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Molecular Analysis by Mass Spectrometry

Modern versions of the Wien-Thompson instrument provide a powerful tool for molecular analysis.

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Summary. New ionization methods combined with powerful analyzers, detectors, and data systems have made mass spectrometry a versatile tool for molecular analysis. Samples consisting of nanogram quantities of hundreds of unique components are routinely analyzed. In favorable cases samples as small as 2.5×10^{-14} gram and samples with masses of more than 3000 atomic mass units have been successfully examined.

The analysis of a substance by mass spectrometry involves conversion of atomic or molecular species into ions and subsequent separation of these ions on the basis of their mass-to-charge ratio by magnetic or electrostatic means. The distribution of mass analyzed ion beams thus obtained is recorded as a function of abundance, and the resulting spectrum is characteristic of the original specimen.

This definition of mass spectrometry, while accurate, fails to convey either the elaborate combination of technologies necessary for instrument construction or the unusual diversity of scientific disciplines within which these instruments find application. A modern mass spectrometer is constructed from elements which approach the state-of-the-art in solid-state electronics, vacuum systems, magnet design, precision machining, and computerized data acquisition and processing. The instruments find application in studies as diverse as biomolecules, petrochemicals, pharmacology, geochemistry, forensic chemistry, and the environment. A mass spectrometer was even included in the scientific package of the Mars lander (*1*).

Early mass spectrometers (*2*) were devoted almost exclusively to studies of permanent gases and gradually evolved to include the general field of atomic analysis, which still represents an important application. The recent strong growth in mass spectrometer use has been based, however, almost exclusively on advances in molecular analysis. In this article I discuss in some detail how the rather special demands of molecular analysis have influenced spectrometer design and describe how modern mass spectrometers have dramatically influenced experimental methodology in a wide variety of fields.

Mass Spectrometer Components

The fundamental challenge of molecular analysis by mass spectrometry centers on the successful formation of ions from molecules without undue degradation of frequently delicate molecular structures. The mass spectrometer ion source and its associated sample introduction systems are responsible for this function. Accordingly, these two components are by far the most critical to successful molecular analysis. Other major components, however, including in particular the mass analyzer, the detector, and the vacuum system must also meet rather special demands. These last components, although less critical, nevertheless constitute the basic hardware that must be present to accomplish mass analysis regardless of the source of ions. Therefore, since they truly form the "foundation" of the instrument these components are discussed first.

A number of schemes involving magnetic fields, electric fields, or a combination of the two have been used to separate composite ion beams into their components. Modern commercial mass spectrometers utilize one of four basic analyzer types: the single or double focusing magnetic analyzer, the quadrupole analyzer, or the time-of-flight analyzer. Both currently and historically, the magnetic analyzer is used in most spectrometers.

The magnetic analyzer. Figure 1 demonstrates the action of a magnetic analyzer. The motion of ions through the analyzer is described by the equation

$$r_m = (2Vm/ZH^2)^{1/2} \quad (1)$$

where V is the accelerating voltage used to extract the ions from the source, m is the mass of the ion, Z is the charge on the ion, H is the magnetic field, and r_m is the radius of the circle through which an ion will travel under these conditions. Ions of different mass M_1 and M_2 will follow different paths when other variables are held constant. In such an analyzer it is usually impractical to have a separate detector for each mass and therefore the magnetic field or, less frequently, the accelerating voltage are scanned to bring the various beams sequentially to a single fixed detector. Alternatively, a photoplate may sometimes be used as a detector over a fairly wide mass range.

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An important parameter to consider in evaluating any analyzer is its resolution. Resolution refers to the ability to separate ions having small differences in mass and is defined as the mass of a given peak divided by its separation in mass from an adjacent peak, with the masses being expressed in atomic mass units (AMU).

By convention, two beams that are separated by a valley 10 percent of their height are considered resolved. This means that if a peak of mass 600 is separated by a 10 percent valley from a peak of mass 601 then the resolution is 600, because the mass is 600 and the separation is 1. In molecular analysis where a large number of different combinations of elements can potentially give the same unit mass, high resolutions may be required. For example, the resolution required to separate the molecular ion of dibutylnitrosamine, an important environmental pollutant (mass = 158.1419) from a possible aromatic hydrocarbon contaminant of formula $C_{12}H_{14}$ (mass = 158.1095) is about 5000.

A number of factors influence the resolution of a magnetic analyzer. The first of these is the ability to compensate for the ion beam divergence. The sources described later all have the common feature that beams of ions emerge from them with a relatively narrow but finite angular distribution. This divergence could be reduced by collimation at a great loss in sensitivity; however, Dempster (*3*) recognized that a magnetic analyzer can have a lens-like action whereby ions with the same energy and mass but traveling in slightly different directions can be brought to focus at a single point. This is shown in exaggerated form in Fig. 1. This process, called direction focusing (*4*), requires accurate positioning of the source and detector with respect to the magnet. Without it, the use of a divergent beam results in much reduced resolution since the spreading of the unfocused beams can become wider than the separation observed between them at the collector slit.

A second factor, called the mass dispersion, also affects resolution. Dispersion describes the separation obtained between ion beams on passing through the analyzer. In general it is directly proportional to the radius of the ion path such that a greater radius gives a greater dispersion and therefore a higher resolution. A greater radius generally requires a larger magnet which, although capable of higher resolution, may dictate somewhat slower scans. A velocity distribution in the ion beam resulting from the random motions of the molecules prior to ionization and acceleration also affects the resolution in a magnetic analyzer. With respect to the analyzer, this is equivalent to saying that the accelerating voltage has a distribution of values. It is clear from Eq. 1 that the radius is dependent both on mass and accelerating voltage, so that variations in V necessarily degrade the resolution. The effect of this problem can be minimized by using very high accelerating voltages which in turn make the initial variation a smaller fraction of the total value. In general, instruments with higher accelerating voltages are capable of higher resolutions. This approach is limited by the need also to use magnets with proportionally higher fields.

Although they should be considered when evaluating an instrument, the parameters discussed so far are not usually controllable by the user. They determine ultimate resolution but seldom determine (and should not determine) working resolution. The working resolution is usually set by the operator by his choice of source slit size. Within limits the resolution will necessarily go up as beam size goes down since the beam size must necessarily be less than the separation (dispersion) obtainable. A point is always reached at which closing the beam defining slit further will not improve the resolution. This limit is usually determined by the distribution of velocities in the ion beam. For a well-optimized magnetic analyzer with a large radius, the limiting resolution is about 7000, with truly useful sensitivity only to about 3000. In order to obtain higher resolutions, it is necessary to take steps to obtain velocity focusing in much the same way that errors in beam direction are corrected by direction focusing.

