Mass Spectrometry: Analytical Capabilities and Potentials

R. G. Cooks, K. L. Busch, G. L. Glish

Summary. The mass range of mass spectrometers has been extended by almost an order of magnitude in the past decade, ionization procedures have been introduced which allow ionic, nonvolatile compounds to be examined, and new capabilities have been achieved through the successful integration of separation and analysis techniques. In combination with other techniques, mass spectrometry has been used in biological and environmental research to characterize constituents of mixtures, including those present in trace amounts; in metabolic profiling, where high throughput and large dynamic range are important; and in protein structure determinations. Measurements of stable isotope abundances by mass spectrometry have been used in enzymology, studies of photosynthesis, and carbon dating. Outside the area of chemical analysis, mass spectrometry has been used to study gas-phase acidities and basicities and to study organic reaction mechanisms in the gas phase. Trends in mass spectrometry include multidimensional experiments, use of ionization methods, direct mixture analysis without extensive sample preparation, and the development of advanced instrumentation including an ion trap and an inductively coupled plasma mass spectrometer. It is likely that mass spectrometry will come to be much more widely used and that data will increasingly be other than conventional mass spectra.

Mass spectrometry is rich in history and in potential. It has drawn from and influenced many fields, including atomic physics, reaction kinetics, geochronology, chemical analysis, and, most recently, biomedicine. Advances in reaction kinematics and dynamics, data acquisition and reduction, isotope separation, and stable isotope labeling have depended on this instrumental method. Practitioners of mass spectrometry have often been specialists, immersed in their subject and almost insulated by its momentum. This group and biologists, among others, are now in the process of mutual discovery; a valuable outcome calls for a familiarity with the capabilities of each. In this article we seek to provide information on recent developments in mass spectrometry. We also seek to emphasize the potential of a subject which we see as a blunt tool, available and needing only to be sharpened for particular tasks.

Three new capabilities epitomize the present state of mass spectrometry and point up its potential for far-reaching contributions in biology and medicine. The mass range of mass spectrometers has been extended by approximately an order of magnitude in the past decade. While other types of mass analyzers have been used to reach considerably higher masses [for instance, 150,000 in some quadrupole experiments (*1*)], progress in the well-established sector magnet technology best illustrates this trend. Commercial instruments are now available with mass ranges, at full accelerating voltage, of 7500 compared with 1000 just a few years ago (*2*). A second capability is desorption ionization, a family of procedures [including fast atom bombardment (FAB), secondary ion mass spectrometry (SIMS), and fission fragment methods] which allows ionic, nonvolatile compounds to be examined by mass spectrometry. These capabilities were reviewed last year in this journal (*3, 4*). The third notable capability is the successful integration of separation and analysis techniques represented by tandem mass spectrometry (MS-MS) (*5, 6*) and the much improved liquid chromatography–mass spectrometry (LC-MS) interfaces (*7*). The latter capability should have an impact comparable to that of gas chromatography–mass spectrometry (GC-MS), but without the limitation to volatile, low-mass compounds. MS-MS is a two-dimensional form of spectrometry which often improves signal-to-noise ratios as well as providing entirely new capabilities, such as that of scanning a mixture for all constituents having particular structural subunits.

Comparable in importance to these new capabilities are the texture of the subject and the attitudes of its practitioners. A mass spectrum is a chemical product distribution, not a representation of electromagnetic transitions. A mass spectrometer is appropriately considered as a chemical reactor, a type of chromatograph, or a spectrometer. These roles, together with the fact that mass analysis can be implemented by use of several different physical principles, distinguish mass spectrometry from other forms of spectroscopy. This latitude in methodology, and characteristics of the hardware which demand interaction with the equipment, have made mass spectroscopists likely to modify instrumentation or to develop entirely new instruments. They constitute a reservoir of talent and experience which can be directed at significant problems in collaboration with the informed biologist. Absence of such interactions in the past is surely responsible for the lack of some desirable capabilities. For example, microscopy is largely a morphological technique, with supporting elemental analysis capabilities. One might have expected that information on molecular distributions would have been a high priority, especially in biological specimens. Development of a capability for molecular microscopy, based on desorption ionization techniques, appears to be feasible.

This paper has three subsequent parts. The first is a summary of some notable recent achievements of mass spectrometry, covering a range of applications. The second seeks to identify and illustrate the forces driving various areas of activity in mass spectrometry. Finally, some views on future directions are given.

Typical Recent Achievements

This section is designed to provide information on the range and significance of work being done with mass spectrometry through selected examples. Other work of equal merit could have been chosen to make similar points.

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The GC-MS combination has had a considerable impact on biological and environmental research, providing a specific means of characterizing constituents of mixtures and having the necessary sensitivity and quantitative accuracy to be applicable to trace constituents. This established procedure continues to yield valuable results, as illustrated by the recent discovery of the presence and uneven distribution of the neural excitotoxin, quinolinic acid, in the mammalian brain at the level of nanomoles per gram (*8*). The selective excitatory activity of this molecule suggests that it may be a neurotransmitter. Detection of the acid was achieved by GC-MS of the volatile hexafluoroisopropanol diester derivative with electron impact ionization. Quantitation was based on standard addition and single-ion monitoring. Concentrations of both 5-hydroxytriptamine and quinolinic acid increase after tryptophan administration, although the distributions of the two metabolites are quite different. These important results were obtained by using classical ionization (electron impact) and separation (GC) procedures.

Mass spectrometry has many qualities that make it suitable for metabolic profiling, for example, in measuring the distribution of urinary acids or steroids in individuals (*9*). Such measurements can have immediate diagnostic value, as in the detection of particular genetic diseases, or they may be aimed at chemical diagnosis of predisease states by comparing profiles with those of normal individuals. A large number of samples must be measured, so high throughput is important, and dozens of compounds must be monitored over a wide range of concentrations, so the method must be widely applicable and have a large dynamic range. GC-MS used with electron ionization has proved equal to this task and has contributed significantly to clinical successes in neuroblastoma cases (*10*).

