ions exiting from MS-I before collision. Further, CA is effected by a helium molecular beam so that essentially all of the CA product ions are produced at the focal point of the lens introducing the ions into MS-II. With this interface 25 keV ion collisions can convert >10% of separated ions from MS-I into CA product ions recorded by MS-II. The double-focusing mass spectrometer of MS-II provides a much needed improvement in sensitivity as well as resolution in comparison to the energy analyzer utilized as MS-II in reversed-geometry double-focusing MS–MS instruments. The energy released in CA fragmentations produces a range of kinetic energies ($\pm$ *ca.* 50 eV) in the resulting product ions, so that mass analysis with an energy analyzer can result in only partial resolution of neighboring masses even below m/z 100. The double-focusing mass spectrometer is designed to focus ions of the same mass but differing energies at the same focal point, so that resolution is not only increased to >10,000, but the resulting peak sharpening greatly improves the signal-to-noise ratio (initial experiments indicate this improvement to be at least 10^2). Coupling of both MS-I and MS-II to a dual-processor DEC PDP-11/45 computer for data acquisition and feedback control has greatly improved operational efficiency of the tandem double-focusing MS–MS instrument.

One of the most impressive examples of trace analysis developed in recent years is that for 2,3,7,8-tetrachlorodibenzodioxin (TCDD) using the high-resolution mass spectrometer[22]. This highly stable compound is easily separated by chemical means from most biological matrices, leaving mainly similar compounds such as the polychlorobiphenyls (PCBs) and bis(chlorophenyl)dichloroethylene (DDE). The final separation requiring $\approx$11,000 resolution is done between the TCDD ion at m/z 321.8936 and one from PCB at 321.8677, as well as one from DDE at 321.9292. However, to measure parts-per-trillion (10^{-12}) in samples containing parts-per-million of PCBs, time-consuming chromatographic clean-up procedures are necessary. We have shown[22] that much of this clean-up procedure can be replaced by a separation in MS-II. The base peak in the CA spectrum of the TCDD m/z 321.8936 peak arises from the loss of COCl, which of course is not possible in the CA spectra of the corresponding isobaric peaks of PCB and DDE (which do not contain oxygen), whose closest CA peaks represent the loss of Cl_2. Although we have not demonstrated as yet whether this will allow corresponding ppt sensitivities, the speed of analysis for routine samples should be greatly improved.

Future directions

The increasing application of chromatographic methods has demonstrated the importance of separation techniques for the analysis of complex mixtures encountered in a wide variety of critical problems. The combined separation–identification systems of GC–MS and LC–MS will surely remain the techniques of choice for unknown mixtures in which the identification of all possible components is desired. However, MS as a separation technique does have advantages in comparison to chromatographic approaches for the routine analysis of pre-selected compounds in complex mixtures. The negligible time requirements for MS separation can provide much faster analyses, and the complementary separation capabilities of MS can be advantageous for specific analytical problems. The greatly increased specificity of MS–MS can be achieved with $>10\%$ of the signal intensity of MS alone, using either the low energy quadrupole-confined or high energy molecular-beam collision regions. Development of a viable method for introduction of non-volatile samples would greatle increase the applicability of such MS analyses. Thus we feel that MS will increase even further the importance of separation methods for complex mixture analysis.

EXPERIMENTAL

Pulsed d.c. discharge vaporization

A pulsed d.c. discharge unit producing a 45 kV discharge of approximately $5 \cdot 10^{-6}$ sec duration was built around a commercial automotive capacitively-induced discharge system. Trigger pulses from a 5 V variable-frequency pulse generator were used to initiate the discharge at frequencies between 10 and 1000 Hz. The discharge was coupled to a test cell containing an LC–MS interface[14] and a vaporization monitoring system. The LC–MS interface, made by Hewlett-Packard, produces a jet of 10-μm droplets about 50 μm apart by forcing a solution through a 5-μm orifice. The vaporization monitoring system employed a filtered photomultiplier tube to detect He–Ne laser radiation (632 nm) scattered off the droplets. The discharge occurred between two electrodes spaced 3 mm apart. The jet passed between the two

electrodes and the laser was focused on the jet approximately 2 cm downstream from the electrodes. The system was evacuated by a mechanical pump. At steady gas pressures of less than 300 millitorr the discharge was erratic, forming sharp arcs. At high pressures, up to 2000 millitorr, a diffuse discharge was obtained, visually filling a volume of approximately 1 cm^3. The scattered radiation signal from the jet showed that the discharge vaporized about 100 droplets per pulse. Next the pulsed d.c. discharge unit was coupled to a Finnigan Model 1015 mass spectrometer, fitted with a CI source. Two electrode-source configurations were used. In the first configuration the source block was not modified and the high voltage electrode was placed outside the source volume. The jet passed through the discharge and the source and then struck a surface cooled to liquid nitrogen temperature to minimize the pressure in the system. Ions produced in the discharge were drawn into the source by an electrostatic lens. In the second configuration the source was modified to place the high voltage electrode opposite the ion exit slit. The distance between the high voltage electrode and the nearest grounded point was 3 mm. With the source filament either on or off and solvent present in the jet, solvent ion signals due to MH^+, M^+ and fragment ions were observed. However in either electrode configuration the discharge produced large amounts of RF noise which severely interfered with the detection system of the mass spectrometer. Attempts to shield the electron multiplier and electronics were unsuccessful. As a result, the usable gain in the multiplier was very low. Attempts to observe involatile sample ions from raffinose pentahydrate as the solute were not successful. The relatively high pressures needed to obtain a diffuse discharge lead to high pressures in the analyzer region of the mass spectrometer, also reducing the probability of observing solute ions. Even though the pulsed d.c. discharge is effective in vaporizing the jet, RF noise and high pressures posed severe operational problems for this system.

Ion impact vaporization

Inelastic collisons between high energy ions and jet droplets can result in heating and subsequent vaporization. Rough calculations indicate than an ion beam of approximately 1 mA of 1 kV ions would vaporize the 10-μm droplets in the jet. To test this approach a simple ion gun was constructed. The gun was basically a hollow cathode discharge (inner tube: 20 × 300 mm; outer tube: 22 × 350 mm) fitted with a coarse stainless-steel screen at the open end. The screen was in contact with the outer tube (ground potential) and was spaced 1 mm from the end of the inner tube (1 to 5 kV potential). A gas at pressures between 300 and 2000 millitorr was introduced through the rear of the gun. The discharge occurred between the inner tube and the screen. The screen was curved, so that those ions passing through the screen were focused to a beam approximately 1 mm in diameter; the focal point distance was a function of the radius of curvature of the screen. Using argon, carbon dioxide and a variety of other gases, beam currents up to 3 mA were measured at the focal point. The gun was placed 2 cm from an ion drawout plate mounted on the front end of the Finnigan quadrupole analyzer (source block removed). The LC–MS interface was placed such that the jet crossed the ion beam at the focal point, approximately 3 mm from the ion drawout plate. As with the pulsed d.c. discharge source, large ion currents from the solvent were observed, but no solute ions derived from raffinose pentahydrate (10^{-2} to 10^{-4} *M* in methanol) were detected. The same problems encountered with the pulsed

d.c. discharge appeared in the ion impact experiments, even though the ion source was operated in a continuous mode. Attempts to shield the ion source and minimize the pressure in the discharge region improved the solvent ion signal intensity but did not produce solute ion signals.

ACKNOWLEDGEMENTS

The generous support of the National Institutes of Health (Grant GM-16609) and the Army Research Office, Durham (Grant DAAG29-79-C-0046) is gratefully acknowledged.

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