Velocity focusing can be obtained by the addition of an electric analyzer in

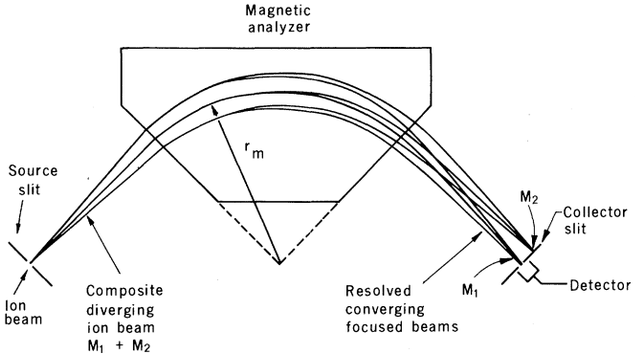

Fig. 1. A 60° magnetic analyzer illustrating mass separation and, in exaggerated form, direction focusing.

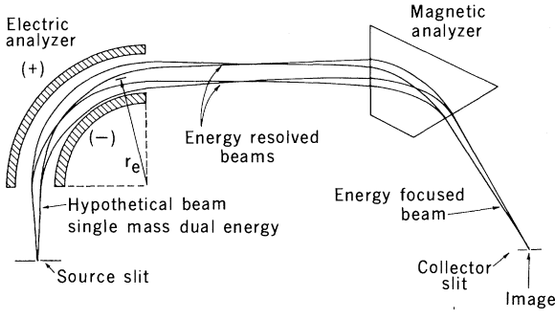

Fig. 2. A double-focusing mass spectrometer with Nier-Johnson geometry (*6*). Direction and velocity (energy) focusing are shown in exaggerated form.

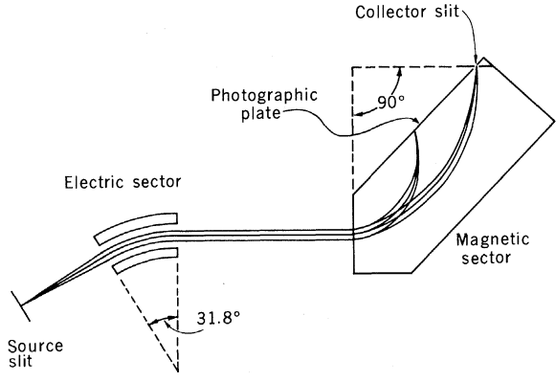

Fig. 3. A double-focusing mass spectrometer with Mattauch-Herzog geometry (*5*).

tandem with a magnetic analyzer as shown in Fig. 2. The radius of an ion beam in a radial electric field is given by Eq. 2

$$r_e = 2V/E \quad (2)$$

where V is the accelerating voltage and E is the voltage across the electric analyzer plates. This analyzer is referred to as a kinetic energy or velocity analyzer because it disperses ions according to their velocities as determined by the combination of accelerating voltage (V) and initial molecular velocity. The analyzer has direction-focusing properties similar to those described for a magnetic sector, so that this feature is not lost. When an electric analyzer and a magnetic analyzer are combined in the proper geometry, the spatial dispersion of ions of different energies produced by the electric sector can be compensated by the refocusing of these ions to a single point by the magnetic analyzer. This process is illustrated in exaggerated form in Fig. 2, where two ion beams having different velocity (energy) but the same mass can be seen to be separated by the electric sector and refocused by the magnetic sector. Since in this case both velocity and direction focusing are obtained, such instruments are said to be double-focusing. Only certain geometrical arrangements will provide double-focusing (*5*). Commercial instruments are available with geometries determined by Nier and Johnson (*6*) (Fig. 2), Hintenberger and König (*7*), and by Mattauch and Herzog (*5*) (Fig. 3). The first two are quite similar in that they provide only a single point of double focus. The Mattauch-Herzog geometry, however, provides a plane of focus and may be used with photographic detection.

With the use of these double-focusing arrangements, commercial instruments now provide resolutions in excess of 150,000. It is difficult to appreciate the technical achievement that such ultrahigh resolution spectrometers represent unless one considers the magnitudes of the actual physical parameters involved. For example, at 150,000 resolution, the ion beam width is on the order of 1 micrometer, voltages and fields are controlled within several parts per million, and positions of source, analyzer, and detector are critical in the micrometer range with total physical separations in the 1- to 3-meter range. It is equally important to appreciate that when using such a spectrometer, it is possible to select an ion beam with such precision that interferences in the measurement of that exact molecular species will most likely come only from other species of identical elemental composition and in the worst case will come only from other fortuitous combinations having near identical mass—a relatively unlikely occurrence.

In conclusion, it should be noted that the magnetic analyzer is unambiguously the most powerful presently available in terms of ultimate resolution; it is, however, inferior to the quadrupole analyzer in scan speed and to the time-of-flight analyzer in scan speed and mass range.

Quadrupole analyzer. A quadrupole mass analyzer (*8*) consists of an arrangement of four pole pieces of preferably hyperbolic cross section symmetrically disposed along the direction of travel of the ion beam. A d-c voltage and radio-frequency component that is variable both in magnitude and frequency are imposed on the rods. Rather complex stable paths for ions of various masses can be obtained by appropriate combinations of these voltages. The mathematical expressions describing the operation of this analyzer are complex and will not be discussed here. Since mass selection is accomplished purely by variations in voltages or frequencies, the quadrupole analyzer is characterized by its ability to scan very quickly with minimal hysteresis and to switch rapidly between settings for a number of different ions with negligible dead time. Even with machining tolerances in the micrometer range and state-of-the-art electronics, commercial quadrupole analyzers have a practical resolution limit of about 1000. Furthermore, the analyzer suffers from significant mass discrimination—the failure to pass ions of different mass with the same efficiency. The quadrupole analyzer has the advantage of relatively low cost and is widely used for low-resolution applications, especially in combination with a gas chromatograph where its superior scanning capabilities are utilized. The resolution of this analyzer is easily degraded by the presence of contaminants on the poles. It is well suited to analytical applications where unit resolution to about mass 600 is required.

Time-of-flight analyzer. The time-of-flight analyzer utilizes a pulsed ion source and achieves mass resolution on the basis of differing velocities (and therefore flight times) obtained from ions of differing mass exposed to the same accelerating voltage. Resolution of about 1000 (10 percent valley) has been achieved in commercial instruments and recent work extends the hope of much improved resolution for this analyzer (*9*); however, at present it is the least used in molecular analysis by a wide margin. This analyzer has the advantage of an essentially unlimited mass range; however, advances in ionization techniques have not yet exceeded the capabilities of magnetic analyzers that offer much higher resolutions.

Detection systems. Regardless of the type of mass analyzer employed, it is necessary to provide some form of ion detector at the outlet of the analyzer. Early mass spectrometers utilized fluorescent targets, photoplates, or simple Faraday collectors. Commercial mass spectrometers today most frequently utilize specially designed electron multipliers in which the first dynode converts the energy of incident ions into secondary electrons which are then amplified in a number of stages in the usual manner. Electron multiplier gains of 10^6 to 10^7 are routinely obtained. This means that for every ion incident on the detector, 10^6 to 10^7 electrons appear at the output. This very large amplification allows the detection of single ions.