Challenging structural problems, including protein structure determinations, can sometimes be solved most efficiently by using a combination of techniques. In several recent examples, Edman sequencing has been combined with mass spectrometry to increase the speed of structure determination and to avoid possible misassignments arising from weaknesses in the individual methods (*11–13*). Two mass spectrometric methods were used to deduce the structure of the 112-amino-acid antitumor protein macromomycin, derived from a *Streptomyces* culture. Partial acid digestion gave a mixture of small (two- to six-amino-acid) peptides, which was derivatized and analyzed by GC-MS with electron ionization. This information was interpreted in conjunction with Edman degradation data and FAB spectra, which provide molecular weights for large peptides even when they are present in a mixture and examined without derivatization. Another rapid method of protein structure determination is that in which amino acid sequences are arrived at by determining the base sequence of the gene coding for the protein. Here, too, a single method is usefully supplemented by other data, and it has been shown how frameshift errors in the translation method can be avoided by obtaining complementary mass spectrometric data (*12*). Successes with proteins such as glutamine–transfer RNA synthetase (550 amino acids) have depended on this combination of methods.

Tandem mass spectrometry has been used to discover and establish the structure of metabolites of the drugs primidone, cinromide, and phenytoin in plasma and urine extracts; analyses were performed in less than an hour, using concentrations of 1 to 50 μg/ml (*14*). The study is predicated on the speed and flexibility of MS-MS scans made with a triple quadrupole instrument, and on the realization that metabolites generally retain a large portion of the parent drug structure. Since no other assumptions about metabolite structure are made, no constraints are introduced, and unknown metabolites are quickly identified within a complex mixture.

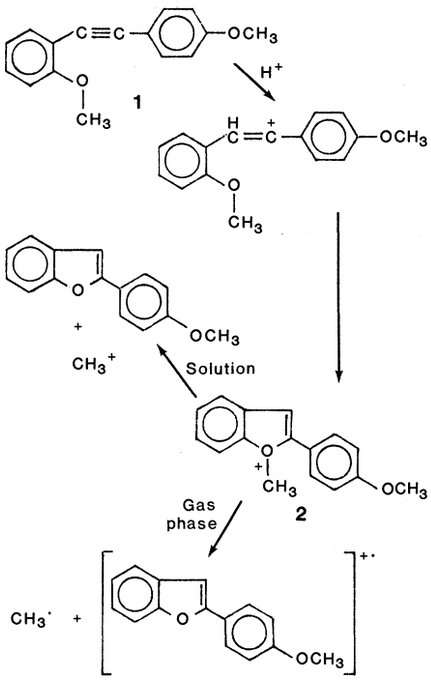
Measurements of stable isotope abundances have been the province of mass spectrometry since the discovery of the isotopes of neon and the early abundance measurements used to establish chemical atomic weights. Heavy atom isotope effects have become a valuable tool in enzymology in the past decade, and even whole plant isotope effects are now under study and are providing insights into photosynthesis (*15*). Kinetic isotope effects are best measured with high-precision isotope ratio spectrometers after chemical conversion of the substrate to CO_2, CO, or N_2. The limitations this imposes in terms of accessibility of the label have been lifted by a technique in which a mixture of compounds is labeled in a remote position and doubly labeled in both the remote position and a position accessible to degradation (*16*). The measured isotope effect is then the product of that at each of the two positions.

Carbon dating is another isotope ratio measurement that has been dramatically improved. In this, and related work on other decay processes, a tandem accelerator is used as an extremely sensitive mass spectrometer. The ability of mass spectrometers to count single ions of ^{14}C, arriving at a rate of one per second or even less, represents an advantage over radioactive decay methods, which can observe only atoms that actually decay during the analysis period. By accelerating the beam to high energies, where interfering polyatomic ions can be completely removed by collision-induced dissociation, $^{12}C/^{14}C$ ratios approaching 10^{15} to 1, corresponding to ages of about 40,000 years, can be measured with a precision of a few percent (*17*). Measurement of isotope ratios with high precision has also figured in the interpretation of an iridium anomaly found in geological sediments (*18*).

We conclude this section with two examples of contributions of mass spectrometry outside the area of chemical analysis. Electron impact and photoionization measurements have long provided valuable thermochemical determinations. The quality of mass spectrometric data has been greatly enhanced since the development of equilibrium methods used with high-pressure (*19*) or long reaction time (*20*) instruments. Measurements of the enthalpy and free energy of naked and partly solvated ions derived from this work have been valuable in understanding solvation. The kinetics of unimolecular decomposition of disolvated ions has been studied by a new procedure to determine gas-phase acidities and basicities (*21*). The method is rapid and is applicable to impure samples; data on aliphatic alcohols show high precision ($\pm$ 0.1 kcal mole^{-1}) and provide new insights into factors such as through-space dipolar interactions which affect gas-phase acidities (*22*).

The second example is from an active area of research: comparisons of organic reaction mechanisms in solution with those in the gas phase. The field embracing ionic structure (*23, 24*), thermochemistry (*25*), and gas-phase reactivity (*26–29*) is important for theoretical chemistry and, because of the overwhelming importance of ionic intermediates in solution reactions, is a natural focus for inquiries into chemical reactivity. Chemical ionization can be used to generate the anionic and cationic species which are the postulated intermediates in solution reactions, and collision-induced dissociation can then be employed to characterize these ions and their gas-phase reaction products. Parallels have been established between gas-phase reactions and the classical Dieckmann ester condensation (*30*), the Beckmann rearrangement (*31*), the Wagner-Meerwein rear-

rangement (*32*), and others (*24*). [Agreement in mechanism includes *anti* rather than *syn* rearrangement in the Beckmann reaction (*31*).] Reaction channels not observed in solution can compete in the gas phase, where the absence of solvent has a considerable effect on the relative stability of particular ionic species. Internal solvation is a key to gas-phase reactivity and is the underlying cause of divergence from reactivity in solution, where external solvation is possible. For example, the acid-catalyzed intramolecular cyclization of **1** proceeds via the oxonium ion intermediate **2** which displays quite different behavior in the two phases, yielding CH_3^+ in solution but $CH_3^\cdot$ in the gas phase (*24*).