As has already been suggested, it is frequently important to be able to obtain a mass scan very quickly. Such a fast scan might cover the range from 40 to 400 AMU in 1 second. This means that at a resolution of 400, a given mass-analyzed ion beam would spend less than 3 milliseconds on the detector. With the use of an electron multiplier, peak positions can be found with about 10 ions; consequently, ion currents of about 3000 ions per second can be useful at this resolution and scan rate. Higher resolutions require higher ion currents and decreased scan rates require lower ion currents. An unknown sample with a residence time in the ion source of 1 second (the time of one scan under the above conditions) which cleanly undergoes conversion of each molecule into a single ionic species would need to produce only 3000 ions over the duration of the 1-second scan to have its mass unambiguously determined to $\pm$ 0.5 AMU. If the efficiency of the ion source and ion optical system in producing ions and delivering them to the detector at this resolution is about 8 ppm (a good value these days) then a sample of about 4×10^8 molecules would be sufficient. For a sample of molecular weight 300, this is about 2×10^{-13} grams. Clearly, lower scan rates or selected ion monitoring (all 3000 ions collected without scanning at all) reduces the sample requirements proportionally. However, molecules in the real world do not generally convert cleanly to a single ionic species but usually fragment badly, and it would be difficult routinely to compress sample introduction into a 1-second burst closely synchronized with a mass scan. In reality then, ultimate detection limits at a resolution

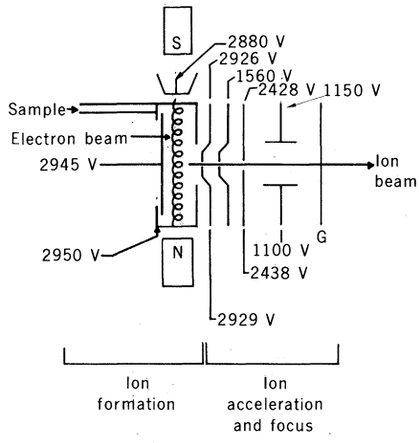

Fig. 4. Electron impact (EI) ion source. *S*, *N*, are the north and south poles on magnets and *G* is ground potential, "0 volts."

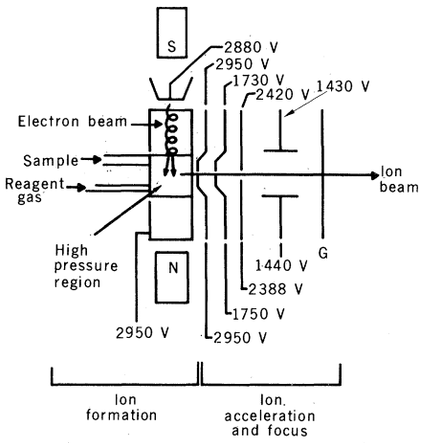

Fig. 5. Combined chemical ionization (CI) and electron impact ion source shown in chemical ionization mode.

of 1000 with scans of 1 to 2 seconds per mass decade (for example, 50 to 500) require about 1 to 50 ng of material when high-efficiency ion sources are used. Under certain conditions, 2.5×10^{-14} g of material has been used with selected ion monitoring (*10*). An important shortcoming of the electron multiplier is its loss of sensitivity at very high mass (*11*). It will become increasingly important to address this problem as new modes of ionization provide ions of higher and higher mass.

A second important detector is the photoplate. At present this detector is used exclusively with double-focusing instruments of Mattauch-Herzog geometry. This detector is used with few exceptions (*12*) for determination of mass at high resolution where numbers of ions are very small and scanning is therefore relatively difficult. The detection limit appears to be on the order of a few nanograms of sample at 40,000 resolution. The photoplate also has potential application with ion sources that produce signals of low intensity over short periods of time, because it has the advantage of simultaneous detection of all ions. A special detector is being developed for Mattauch-Herzog instruments that is essentially a focal plane multichannel electron multiplier that records all ions simultaneously and electronically (*13*). At present, the spatial resolution of this detector limits mass resolution to about 1000. Because this detector can record an entire spectrum with the same advantage as that noted for selected ion monitoring, it has the potential to improve spectrometer sensitivity several orders of magnitude.

Vacuum system. An important component of any mass spectrometer is its vacuum system. In many ways a mass spectrometer can be accurately described as a large, rather elaborate high-voltage vacuum tube. It would be heresy even to consider introducing any material into such a vacuum tube that could degrade the vacuum, compromise the insulators, or form insulating coatings on the grid surfaces. Unfortunately, this is exactly what is done whenever a molecular sample is introduced into a mass spectrometer. Accordingly, a mass spectrometer has rather stringent vacuum requirements. In magnetic instruments the analyzer section should be maintained at pressures of 10^{-7} torr or less in order to minimize resolution losses arising from collision of the ion beam with neutral molecules. Further, the source region must have exceptionally fast pumping in order to avoid memory effects. It is common for the source and analyzer regions to be differentially pumped with the only connection between the two regions being a channel just large enough to pass the ion beam. This practice prevents the sometimes high pressures in the ion source from degrading the vacuum in the analyzer.

High vacuum in mass spectrometers has usually been produced by diffusion pumps, although ion pumps have occasionally been used in the analyzer region. Recently, turbomolecular pumps have been introduced by a number of manufacturers. These pumps provide an ultraclean relatively robust source of high vacuum at pumping speeds of several hundred liters per second and, further, they require no cold traps. The availability of this completely mechanical source of high vacuum represents a major advance in mass spectrometer design. Finally, it is important that the entire vacuum enclosure of a mass spectrometer and especially the source region be fully heat resistant so that accumulated adsorbed samples can be removed by baking.

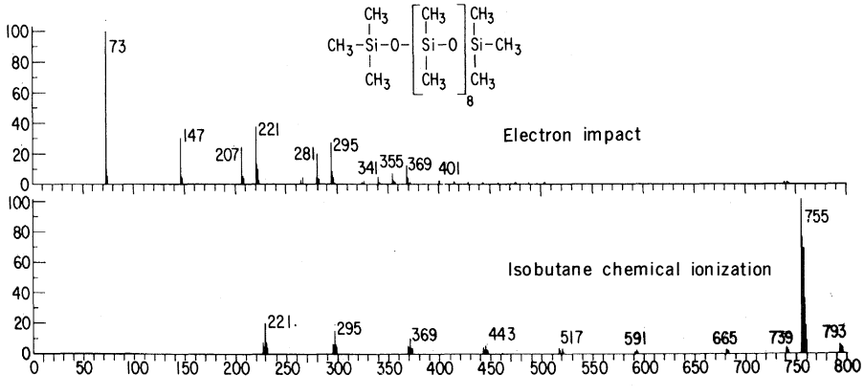

Fig. 6. Electron impact and chemical ionization mass spectra of a trimethyl silyl end-capped dimethyl silicone oligomer. Instrument: Varian-MAT 311A.

Techniques of Molecular Analysis

As noted earlier, the essence of molecular analysis is the production of ions from molecules without degradation of molecular structure. An ionization technique that provides no molecular ions (ions derived from the intact molecule without fragmentation) is useful for analysis of unknown materials only if reference spectra are available or if the array of fragments can be interpreted to imply a particular molecular structure. As a result, ionization techniques that provide reasonable yields of molecular ions are preferred.

The generation of an ion beam frequently involves two processes because many samples must be both volatilized and ionized. It is important to note that the present limits of molecular mass spectrometry are determined neither by

spectrometer mass range nor by inadequate ionization techniques but rather by an inability to volatilize samples without thermal degradation.

Electron impact. The oldest and still most widely used ionization method is electron impact. A schematic diagram of an electron impact source is shown in Fig. 4. Gas-phase samples are confined in an ionization box together with an electron beam of from 30 microamperes to 3 milliamperes having an energy of 10 to 100 electron volts. The electron beam interacts with sample molecules, dislodging electrons and forming positive ions. The ions thus formed are extracted and focused into a beam by means of the electrodes and voltages shown in Fig. 4.

Ionization by this method results in the transfer of large amounts of energy to the molecule which frequently results in extensive fragmentation. Operation with low electron energy minimizes fragmentation but drastically reduces ion yield. No other ion source provides equal sensitivity combined with reliability and ease of operation. In addition, practically all of the data present in libraries of mass spectra were obtained with this source. The sensitivities quoted earlier as being typical refer to the electron impact source. Recent improvements in this source have been derived largely from increased electron currents, improved ion extraction and focusing, and smaller "tighter" ionization boxes. The more compact ionization box restricts the sample more closely to the region of the electron beam, thereby increasing the ionization efficiency.

Samples are admitted to an electron impact source in a variety of ways. The most common of these include: (i) a batch inlet that consists of a simple heatable vacuum vessel into which samples can be placed, vaporized, and subsequently leaked into the mass spectrometer; (ii) a solids probe that consists of a rod with a heated tip which can be introduced through a vacuum lock into the mass spectrometer into a position where relatively high-boiling samples placed on the tip can be warmed and vaporized very near the ion source; or (iii) a gas chromatograph either connected directly to the source when sufficient pumping capacity is available or connected via a variety of molecular separators (*14*) which serve to remove the carrier gas. It has also been demonstrated that heating small amounts of sample directly adjacent to the electron beam sometimes allows the analysis of species with very low vapor pressures without thermal decomposition of the sample

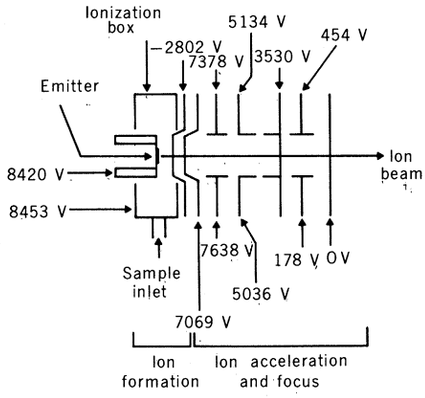

Fig. 7. Field ionization-field desorption ion source.

(*15, 16*). In any event, samples must always be in the gas phase in order to be ionized by this source. For this reason, the source has limited general application for molecules with molecular weights greater than about 1000, because of their low vapor pressures, and for molecules such as sugars which combine thermal lability with low vapor pressures.

The electron impact ion source is most commonly operated in such a way that it produces a beam of positive ions. By reversing the polarity of the accelerating voltage, it may also be used to produce a beam of negative ions. In the negative ion mode, the sensitivity observed is related to the ease with which a sample molecule can capture electrons. In this mode, therefore, this source has exceptionally good sensitivity for molecules such as polyhalogenated aromatics (*17*), and notably poor sensitivity for materials with lesser electron affinities.

Chemical ionization. Many molecules, especially those largely aliphatic in nature, exhibit extensive fragmentation under electron impact and frequently fail to give molecular ions. This shortcoming of the electron impact source has led to the development of the chemical ionization source (*18, 19*) (Fig. 5).

This source differs from the electron impact source in Fig. 4 only in that the ionization chamber is now very tight so as to allow the generation of pressures of several torr of an externally added reagent gas. This high-pressure reagent gas is converted to a plasma by an electron beam. Sample molecules admitted to this plasma are ionized by the plasma according to mechanisms that are dependent both on the reagent gas and on the sample. The addition of a proton from the plasma is the most common mode of ionization observed with the chemical ionization source. The advantage gained by the use of chemical ionization is well demonstrated in Fig. 6, in which an electron impact mass spectrum of a silicone is compared with a chemical ionization mass spectrum of the same molecule when isobutane is used as the reagent gas. No molecular ion is obtained at all by electron impact, but a strong protonated molecular ion (m/Z = 755) is obtained by chemical ionization [the distribution of interactions of various reagent gases and sample combinations are discussed in (*20*)]. The inherent difficulty in predicting the nature of the interactions between a given reagent gas and a sample makes the analysis of complete unknowns somewhat difficult.

As in the electron impact source, the chemical ionization source suffers from the disadvantage that the ionization occurs from the gas phase. Samples may be admitted to the chemical ionization source by all of the methods described for the electron impact source. Materials with very low vapor pressures can frequently be run by heating the sample directly in the ionization region (*21*) in a manner similar to that described for electron impact. Use of a liquid chromatograph with a mass spectrometer has been demonstrated and such an instrument is commercially available. Unfortunately, this combination severely limits the liquid chromatograph since only samples that can be volatilized after reaching the mass spectrometer can be run. Since such volatile samples are frequently run more successfully by gas chromatography, the liquid chromatograph–mass spectrometer (LC-MS) combination has rather limited application.

The sensitivity of the chemical ionization source is dependent on the sample and reagent gas, but in favorable cases it can exceed that observed with electron impact sources. The chemical ionization source can also be used in the negative ion mode (*22*).

Field ionization. A third and fundamentally different type of ion source is the field ionization source (Fig. 7) in which ions are produced by the influence of very high electric fields. The electric field strengths required are generated by the combination of suitably high voltages (10 to 12 kilovolts) and specially formed anodes consisting of one or many very fine points with diameters less than a micrometer. The electric field is concentrated at these points and ionization is considered to occur in the vicinity of the points by way of electron tunneling (*23*). The ions, once formed, are extracted and focused into a beam as in other ion sources. This source has the distinct ad-

vantage that most compounds give molecular ions. This fact arises because relatively small amounts of energy are transferred to the sample molecules and because the time between ionization and detection in this source is much shorter than that for other sources, thereby allowing less time for fragmentation.