Trends in Mass Spectrometry

In this section we identify and illustrate the forces that are driving developments in mass spectrometry. Four factors are discussed: (i) multidimensional experiments, (ii) ionization phenomena, (iii) direct mixture analysis, and (iv) instrument elaboration.

Multidimensional experiments. The trend toward multidimensional forms of spectroscopy is evident in many areas of chemical analysis. In some ways, multidimensional experiments are an alternative to high-resolution ones—detail is sought through examination of a more extensive data array rather than ever-finer exploration in a single dimension. Advances in data acquisition and processing have prepared the way for these experiments. They also make it possible to optimize the sensitivity-specificity relation by examining only the regions of data space that contain the most information.

Resonance-enhanced multiphoton ionization (*33, 34*) is an extremely efficient method for ionizing gaseous molecules, whose ionization cross sections are strongly wavelength-dependent. Samples can be characterized by intensity distributions in a two-dimensional (mass, wavelength) matrix. In addition to its efficiency, the orthogonal nature of the mass and photoionization information makes this a potentially powerful technique, both for pure compounds and for mixtures. Early examples of applications to polynuclear aromatic compounds appear to be fulfilling this promise (*35*).

A related two-dimensional experiment is that in which photodissociation of a mass-selected ion is followed as a function of the photon energy. In effect, the ion is characterized by its breakdown curve—that is, by the internal energy dependence of its mass spectrum—and the isomer specificity reported is better than that achieved with simpler experiments (*36*). Rapid, more approximate methods of obtaining the internal energy dependence of mass spectra are also coming into use. In these methods fragmentation patterns are measured as a function of the angle of deflection for collision-induced dissociation at kiloelectron-volt energies (angle-resolved mass spectrometry) (*37*) or as a function of the collision energy in the electron-volt range (energy-resolved mass spectrometry) (*38*). Breakdown curves calculated from unimolecular kinetics or measured by the precise but tedious photoion-photoelectron coincidence experiment (*39*) agree with those obtained by these newer, faster methods. Breakdown curves established by energy-resolved mass spectrometry have been used to optimize experimental conditions, for example, in quantitation of the drug deacetylmetipranolol in urine (*40*).

The MS-MS experiment, in which one analyzer transmits a reactant ion and the second a product ion, is a unique form of two-dimensional spectroscopy (*5, 6*). The analyzed species are strongly coupled through their reactant-product relation (in contrast with the situation in the photoionization experiments already discussed and in the "hyphenated" chromatography-spectroscopy methods). As a result, several informative scan types are accessible: if one fixes the product analyzer, a scan of the analyzer that selects the reactant records all reagents that yield the chosen product. Such parent scans provide information on all constituents of the sample which yield the selected product—that is, all compounds of a particular chemical type. Scans in which the reagent is chosen and the products recorded are termed daughter scans, and they characterize the individual molecular species selected for examination. One can go further: the two mass parameters being selected, say m_1 and m_2, can be surveyed according to a relation of the type $m_1 = m_2 + c$, where c is a constant. Such an expression has meaning only in a homogeneous multidimensional experiment where the same type of property is being examined. The scan just mentioned is useful; it is termed a (constant) neutral loss scan and records all sample constituents that can react by loss of a fragment of mass c. For example, surveys of coal liquids for all phenols (which lose 18 mass units after protonation) can be made with this type of scan, while partially hydrogenated azaaromatics (important in fuel processing) are among several series of compounds easily recognized in such a screen (*41*).

Ionization. The creation of gas-phase ions is emerging from a period of intense activity during which signal advances were made (*42*). New procedures, such as fast atom bombardment (*43*), have been introduced, and advances in other techniques, such as laser desorption (*44, 45*) and electrohydrodynamic ionization (*46*), should increase their usefulness.

While efforts to detail the events that occur in the several desorption ionization methods and to optimize these methods for individual problems will continue, several major lessons are now evident. First, the controlling factors in the newer ionization methods are chemical rather than physical. This point was initially made for field desorption (*47*), but it is also true for other desorption procedures. A variety of physical processes, including different methods of energy deposition, give strikingly similar mass spectra. On the other hand, changes in sample composition on pretreatment with simple reagents can cause profound changes in the nature and abundance of ions recorded in the spectrum. Second, the newer forms of mass spectrometry are more complex than traditional procedures because they are influenced, not only by the unimolecular fragmentations of gas-phase ions, but by ion-molecule reactions in regions of varying pressure (*48*) and by radical reactions associated with energized condensed-phase material (*49*). Third, it is striking that biomolecules are rugged enough to survive the vigorous conditions (such as bombardment with ions at

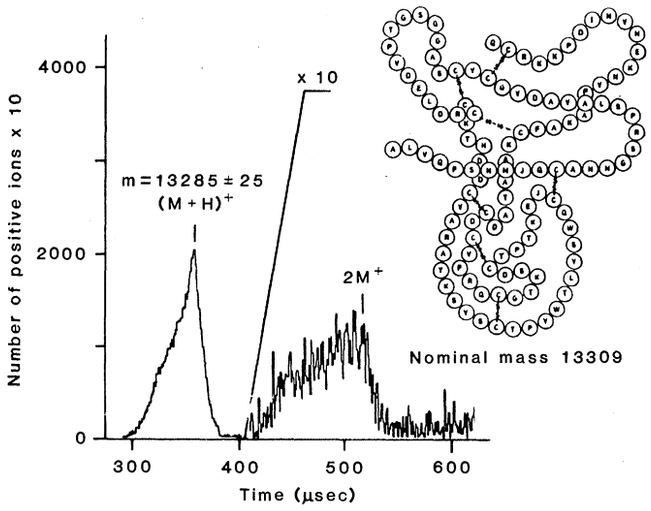


Fig. 1. Molecular weight determination of a tiger snake venom polypeptide. [From (*68*)]

millions of electron volts) used to ionize them. Abundant molecular ions are the rule and structure-spectrum correlations are rapidly being developed. These generalizations do not hold with the same force for organometallics or inorganic complexes, where reactions such as clustering, transmetallation, or ligand interchange can often occur (*50, 51*).