This source has the disadvantages that like electron impact and chemical ionization, samples must be added in the gas phase, and that the sensitivity is on the order of a factor of 1000 less than an electron impact source. The frequent absence of fragmentation can also be a disadvantage since structural information implicit in such degradations is lost. An additional problem is the necessity to construct reproducibly appropriate field anodes. Schulten and Beckey (*24*) have described a method for growing carbon dendrites by pyrolysis of benzonitrile in high electric fields for use as field anodes. A micrograph of a field emitter prepared according to these authors is shown in Fig. 8. This method requires about 24 hours of preparation time, but recently, less time-consuming techniques have been described (*25*). Other approaches to this problem have also been discussed (*26*). One further difficulty encountered with field ionization is the variation in ion yields depending on the history of the anode or even the particular combination of components in a sample mixture. This problem appears to arise from alterations in the anode surface by adsorption of sample. When samples are run sequentially, this problem can be minimized by strong heating of the anode between samples.

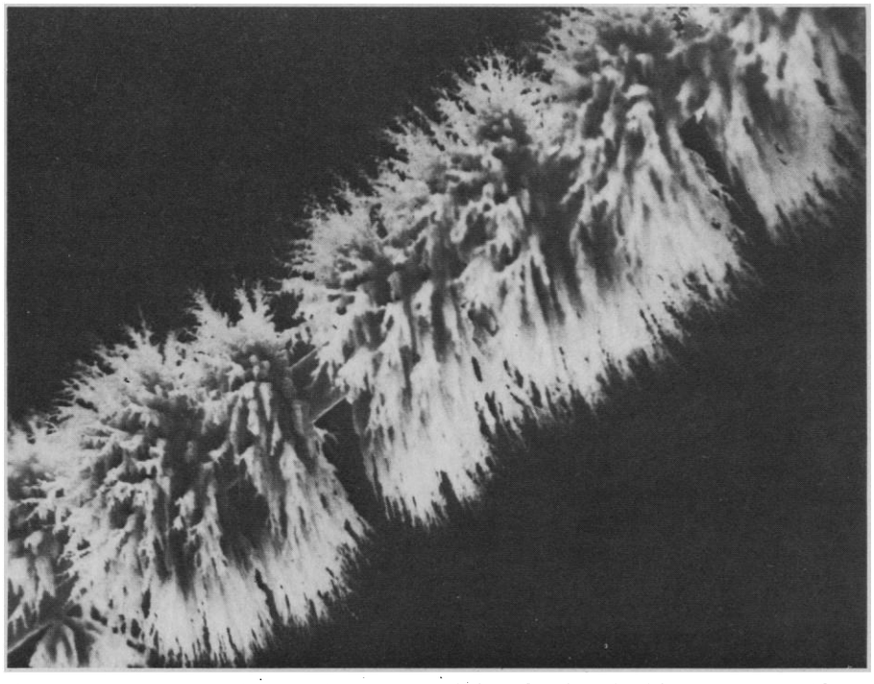

Fig. 8. A photomicrograph of a field anode consisting of carbon dendrites grown on a 10-μm tungsten wire by the method of Schulten and Beckey (*24*) (× 350). [Photograph by E. Lifshin]

A modification of the field ionization source, in which the field anodes are removed from the ion source coated with sample by deposition from solution and are subsequently remounted in the ion source, is called field desorption. This technique developed by Beckey (*27*) has wide application to the analysis of thermally labile samples and samples of very high molecular weight. This technique has been particularly useful in studies of biologically significant molecules, providing in many cases the first unequivocal verification of structure. The technique has been applied to antibiotics, peptides, nucleosides, nucleotides, oligosaccharides, and carotenoids to name only a few. Figure 9 shows the field desorption spectrum of sucrose. Useful spectra of this molecule are not obtained by conventional electron impact or chemical ionization techniques.

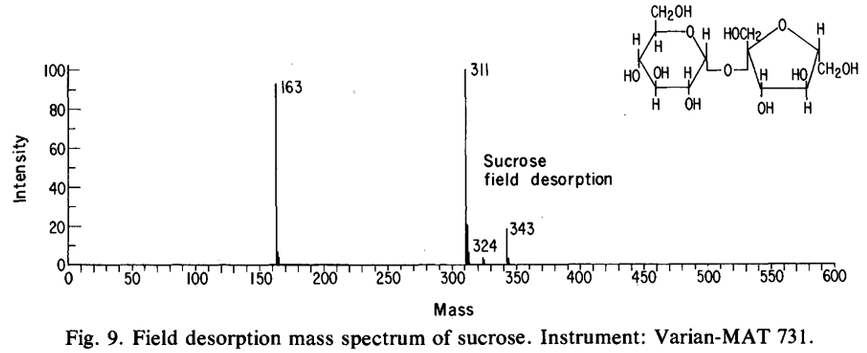

Fig. 9. Field desorption mass spectrum of sucrose. Instrument: Varian-MAT 731.

The exact nature of the ionization process in field desorption is a matter of intensive study. In at least some instances, it appears that coating the anode with sample simply serves to provide a reservoir of sample very near the site of ionization and that ionization occurs as in

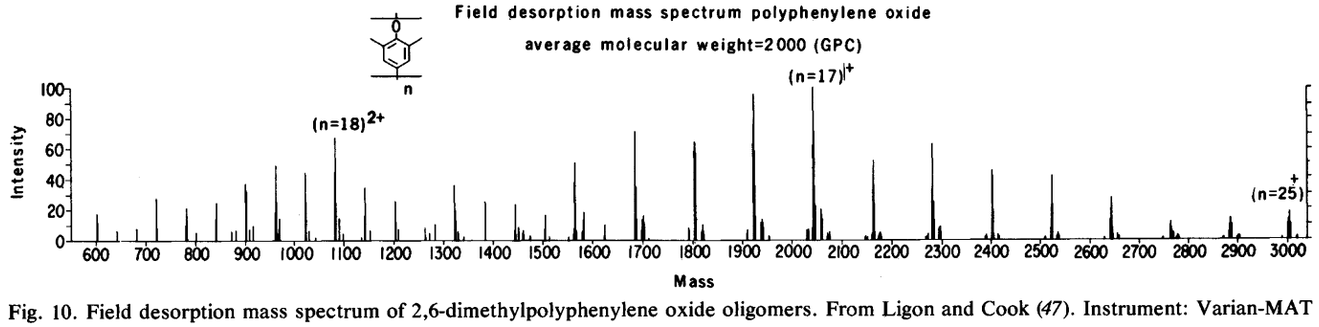

Fig. 10. Field desorption mass spectrum of 2,6-dimethylpolyphenylene oxide oligomers. From Ligon and Cook (*47*). Instrument: Varian-MAT 731.

field ionization. In these instances the ability to run samples of very low vapor pressure appears to result simply from the proximity of the sample to the site of ionization as described for electron impact. This interpretation is supported by the observations that heating of the anode is frequently required to obtain spectra, and that disappearance of the sample from the anode under heating appears to occur at the same rate independent of the application of high voltage (*28*).

A number of other surface-related ionization mechanisms have, however, been shown to occur under substantially the same conditions. For example, the addition of alkali metal ions to samples deposited on the anode leads to the observation of strong ion currents of alkali metal ions solvated by the organic molecules present. It has further been shown that field anodes treated with saponified alkali metals can produce ion beams of alkali metals solvated by organic samples admitted from the gas phase (*29*). A similar process has been demonstrated for anodes treated with sources of protons, and this process has been shown to be related to ease of protonation and not ionization potential (*30*). These processes appear to occur from liquid phases and to be most apparent in the absence of the usually required anode microstructure. It appears likely that these processes are occurring from field-induced conical deformations of the liquid surface which concentrate the field in the same way as the dendritic microstructures used in field ionization. In this case the high field has the effect of extracting charged components from the liquid and does not result in classical field ionization. These processes appear closely related to electrohydrodynamic ionization, a technique in which charged species are field-extracted from glycerol solution (*31*).

It has been discovered empirically to be occasionally advantageous to encourage these alternative ionization mechanisms by, for example, adding lithium ion to a sample, because improved sensitivity may result (*32*).

In terms of a capability to obtain mass spectra of compounds having very high molecular weights and very low volatilities, the field desorption source represents the state-of-the-art in commercial instruments. Figure 10 demonstrates the high mass capability of the technique. As noted under field ionization, largely unpredictable modifications of the anode surface by samples and sample impurities can dramatically alter ion yields. This problem is accentuated under field desorption conditions because of the wide variety of largely uncharacterized materials which may be deposited from solution as part of the sample. It is not unusual for such "impure" samples to yield no information whatever, even when compounds amenable to field desorption are known to be present.

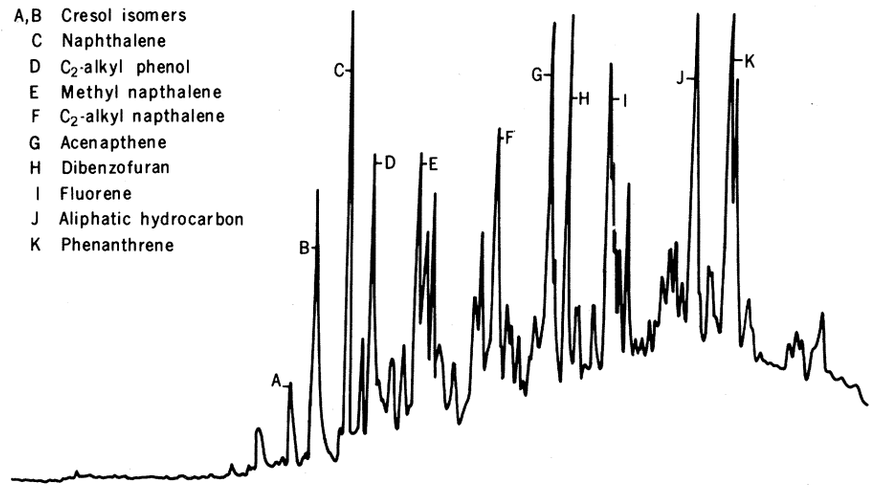

Fig. 11. Gas chromatography–mass spectrometry analysis of condensible fraction from experimental coal gasification procedure. Instrument: Varian-MAT 111.

Other ion sources. A number of other ion sources have been reported and used over the years, and some are available in commercial instruments. These include, for example, photoionization, surface ionization, and atmospheric pressure ionization sources. Space does not allow the discussion of these less widely used sources here.

An important trend in ion source design is based on the realization that very large thermally labile molecules can frequently be ionized and analyzed intact if very strong heating is applied very quickly. This has been demonstrated both by fission fragment impact heating (*33*) and by laser pulse heating (*34*). A commercial instrument utilizing laser pulse heating and a time-of-flight analyzer has recently appeared.

Gas Chromatography–Mass Spectrometry

The use of a mass spectrometer as a sophisticated detector for a gas chromatograph is by far the most important specialized application of the instrument both in terms of broad scientific impact and impact on instrument development. This combination, first described by Holmes and Morrell (*35*), has directly resulted in an almost exponential growth in the use of mass spectrometers. The exceptional power of this combination arises from its ability to provide unambiguous qualitative and quantitative analyses of submicrogram quantities of materials present in extraordinarily complex mixtures. Figure 11 summarizes in part the results of the GC-MS analysis of a particularly complex mixture. The components indicated represent only a small fraction of the nearly 150 species identified in this sample. The total sample weighed more than 100 micrograms.

The development of GC-MS placed a number of new demands on mass spectrometer performance and, because of the great interest in the technique, provided the economic incentive for manufacturers to provide instruments which met these demands. The upshot of this has been a rapid escalation in mass spectrometer performance. The areas most affected have been vacuum systems, scanning capability, sensitivity, and data system development.

A gas chromatograph may be connected by way of a molecular separator that eliminates a major portion of the carrier gas, or it may be connected directly. Early GC-MS combinations invariably utilized molecular separators or sampled only a small portion of the gas chromatographic effluent. This was because of their limitations in vacuum system capacity. Modern instruments allow gas inlet flows of several milliliters per minute without degradation of instrument performance. In addition, pumping is sufficiently rapid that gas chromatographic effluents separated by only a few seconds in time do not exhibit memory effects. Both source and analyzer region are now routinely manufactured with heat-resistant materials from which contamination by gas chromatograph effluents can be removed by heating.

High-resolution gas chromatography columns provide effluents of resolved components to the mass spectrometer during periods of only 5 to 10 seconds or less. This requires that the mass spectrometer be able to scan very quickly indeed. Such fast scans are relatively easy to accomplish with a quadrupole analyzer, and this has led to widespread use of these analyzers in GC-MS applications. Developments in magnet design now provide scans on the order of 1 second per mass decade for magnetic analyzer instruments.

Fast scanning requires higher ion currents to achieve the same signal-to-noise ratio and, therefore, GC-MS has also led to a great increase in instrument sensitivity. Values of 1×10^{-9} coulombs per microgram are typical now, whereas values of 1×10^{-12} or less were typical 10 to 15 years ago. These gains arise largely from changes in ion source design which have been discussed.

Two other challenges inherent in GC-MS which led directly to the development of mass spectrometry data systems are the very large amounts of data generated by the combination (10 to 1000 spectra per sample are common) and the significant background signals encountered in GC-MS which necessitate background subtraction.

Before the advent of data systems, it was common practice for mass spectral data to consist of an oscillographic or strip chart recording of a series of peaks. It was necessary for the operator to determine the identity of some known peak, such as 18, from water and manually count integral masses from that point out to the mass of the sample. In addition, it was necessary to obtain intensities by direct measurement, and background subtraction was accomplished by direct one-for-one manual subtraction of these intensities. Such a procedure was practical for small numbers of spectra but was simply prohibitive for the volumes of data provided by a GC-MS instrument. Data systems are now available which accept data at the rate necessary for GC-MS and provide rapid output by electrostatic plotting of spectra which include a mass scale, normalized intensities, and background subtraction. Numerous other functions are also now provided by the data system including plots of selected ion intensities against spectrum number (*36*), correction for changes in sample quantity present during a scan, deconvolution of overlapping gas chromatography peaks (*37*), library search, and in the case of quadrupole analyzers, frequently instrument control. So important is the data system to a modern GC-MS that the combination is frequently referred to in the literature as GC-MS-DS. Direct GC-MS combination has been demonstrated with all of the ion sources discussed.