There are corollaries to each of these observations. First, to examine biomolecules by desorption ionization mass spectrometry, it is useful to derivatize them to convert them into an ionic form (*52*). Formation of gas-phase ions then requires a simple phase transfer, which is often very efficient. This approach is the reverse of that traditionally used in mass spectrometry, where volatile and nonionic derivatives have been the objective. It is also much easier to effect; simple acid or base treatments often suffice. The second generalization, regarding the complex origin of ions and often poor signal-to-noise ratios in desorption ionization, represents a problem to which MS-MS is a readily available solution. Combinations of MS-MS, particularly with SIMS, FAB, and laser desorption, have resulted in solutions to problems in alkaloid (*53*), macrocyclic antibiotic (*54*), and macrocyclic peptide characterization, including corrections of earlier proposed structures (*55*). Not only is signal-to-noise ratio improved, but structurally diagnostic fragmentations are recorded. The third generalization, that covering analyte reactions during ionization, has led to the use of glycerol and other liquid matrices to reduce such interactions. The problem is evident in exaggerated form in work on frozen nitrogen oxides, where, even under low-flux conditions, cluster ions are generated with stoichiometries different from that of the analyte (*56*). An alternative solution, which has been used in examining organometallics and inorganic complexes, is the use of solid-salt matrices (*57*).

Activity in ionization continues and the following particulars are worth mention. The bombarding species in desorption ionization can be a metal ion such as Hg^+ (*58*), an organic ion (*59*), or even a dust particle in the 10^9- to 10^{15}-dalton range (*60, 61*). Field emission metal ion sources are also useful (*62*), especially because they do not contribute gas loads to the source and because their intense beams can be finely focused. Many workers have explored liquid matrices in FAB (*63*), including the use of added noble metal salts to act as cationizing agents (*64*). This approach, especially with polyisotopic metals, enhances the ability to identify ions of the analyte.

Plasma desorption (PD), originally achieved with ^{252}Cf fission fragments (*65*), is now often done with beams extracted from nuclear accelerators. The successes of PD are duplicated by particle bombardment sources (SIMS and FAB), which operate in the keV range and are easily retrofitted to commercial mass spectrometers. The time-of-flight mass analyzer used in PD has the advantages of simplicity and a large mass range, and it serves to integrate the low signals available in this experiment. However, it has very low resolution and data acquisition times are usually several hours (*66, 67*). Nevertheless, PD continues to produce molecular weight data on more massive biological compounds than can be measured by other techniques. For example, the molecular weight of a tiger snake venom peptide was measured as 13,284 ± 25 daltons (Fig. 1) (*68*). Fragment ions formed in PD also provide valuable information, as in sequence studies on protected oligonucleotides (*69*) and polypeptide antibiotics (*70*). Intercalation of DNA by polycyclic aromatic compounds may be another area in which this method can make significant contributions (*71*).

Applications of the other desorption ionization methods usually give much higher quality mass spectra. FAB has been used for real-time monitoring of protein digestion (*72, 73*). The enzyme and protein are mixed in a glycerol matrix, and intermittent exposure to the atom beam allows peptide production to be monitored for up to 20 minutes inside the mass spectrometer. The observation of the molecular ion from bovine insulin by plasma desorption (*74*) was followed shortly by the same accomplishment at higher resolution with atom bombardment (*75*), and the spectrum of human proinsulin with the intact molecule at 9390 has now been reported (*76*) (Fig. 2). This type of accomplishment shows the promise of mass spectrometry as a tool in genetic engineering.

Field desorption continues to be important in the analysis of biological molecules. This area has long been its forte, although it has also been useful for trace metal, inorganic, organometallic, and isotopic analyses (*77*). Although the technique has a reputation for irreproducibility, this is countered by the excellent results consistently produced by laboratories that pursue the technique.

A recent application of field desorption mass spectrometry (FDMS) has been in the structural analysis of human hemoglobin variants (*78, 79*). FDMS pinpoints the single amino acid substitution which often creates an abnormality but is not detected by electrophoresis or liquid chromatography. The experiment uses a few micrograms of tryptic hydrolysates of the purified hemoglobins. Molecular weights of peptide residues are assigned from the protonated molecular ions; for peptides of mass higher than about 2100 daltons, doubly charged ions $(M + 2H)^{2+}$ are used. Shifts in the amino acid composition of a peptide are identified by the mass shifts between the expected protonated ion and its replacement. For example, the hemoglobin variant that causes sickle cell anemia involves substitution of valine for glutamic acid in a residue and is easily pinpointed (Fig. 3). FAB has also been used (*79*) for the analysis of hemoglobin variants. The results were roughly comparable with the following exceptions: (i) the FAB spectra contained interfering chemical noise below mass-to-charge ratio (m/z) 500, and (ii) the greater intensity of doubly charged ions in the FD spectra increased the accessible mass range and provided a

second confirmation of peptide molecular weights. Balanced against this is the relatively short period (about 20 seconds) of stable ion emission in FD as compared to the several minutes of ion emission in FAB.

Direct mixture analysis. In many laboratories, much effort is spent on preparing samples for measurement through extraction, centrifugation, and chromatography. Procedures that reduce this effort deserve attention. MS-MS and LC-MS offer this capability and both are undergoing rapid development.