It is difficult to overemphasize the impact of GC-MS in terms of the sheer number of scientific disciplines affected. The power of this technique in mixture analysis has allowed major advances in our understanding of flavors and fragrances, environmental pollution, drug metabolism, and geochemistry, to list only a few.

High-Resolution Mass Spectrometry

For most analytical techniques, operation at a high resolution simply implies the ability to accomplish a given task with greater accuracy and precision; this is also true for mass spectrometry, but high-resolution mass spectrometry carries with it other implications as well. Operation at low resolution most frequently involves scanning of the instrument through its mass range and making a recording of the integral masses and intensities of the resolved ion beams. The high-resolution mode of operation is in general much less concerned with recording an entire spectrum or with the relative intensities of the ions but rather with the determination of mass particularly for molecular ions to a high degree of accuracy. Such accurate mass measurements are extremely useful, since by application of tables or simple iterative procedures they can lead to assignment of elemental composition. These measurements are always made relative to an internal standard, and are usually conducted in one of two ways. In the first procedure, called peak matching (*38*), the exact ratio of a sample and reference beam is determined by attenuating the accelerating voltage with a calibrated precision voltage divider such that the higher mass signal can be superimposed on the low mass signal. The ratio is simply read off the voltage divider and used to relate the sample and reference masses.

In the second procedure a spectrum is acquired by means of a data system; the data system calculates peak centroids, locates the peaks of the reference compound, and finally, because the scan function is known, interpolates the exact mass of the sample peaks. As part of the same procedure the data system can iterate through the various possible elemental compositions and assign these values as well. Because of the greatly reduced ion currents at high resolution which make scanning difficult, high-resolution spectra are also frequently recorded on photoplate. In this case the digitization and mass determinations are done offline in a subsequent step with a microdensitometer and computing techniques similar to those already described.

High resolution per se is not required for accurate determination of mass since peak centroids can be found accurately by a data system for relatively broad signals. It is necessary, however, that the peak of interest be clearly resolved, and high resolution is generally required to accomplish this.

Instruments having the highest resolutions have usually been used to determine the composition of petroleum oils (*39*) and, recently, for coal liquification products (*40*). It is necessary in these cases to determine both intensity and position, because the intensity is used to provide quantitative analysis. Unusually high resolution is required to resolve ion beams which vary in composition only by exchange of O_2 for S, both of which may be present in fossil fuel samples. This mass difference at mass 500 requires a resolution of 28,000. The most difficult separation normally encountered is exchange of H_2 for deuterium. A resolution of $> 322{,}000$ is required at mass 500.

Other applications include selected ion monitoring at high resolution in combination with gas chromatography. This procedure has the advantage of being sensitive to only one particular elemental composition eluting from a gas chromatograph and is, therefore, very resistant to interferences. High-resolution measurements of fragment ions are also useful since they can help in determinations of molecular structure based on interpretation of fragmentation patterns. High-resolution measurements are difficult with ion sources that produce low-intensity ion beams, such as field ionization.

Analysis of Metastable Transitions

It is difficult to ionize molecules without causing fragmentation. As a result, most molecular mass spectra exhibit fragment ions. It was realized very early that fragmentation could be predicted qualitatively on the basis of molecular structure and that, conversely, molecular structure could be deduced from fragmentation patterns. Such interpretation of mass spectra constitutes an independent discipline for which there is a large body of literature (*41*). Experience has shown that fragmentations may be quite

complex, often passing through numerous intermediates. Since these fragmentation pathways may not be obvious from the spectra, it is often important to study ion degradation schemes instrumentally. This can be done by analysis of metastable transitions. A metastable ion is defined as an ion arising from a fragmentation occurring outside the source in a field-free region. Such fragmentations, although slower, are generally assumed to mimic those occurring in the source. Such ions are accelerated in the ion source to the velocity correct for their precursor, but they undergo mass analysis at their degraded mass. These ions, therefore, when mass analyzed occur in the scan at mass values below their real value. Also, metastable ions fail to pass electric analyzers because they have incorrect kinetic energies.

A large number of schemes have been developed to observe these ions and to relate them to their precursors (*42*). One of these, called mass-analyzed ion kinetic energy spectrometry (MIKES) (*43*) deserves special mention. This technique is carried out on double-focusing magnetic sector mass spectrometers which have the magnetic sector preceding the electric sector. The analysis is done in the following way. The magnetic sector is tuned to the mass of an ion for which it is of interest to determine the daughters. Under these conditions this particular ion beam and no other is passing through the field-free region between the magnetic sector and the electric sector. If part of those ions undergo metastable decompositions, then the resulting daughter ions fail to pass the subsequent electric sector because they have incorrect kinetic energies. If, however, the voltage on the electric sector is scanned, then these ions can be made to strike the detector. The voltage at which the ions strike the detector can be related to mass by the following expression:

$$m_2 = (E_2 m_1)/E$$

where m_1 is the precursor mass, E and E_2 are the nominal and reduced sector voltages, respectively, and m_2 is the mass of the daughter ion. It is possible, therefore, to determine directly and unambiguously the mass of all of the daughter ions of a given precursor. This procedure can be repeated for all of the ions in a spectrum if desired, and entire fragmentation patterns can be mapped.

If a mass-analyzed ion kinetic energy spectrum is recorded of the molecular ion, this spectrum is as characteristic of the molecule as is a conventional mass spectrum. It will differ, however, from the conventional mass spectrum because the ions passing through the second field-free region have different internal energy distributions from those fragmenting in the ion source. MIKE spectra recorded on fragment ions may be compared with spectra of the same ion derived from a different molecule. In this way correlations of ion structure may be obtained.

If a small region of high pressure is provided in the field-free region, the ions may be induced to decompose as a result of collisions with neutral molecules. This procedure, called collision-induced dissociation (CID) (*44*), results in improved metastable intensities but the transitions observed may differ from those which occur spontaneously.

A particularly powerful aspect of MIKES is that it can be used to obtain spectra representative of pure compounds from very complex mixtures (*45*). This is possible because the magnetic sector serves as the separation device and spectra are obtained by scanning the electric sector. In order to be successful, the nominal mass selected must be monoisotopic, although unique daughters can be monitored without interference even in this case.

Conclusion

Mass spectrometry is undergoing an extremely rapid development which shows little indication of abating either in the areas of instrument refinement or of extended applications. It is interesting to consider that if combined ionization and ion optical efficiencies could be improved from the 8 ppm typical today to perhaps 10 percent, then an unknown sample would need to consist of only about 30,000 molecules. If selected ion monitoring could be used, perhaps 100 molecules would be sufficient. It is also interesting that even if attainable this rather remarkable sensitivity would still be at least an order of magnitude worse than the detection limit of the silk moth for its pheromone, bombykol (*46*).