The mass spectrometer is usually thought of as an analytical device rather than a separator, but the two functions are intimately connected. Large mass spectrometers, known as calutrons, have been used for 40 years to separate and isolate macroscopic amounts of particular elements (*80*). By linking two mass spectrometers in tandem it is possible to employ the first as a separator and the second as an analyzer, and so to perform direct analysis of mixtures. The two main advantages of the method can be illustrated (*81*) by considering a complex coal liquid mixture, the mass spectrum of which is shown in Fig. 4a. The signal due to a dioxin spike is lost in the chemical noise from the other constituents and single-stage mass spectrometry is not capable of analyzing for it. MS-MS filters against chemical noise and allows a high-quality spectrum of the dioxin to be recorded (Fig. 4b). In addition to improving detection limits in this way, tandem mass spectrometry provides alternative scan modes which can be employed to efficiently search the data domain for particular information. For example, chlorinated dioxins are characterized by the loss of $COCl^{\cdot}$, so a scan for this reaction reveals all dioxins present in the mixture (Fig. 4c).

The enhanced detection limits that can be achieved by MS-MS over single-stage mass spectrometry are further illustrated by data on nucleosides. In one case (Fig. 5), 36 pmole of a nucleoside gave a spectrum in which the ion due to the analyte was lost in the chemical noise; however, the same sample gives an MS-MS spectrum that has an excellent signal-to-noise ratio and is a good match to that of the authentic compound (*82*).

These types of capabilities are beginning to have an impact on pharmacokinetics, where GC-MS-MS can decrease GC-MS detection limits by an order of magnitude. Isosorbide-5-mononitrate, a coronary vasodilator, is metabolized to the glucuronide, which can be determined in urine by a simple MS-MS procedure to 0.1 ng/ml (*40*). The improved

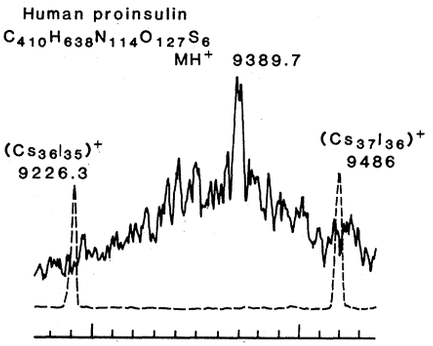


Fig. 2. Molecular ion region of human proinsulin with inorganic cluster ions used to establish mass scale. [From (*76*)]

detection limit in the MS-MS experiment is the direct result of minimizing interferences; one follows a reaction and not simply the product of a reaction.

The search for new compounds by MS-MS, as opposed to analysis of targeted compounds, is a new and rapidly evolving field. In one approach, illustrated by work on alkaloids, daughter MS-MS spectra are taken on all major ions in the chemical ionization (*83*) or laser desorption (*53*) mass spectra. By using only a few grams of plant material it has proved possible to discover new alkaloids in several plant species by careful interpretation of the MS-MS spectra. An alternative approach is to use parent scans to search for structural units expected to be present in the compounds of interest.

Both the sensitivity and the speed of analysis available with MS-MS are noteworthy. Tetrahydrocannabinol administered in doses of 0.1 mg/kg can be followed for 8 days down to 10^{-11} g/ml by using a combination of GC-MS with simple MS-MS to avoid extensive sample cleanup (*84*). Sensitivities in the low parts per trillion have been reported in MS-MS studies on animal tissue (*85*). High-resolution mass spectrometry and MS-MS have been used to achieve absolute detection limits of less than 1 pg for tetrachlorodibenzodioxin (*86*), and a GC-MS-MS combination has achieved good signal-to-noise ratios with 250-fg samples (< 20 parts per trillion) analyzed in a mobile laboratory at the rate of 30 samples per day (*87*). In terms of sample throughput, the determination of trichlorophenol in serum at concentrations as low as 1 ppb and a rate of 90 samples per hour is noteworthy (*88*), as is characterization of the foodstuff contaminant (and chemical warfare analog) vomitoxin at 25 pg in wheat at a rate of 10 minutes per sample (*89*). For the latter analysis, gas chromatography with electrochemical detection has comparable sensitivity but is slower and requires prior sample cleanup.

A different approach to the characterization of mixtures of nonvolatile compounds is LC-MS. The first practical LC-MS interface was based on complete removal of the solvent, and temporary storage of the solute during transport by a moving belt or wire into the ion source (*90*). In the source the sample is either thermally desorbed and ionized by electron or chemical ionization, or the belt is bombarded by an energetic beam to create secondary ions (*91, 92*). Improved techniques are based on direct introduction of some or all of the eluant into the source. The large pumping capacities of chemical and atmospheric ionization sources makes it possible to work at flow rates consistent with normal column operation, for example, 2 ml of aqueous mobile phase per minute (*93–97*). The

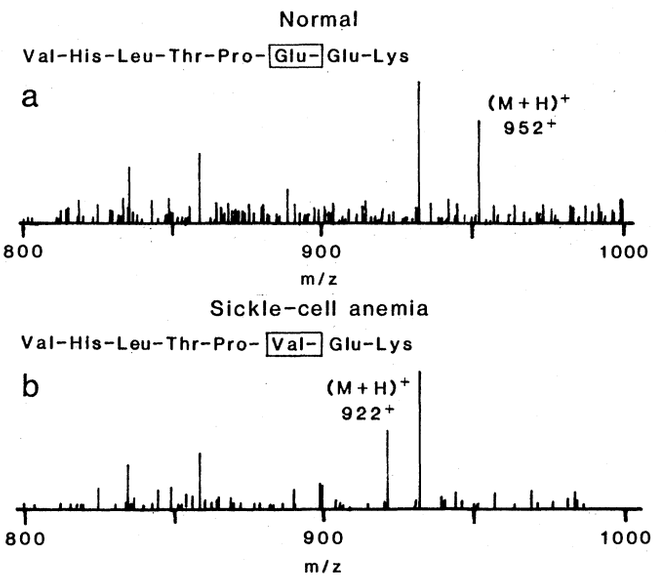


Fig. 3. Comparison of field desorption mass spectra, showing amino acid substitution in hemoglobin. [From (*78*)]