References and Notes

1. K. Biemann *et al.*, *Science* **194**, 72 (1976); K. Biemann, T. Owen, D. R. Rushneck, A. L. Lafleur, D. W. Howarth, *ibid.* p. 76.
2. W. Wien, *Ann.. Phys.* **65**, 440 (1898); J. J. Thompson, *Philos. Mag.* **21**, 225 (1911); F. W. Aston, *ibid.* **38**, 707 (1919).
3. A. J. Dempster, *Phys. Rev.* **11**, 316 (1918).
4. J. Classen, *Jahrb. Hamb. Wiss. Anst. Beiheft* (1907); *Z. Phys.* **9**, 762 (1908).
5. J. Mattauch and R. Herzog, *Z. Phys.* **89**, 766 (1934).
6. E. G. Johnson and A. O. Nier, *Phys. Rev.* **91**, 10 (1953).
7. H. Hintenberger and L. A. König, *Advances in Mass Spectrometry Proceedings Conference, 1958*, J. D. Waldron, Ed. (Pergamon, New York, 1959), p. 16.
8. W. Paul and H. Steinwedel, *Z. Naturforsch. Teil A* **8**, 448 (1953).
9. B. A. Mamyrin, V. I. Karataev, D. V. Shmikk, V. A. Zagulin, *Sov. Phys. JETP* **37**, 45 (1973); W. A. Thomas, R. L. Miller, G. Sanzone, in *Proceedings of the 25th Annual Conference on Mass Spectrometry and Allied Topics*, A. G. Harrison, Ed. (1977), p. 93.
10. D. F. Hunt and F. W. Crow, *Anal. Chem.* **50**, 1781 (1978).
11. R. J. Beuhler and L. Friedman, in *Proceedings of the 25th Annual Conference on Mass Spectrometry and Allied Topics*, A. G. Harrison, Ed. (1977), p. 252.
12. B. W. Wilson, C. E. Costello, N. R. Mancuso, K. Biemann, in *Proceedings of the 26th Annual Conference on Mass Spectrometry And Allied Topics*, A. G. Harrison, Ed. (1978), p. 175.
13. C. E. Griffin, *Int. J. Mass Spectrom. Ion Phys.* **15**, 437 (1974).
14. W. H. McFadden, *Techniques of Combined Gas Chromatography/Mass Spectrometry: Applications in Organic Analysis* (Wiley, New York, 1973), pp. 164–220.
15. W. R. Anderson, Jr., W. Frick, G. D. Daves, Jr., *J. Am. Chem. Soc.* **100**, 1974 (1978).
16. B. Soltman, C. C. Sweeley, J. F. Holland, *Anal. Chem.* **49**, 1164 (1977).
17. J. H. Bowie and B. D. Williams, "Negative ion mass spectrometry of organic, organometallic, and coordination compounds," in *Mass Spectrometry, International Review of Science, Physical Chemistry*, A. MacColl, Ed. (Butterworths, London, 1976), series 2, vol. 5, pp. 89–127.
18. M. S. B. Munson and F. H. Field, *J. Am. Chem. Soc.* **88**, 2621 (1966).
19. F. H. Field, *Acc. Chem. Res.* **1**, 42 (1968).
20. B. Munson, *Anal. Chem.* **49**, 772A (1977).
21. M. A. Baldwin and F. W. McLafferty, *Org. Mass Spectrom.* **7**, 1353 (1973).
22. K. R. Jennings, in *Mass Spectrometry*, R. A. W. Johnstone, Senior Reporter (Specialist Periodical Reports, Chemical Society, London, 1977), vol. 4, pp. 203–215.
23. H. D. Beckey, *Principles of Field Ionization and Field Desorption Mass Spectrometry* (Pergamon, New York, 1977).
24. H. R. Schulten and H. D. Beckey, *Org. Mass Spectrom.* **6**, 885 (1972).
25. H. B. Linden, E. Hilt, H. D. Beckey, *J. Phys. E* **11**, 1033 (1978).
26. T. Matsuo, H. Matsuda, I. Katakuse, *Anal. Chem.* **51**, 1 (1979).
27. H. D. Beckey, *Int. J. Mass Spectrom. Ion Phys.* **2**, 500 (1969).
28. J. F. Holland, B. Soltman, C. C. Sweeney, *Biomed. Mass Spectrom.* **3**, 340 (1976).
29. F. W. Rollgen and K. H. Ott, *Z. Naturforsch. Teil A* **33**, 736 (1978).
30. W. V. Ligon, *Science* **204**, 198 (1979).
31. B. P. Stimpson and C. A. Evan, Jr., *J. Electrostat.* **5**, 411 (1978).
32. H. J. Veith, *Tetrahedron* **33**, 2825 (1977).
33. R. D. MacFarland and D. F. Torgerson, *Int. J. Mass Spectrom. Ion Phys.* **21**, 81 (1976).
34. A. Posthumus, P. G. Kistemaker, H. L. C. Meuzelaar, C. Ten Noever de Brauw, *Anal. Chem.* **50**, 985 (1978).
35. J. C. Holmes and F. A. Morrell, *Appl. Spectrosc.* **11**, 86 (1957).
36. R. A. Hites and K. Biemann, *Anal. Chem.* **42**, 858 (1970).
37. J. E. Biller and K. Biemann, in *Proceedings of the 22nd Annual Conference on Mass Spectrometry and Allied Topics*, J. L. Margrane, Ed. (1974), p. 430.
38. K. S. Quisenberry, T. J. Scolman, A. O. C. Nier, *Phys. Rev.* **102**, 1071 (1956).
39. H. E. Lumpkin, *Anal. Chem.* **36**, 2399 (1964).
40. T. Aczel, *Reviews in Analytical Chemistry* (Freund, Tel Aviv, 1972), p. 226.
41. H. Budzikiewicz, C. Djerassi, D. H. Williams, *Mass Spectrometry of Organic Compounds* (Holden-Day, San Francisco, 1967); M. C. Hamming and N. G. Foster, *Interpretation of Spectra of Organic Compounds* (Academic Press, New York, 1972).
42. R. G. Cooks, J. H. Beynon, R. M. Caprioli, G. R. Lester, *Metastable Ions* (Elsevier, Amsterdam, 1973).
43. J. H. Beynon *et al.*, *Anal. Chem.* **45**, 1023A (1973).
44. J. H. Beynon, M. Bertrand, E. G. Jones, R. G. Cooks, *Chem. Commun.* **341** (1972).
45. R. W. Kondrat, R. G. Cooks, *Anal. Chem.* **50**, 81A (1978).
46. K. E. Kaissling and E. Priesner, *Naturwissenschaften* **57**, 23 (1970).
47. W. V. Ligon and J. C. Cook, unpublished data.