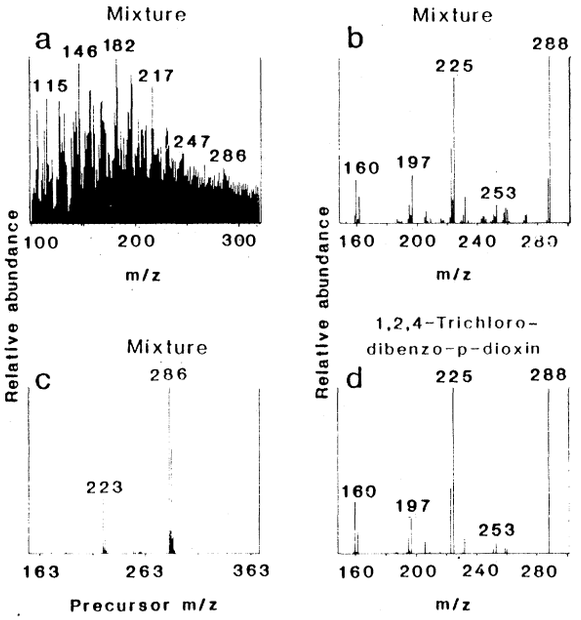


Fig. 4. 1,2,4-Trichlorodibenzodioxin spike in a coal liquid is lost in the chemical noise in the mass spectrum (a) but clearly revealed by the MS-MS daughter ion spectrum of the parent ion 288^+ in the mixture (b), which matches that of the authentic compound (d). A survey of the mixture for chlorinated dioxins is achieved by a neutral loss scan (c). [From (*81*)]

solvent itself acts as the reagent gas in these experiments. An alternative, the thermospray procedure (Fig. 6), does not employ any external ionization technique (*94*). An aerosol generated in the interface is evaporated, and separation of charges present in the nominally neutral solution allows positive- and negative-ion mass spectra to be recorded. Performance, which is still being improved, is illustrated by detection limits of 10 pg (selected ion monitoring) or 1 ng (full spectrum) for β-hydroxyethyltheophylline and by the observation of the protonated molecule of an underivatized decapeptide. The method involving direct liquid introduction and chemical ionization gives comparable data; for example, 50 ng of vitamin B_{12} gives a negative-ion spectrum of high quality (*98*).

Even in the area of elemental analysis, the tendency to minimize separations is being strongly felt. Resonance ionization mass spectrometry (RIMS), one of the most exciting advances in inorganic mass spectrometry of the past decade, employs multiphoton techniques to selectively ionize particular elements (*99*). In one application of the technique, neodymium, a nuclear fuel by-product used in evaluating reactors, is measured in the presence of samarium. Chemical separation of these elements is difficult and several isotopes interfere isobarically, thus precluding thermal ionization from giving accurate isotope ratios for neodymium (*100*).

Instrumental elaboration. Instrumental developments do not only occur in response to perceived needs; capabilities are sometimes in place before appropriate problems are conceived. The past 12 months have seen the introduction or delivery of the following new commercial instruments: (i) an ion trap, a sophisticated but inexpensive three-dimensional quadrupole which forms the basis for a GC-MS instrument, (ii) an inductively coupled plasma (ICP) mass spectrometer, which shows considerable improvements in performance over previous (spark source) mass spectrometric methods of trace metal analysis, (iii) Fourier transform mass spectrometers, which provide high resolution and mass range, MS-MS capabilities, and unrivaled abilities to explore ion-molecule reaction chemistry, (iv) hybrid mass spectrometers, in which a multiple quadrupole section is added to a high-resolution sector instrument to provide both exact mass measurement and standard mass spectrometric capabilities in a single versatile instrument (see cover photograph).

Fourier transform mass spectrometry (FTMS) (*101*) illustrates the speed with which instrumental developments are transforming mass spectrometry. The method has arisen from ion cyclotron resonance experiments, which, in turn, derive from the cyclotron principle of mass-to-charge ratio analysis. The high resolution of FTMS is probably its most discussed property. Impressive performance data have been reported, such as a resolution of 1.4 million for *m/z* 166 from tetrachloroethane (*102*) and $> 10^8$ for *m/z* 18 from water (*103*). FTMS instruments are also capable of performing MS-MS experiments (*104–106*). Unlike a conventional MS-MS experiment, where the different stages of analysis are separated in space, the separation here is achieved in time. This allows the extension of the experiment to three (MS-MS-MS) or more stages (*107, 108*). Choice of ionization procedures in FTMS is constrained by the powerful magnetic field; laser desorption is more readily implemented than the particle desorption methods (*109, 110*). FTMS instruments require very low pressures for optimum performance. This has made interfacing with chromatography difficult, although a GC-MS combination has been reported (*111*). A possible solution to the pressure limitations is the use of a quadrupole mass filter as a device for injecting ions into the FTMS (*112*). All the "dirty" work can then be done in an environment removed from the FTMS.

The main successes of FTMS have been in the area of ion-molecule reaction chemistry, including gas-phase metal ion chemistry (*113, 114*). Ions may be stored, reacted, and characterized in the cell, using computer-selected reaction times and controlled translational energies. The effect of sulfur on Co^+-butane

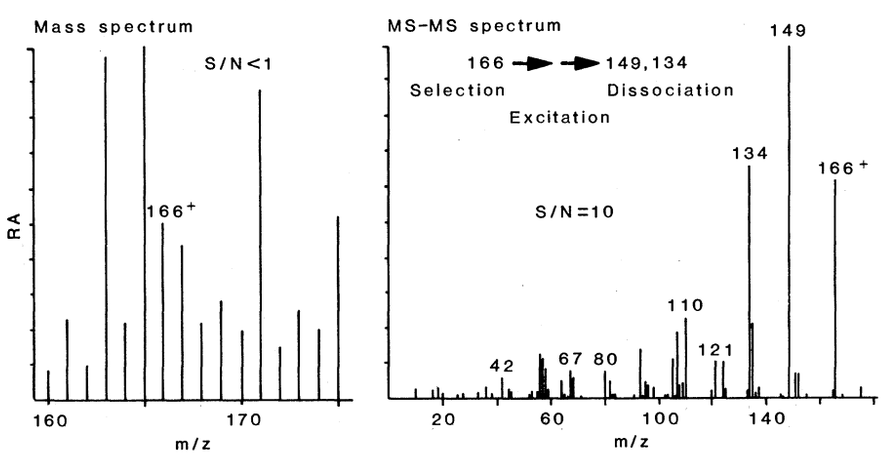


Fig. 5. Improved detection limits in MS-MS over single-stage mass spectrometry for 10 ng of the alkylated nucleoside O^6-methyldeoxyguanosine. [From (*82*)]

chemistry illustrates some of these capabilities. Figure 7a shows a series of peaks corresponding to CoS_n^+ ($n = 0$ to 3) generated sequentially by sulfidation of the metal. Ethylene sulfide is added at a peak pressure of 10^{-5} torr and permitted to interact with Co^+ for about 250 msec. The desired product, CoS^+, is then specifically selected by removing all of the other ions from the cell. The CoS^+ is permitted to interact for approximately 1 second with butane at 10^{-7} torr and the appearance of two products is observed (Fig. 7c). Finally, in Fig. 7d the structure of the $CoC_4H_8^+$ product is examined by collision-induced dissociation. The products observed are indicative of a linear Co^+-butene structure resulting from C–H bond activation.

Instruments that go by the name of mass spectrometer are appearing in ever-increasing variety with an astonishing range of applications. Portable instruments of fairly conventional design have long been used for atmospheric and planetary exploration (*115*), and the Viking mission's life probe included a GC-MS which provided the hard numbers (and bad news) regarding life on Mars (*116*). Environmental monitoring in real time with mobile mass spectrometers is an established technology (atmospheric pressure MS-MS) (*117*) and MS-MS procedures used to automate and greatly speed searches for priority and other pollutants have been reported (*118*). On-line instruments are increasingly used in quality control in industrial plants. A method is no sooner established than entirely new procedures are developed to effect it. This is evident in the use of linked scans of sector analyzers (*119, 120*) and the measurement of flight time during passage through a single analyzer (*121*) to effect MS-MS. Naturally, attempts have been made to maximize, in single high-performance instruments, all the capabilities important in organic analysis. The hybrid instruments that combine sectors and quadrupoles typify this attempt. Commercial versions of these instruments offer exact mass measurements, MS-MS spectra, GC-MS, IC-MS, and various ionization procedures, and they facilitate studies on such fundamental topics as kinetic energy releases and translational energy dependence of ion-molecule reactions.

Prospects

It can be predicted with some confidence that mass spectrometry will soon come to be much more widely used, that many experiments will be done on types of instruments now being introduced, and that the data obtained from mass spectrometers will increasingly be other than conventional mass spectra. These conclusions are based on the following: (i) Low-cost mass spectrometers of advanced design have recently become available. These systems have limited mass ranges (600 daltons in one commercial instrument) and are restricted to electron impact on GC eluants; however, they have data acquisition and reduction capabilities that give them considerable power and guarantee them wide distribution. (ii) Simultaneously, high-performance mass spectrometers of a number of different types are being introduced. Multiple sector, multiple quadrupole, hybrid sector-quadrupole and sector-time of flight, Fourier transform, and other instruments are available. Many are capable of exact mass measurement and have other special capabilities such as variable scattering angles, collision energies, and reaction times. (iii) Much GC-MS work is already being done with selected ion monitoring, rather than by scanning complete mass spectra. The increased variety of experiments possible in multianalyzer mass spectrometers increases the tendency to employ scans or data acquisition schemes optimized to the problem in hand—most notably scans that are selective for the particular compound or group of compounds of interest.

Much of the recent excitement about mass spectrometry has centered on new ionization techniques and new instrumental configurations. Looking ahead, one can see the possibility of new types of experiments in which mass spectrom-

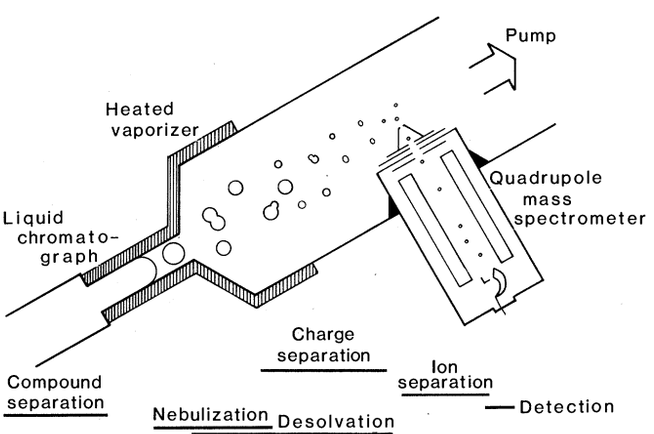
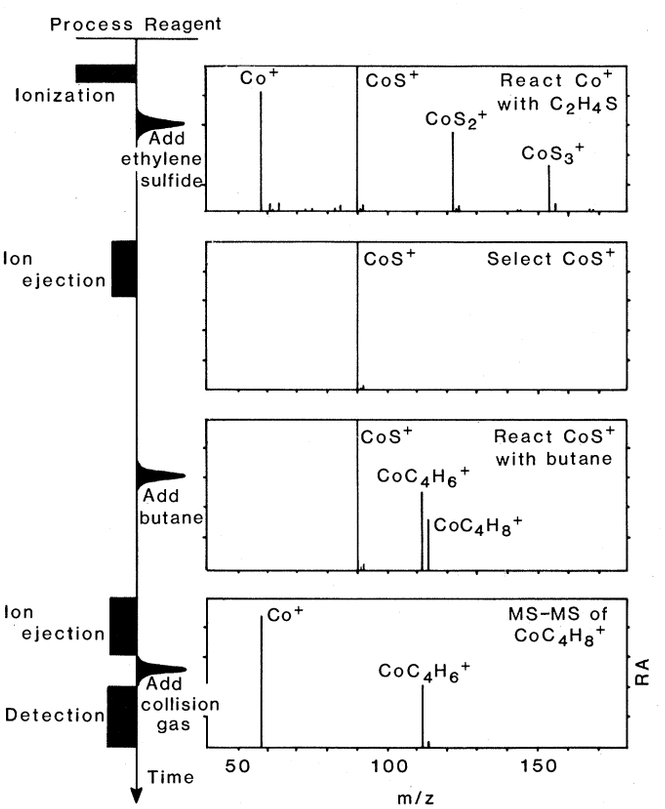


Fig. 6 (left). Liquid chromatograph–mass spectrometer thermospray interface, allowing biomolecules to be examined without an external ion source. [From (*94*)] Fig. 7 (right). Sequence of steps used to prepare ions, react them, and characterize their products in a computer-controlled FTMS experiment. [From T. Carlin and B. S. Freiser]



etry is used. One is the use of molecular ion beams to prepare macroscopic amounts of material, particularly in tailoring surfaces to achieve chemical properties desirable in catalysis (*122*). The variety of chemical species and the control of translational energy and isotopic composition thus available make such synthetic experiments particularly attractive. Ion implantation and metal atom vapor experiments achieve similar objectives, but the even more promising chemical reactions of polyatomic ion beams are essentially unexplored. A second new type of experiment is the imaging of specimens for organic constituents at the micrometer level, using finely focused probe beams in a desorption ionization experiment. Larger scale versions of this experiment have proved successful, including those involving direct examination of the surfaces of paper and thin-layer chromatograms, and of electrophoretograms (*123*). Elemental analysis by analogous procedures is a well-developed procedure (*124*).

The new experiments indicated above exemplify the range of mass spectrometry. We noted earlier that the discipline has been inward-looking, so that some of the more remarkable capabilities of mass spectrometry are little known outside the field. An example is the determination of energy transfers from vibrational to translational modes associated with unimolecular dissociations. These quantities can be measured with extraordinary sensitivity by mass spectrometry; energies less than 10^{-4} eV (about 2 calories per mole) are accessible. For example, α-haloacetophenone molecular ions yield the benzoyl cation in a process releasing 5 to 10 calories per mole (*125*), while charge separation reactions of hydrocarbon dications release up to 3 eV (*126*).

In the biological sciences, applications of mass spectrometry now lag instrumental capabilities, particularly in regard to mass range and ionization. Many classes of compounds for which mass spectrometry has either been awkward or had limited success—steroid conjugates, glycopeptides, phospholipids, and prostaglandins—now fall within its scope. The high sensitivity of the method will continue to make it an important tool for elucidating the structures of new compounds available in trace amounts, as was the case with the endorphins (*127*) and the leukotriene slow-reacting substances (*128*). Clinical applications, as in diagnosing comatose drug overdose patients (*129*) and treating endocrine dysfunctions (*130*), could become much more widespread as instrument costs continue to decline. The capabilities of mass spectrometry in mixture analysis should lead to expanded use in metabolic profiling and pharmacokinetics, and its high molecular specificity, sensitivity, and quantitative accuracy make it particularly useful in such work as the examination of methylated DNA, where the site and degree of alkylation are of concern (*82*), or the direct analysis of antitumor agents in cell cultures (*131*).

Analytical chemistry has a distinct persona, a mode of approaching its science, which is difficult to define but well illustrated through current developments in mass spectrometry. Creativity is expressed in the architecture of new types of instruments, in new scan and data manipulation modes, in optimizing the examination of multidimensional data domains. The biological scientist should seek familiarity with the underlying framework, as well as utilize the techniques and capabilities which are its most recent products. An active partnership is necessary if we are to continue to adapt the mass spectrometer to new ends, if we are to march, rather than stumble forwards.

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Hyphenated Techniques for Analysis of Complex Organic Mixtures

Charles L. Wilkins

Techniques in which a separation device is combined with a detector, such as gas chromatography–Fourier transform infrared spectroscopy (GC-FTIR) and GC–mass spectrometry (GC-MS), have already become well accepted analytical tools. Accordingly, the present article will be confined to those techniques in which two or more detectors are used in addition to a separation device. The vehicle for this discussion will be the relatively new method of GC-FTIR-MS.

About 15 years ago, in one of the earliest papers on the GC-FTIR technique, Low and Freeman (*1*) made the suggestion that addition of a mass spectrometer might yield an even more valuable tool for the analysis of mixtures. Practical realization of this suggestion was delayed until advances in computer technology permitted it. One reason was the demanding data acquisition requirements of on-line spectrometers (signal digitization rates of 30 to 100 kHz for GC-IR and 100 kHz to 5 MHz for GC-MS) and the equally demanding data reduction needs. Furthermore, significant sample mismatch problems arose because of the substantially different requirements of the gas chromatographic separation method and each of the two spectrometric techniques. Thus, it is not surprising that 8 years passed before an analytical system resembling that suggested by Low and Freeman was demonstrated. Even then, the system described (*2*), which included pyrolysis GC, mass chromatography, elemental analysis, and infrared spectrometry "on-the-fly" (that is, with the sample passing through) was not the general-purpose tool for mixture analysis visualized by the earlier workers. In fact, it was 1980 before there was a successful demonstration of a GC-FTIR-MS linkage providing full mass and infrared spectral information on eluting mixture components (*3*, *4*).

For obvious reasons, methods involving integration of multi-instrument arrays have recently come to be known as hyphenated techniques (*5*, *6*). The GC-MS (*7*) and subsequently the GC-MS-COM, when a laboratory computer was added, probably provide the earliest and best known example. It is interesting that computers, and even multiple computers, have become ubiquitous and that their presence in linked analysis systems is no longer noted in the hyphenated

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